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Dear Colleagues, Passionate researchers.

in my, albeit brief, experience as Editor-in-Chief of the journal Annali di Botanica I wanted to make an active contribution to its development by trying to bring to light important elements that have characterized the journal from the beginning.
It should not surprise you that I am a physical chemist, because several characteristics of Annali di Botanica can be traced back to the systemic vision that characterizes this discipline, which make it a reference not only for the study of ecosystems, but also for issues relating to solutions "nature based", widely cited in terms of conservation, planning and restoration of territories in a sustainable way as also underline by the strategies to reach different Sustainable Goals of the UN Agenda 2030.
Thus, together with the Editorial Committee, I wanted to give, already with the 2023 issue and now with this one which collects the 2024 articles, a multi-dimensional vision of this reality already "distilled" by the subtitle of the magazine "Coenology and plant ecology": defining " new trajectories" with respect to which to collect contributions from the various authors.
In fact, as in many articles appeared on Annali di Botanica during its prestigious history it is reported, starting from the description of the communities by the method of the phytosociology, we can capture the essence of the meaning of the "interaction" between plants and territories: in my vision, to construct multidimensional landscapes that cannot exclude humans.
So, "the effects of landscape plants on public Health; Historical use of medicinal plants and future potential: from phytotherapy to phytochemicals"; "Relevance of public active participation to address the complexity of science-based issues, such as biodiversity loss" (All titles of articles in 2024 issue) became very interesting subjects to construct a new approach to the study of the Nature from the point of view of Annali di Botanica.
As well shown in the past also in this journal by the articles of Prof. Sandro Pignatti (the Professor was in the editorial board from 1983, then he was co-editor with Prof. G. Mazzolani and from 1990 to 2007 his role was Managing Editor) and his research group, it is necessary "a thermodynamic approach to interpret the ecosystem Complexity".
In these terms he developed his scientific route on the path of Elya Prigogine, the physical chemist noted for his work on dissipative structures, complex systems, and irreversibility. Nobel Prize in Chemistry in the 1977.
To use Pignatti's words of absolute modernity, written in 1997 in Annali di botanica (Annali di Botanica, Vol. 55, 1997):
"When the association is described only by floristic data, it appears as a chaotic system. New methods for vegetation analysis are described, based on the complementarity of floristic and gynecological data. The use of the fingerprint approach to express ecological factors is discussed."
So, to green up Annali di Botanica, that dates back to 1884 when Pietro Romualdo Pirotta, Professor in Botany at the University of Rome, founded the Journal under the name "Annuario del Regio Istituto Botanico di Roma" (Volumes I-X, 1885-1901), which was later changed in 1902 to Annali di Botanica [Ann. Bot.(Roma)] we chose to celebrate in this issue the contribution of professor Sandro Pignatti, presenting the work of Tiziana Babusci and the past director of the Department Milena Altamura to concentrate and to catalogue the major part of his passionate career of research in the library of the department of Environmental Biology of Sapienza University of Rome, where he has worked. Tiziana is the Librarian of the beautifully space dedicate to the books in the jewel designed by the genius of Architect Giuseppe Capponi in The Institute of Botany and Pharmaceutical chemistry building built with futuristic solutions in fact of "natural" energy use in the period 1932-1935 into University campus "Citta universitaria di Roma".
The traces on which we develop our paths are based on our history, which is intertwined with that of the places in which we live and plants are always witnesses of this. For this reason, we also opened the Journal to the dimension of time with the article from the last issue by the prof. Nigro "The plants of Jericho. The earliest cultivars between symbiosis and domestication".
Starting from the researches of prof. Pignatti, his work was developed for many years into the laboratories at Botanical Garden of Rome, buttonhole of the Department of Environmental Biology of Sapienza University of Rome, and introducing an article about "Phytochemical analysis on the leaves of teucrium capitatum l. subsp. capitatum collected in the Botanical Garden of Rome".
Prior to my time as editor-in-chief, Prof. Fausto Manes was editor-in-chief of the journal for many years. He did a commendable job, leading the journal to acquire its first impact factor and thus gain international prominence. He went through difficult years for the scientific publishing

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sector and handed over to me a well-kept and healthy journal, for which I thank him from the bottom of my heart. I pass the baton to prof. Fabio Attorre, author with his colleagues of the article in this issue, Director of the Botanical Garden of Rome, as next Editorial-in-chief of the Annali di Botanica, thanking Dr. Clelia Palanza, Assistant editor, who with her precious work ensures continuity in "the care" and the creation of the journal.

Cesare Manetti

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ASSESSMENT OF CURRENT STATUS AND CONSERVATION STRATEGIES OF SOME HIGH VALUED MEDICINAL PLANTS FROM HIMALAYAN REGIONS

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Abstract – The Himalayan region is well known for its abundant biodiversity, especially for the wide variety of highly valued medicinal plants that are native to the region and have been utilized for generations in traditional healing practices. The current status and strategies for conservation of selected high-value medicinal plants from the Himalayan areas are evaluated in this review investigation, with an emphasis on the plant ecological significance, threats, conservation difficulties, and sustainable management techniques. It explores the risk factors that these plants encounter, which include habitat loss from deforestation, overharvesting, unethical collecting methods, the effects of climate change, and invasive species. The medicinal plants are threatened due to the overexploitation and being categorized into the various categories by IUCN. The management of medicinal plant resources is therefore so much crucial in the current time. There are a number of medicinal plants that grow in the Himalayan region that possess excellent medicinal properties. Medicinal plants like *Rhododendron* sp., *Aconitum* sp., *Bacopa monnieri, Glycyrrhiza glabra, Picrorhiza kurroa, Juglans regia* and other important medicinal plants mentioned in this review paper are of great importance. To conserve these medicinal flora and sustainable future availability, various *in situ* and *ex situ* approaches are considered, among them the most advanced are biotechnological approach like tissue culture is an advanced techniques that enables mass cultivation of medicinal plants. These techniques have the potential to meet the growing demand for plant derived medicines. The application of plant tissue culture approaches has resulted in substantial research into medicinal plant biodiversity conservation. *In vitro* regeneration is an extremely powerful biotechnological tool for propagating medicinal plants and increasing their bioactive compounds. The aim of this review is to provide an overview on the assessment of the current status and conservation challenges relating to high-valued botanical species in the Himalayan region. To ensure the sustainment of these species for future generations, conservation strategies must take into account their ecological, economic, and cultural dimensions.

Keywords: biodiversity, conservation, himalayas, medicinal plants, threats, secondary metabolites.

Introduction

Indian sub-continent and the Eurasian continent have collided constantly throughout history creating the Himalayan range. As a result of the varied habitats of the Himalayas, the region has a rich biodiversity spanning 3000 km from Northern Pakistan, Nepal, Bhutan, and the North-Western and North-Eastern parts of India. Lower Himalayas nurture luxuriant trees including *Pinus roxburghii* and *Alnus nitida* along the slopes, while moist terraces support *Alnus nepalensis*, *Quercus leucotrichophora*, *Rhododendron arboreum* etc. A higher altitude zone of the Lower Himalayas is characterized by forests of *Quercus semecarpifolia*, *Quercus floribunda*, and *Pinus wallichiana*. Several species of trees exist in the Great Himalayan range, including *Abies* sp., *Betula utilis*, *Rhododendron campanulatum*, and *Juniperus communis*. A unique assemblage of herbs can be found in the alpine meadows and grasslands at higher altitudes (above 3600

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meters). Some of the vital plants of these heights are the herbs of genera *Aconitum*, *Picrorhiza*, *Rheum*, *Meconopsis*, and the scrub species *Rhododendron anthopogon* and *Rhododendron lepidotum*. Some of the most endemism-rich Himalayan families are Rosaceae and Umbelliferae. A total of 3160 endemic plant species have been recorded. In all, over 10,000 plant species are used in medicine at some time or another with close to 18 % of the 70,000 known plant species having known therapeutic properties. It is mentioned in the ancient Indian texts that herbs have been growing in the Himalayan region for centuries. The ancient system of Ayurveda is a system of health and well-being. The repository still contains antiquated information (Kala, 2000; Patil et al., 2019; Sharma et al., 2022). According to Atharveda, countless herbs have medicinal properties for curing various maladies. The use of herbal plants as a remedy for a sick person is completely safe, as there are no side effects on the body. Due to their harmony with nature, herbal plants are preferred over synthetically treated items and manufactured drugs (Sharma et al., 2020; Thakur et al., 2020). Ayurvedic herbs possess a greater capacity to provide holistic healing to individuals in the long run compared to other drugs and medications (Khalsa, 2008). According to Rahman et al., (2011) the herbs can not only be used for producing medicines for humans but also be utilized as a source of bio-pesticides (Castor plant), dyes (Rattan jot), brews (*Rhododendron*) and perfumes (*Jatamansi*) (Sharma et al., 2024). A number of studies revealed there has been an increase in people turning to natural cures for a wide variety of ailments. Current healthcare systems around the world rely heavily on plant-based drugs. The majority of the world's population uses locally available herbs for medicinal purposes (Dubey et al., 2004; Sharma et al., 2023). Therefore, keeping in mind the severity of medicinal plants, this review paper aimed to review an important medicinal plant of the Indian Himalayas and discussed their IUCN status, description, distribution pattern, phytochemistry, medicinal properties, as well as the threats and conservation practices associated with them. This review paper attempt to assess the current status and conservation strategies of selected high-valued medicinal plants from the Himalayan regions.

Current status of medicinal plants

Only about 1% of the estimated 5,00,000 plant species have been investigated phytochemically, proving that these plants hold great potential for discovering new bioactive compounds. Traditional medicine provides primary healthcare to approximately 82% of the population in developing countries. In terms of area, India makes up 2.4% of the global biodiversity with 8% of the world's biotic resources, making it one of the 12 mega biodiversity hotspots of the world (Sharma et al., 2020; Thakur et al., 2020). Approximately 17500 species of high plants are found in India, including 64 gymnosperms, 1200 pteridophytes, 2850 bryophytes, 2021 lichens, 15500 fungi, and 6500 algae (Sanjappa, 2005). The Indian subcontinent has a lot of endemic flora or plants that are endemic to the region. Species diversity, genetic diversity, and habitat diversity are all found in India with abundant medicinal plant diversity. Mukherjee & Wahile (2006) concluded that the country has a wide variety of medicinal plants that are rich in all three aspects of biodiversity. It is estimated that 90 percent of India's medicinal plant diversity is found in forests, while only 10 percent of medical plants in India are found in non-forest habitats (Wakdikar, 2004). Out of 18,665 plant species in the world, only 3000 medicinal plants are used in the classic systems of medicine like Ayurveda, Siddha, and Unani (Schippmann et al., 2006; Sharma et al., 2021). Schippmann et al. (1990) estimate that one-fifth of all plants in India have medicinal properties. Approximately 12.5% of plant species in the world have medicinal value, while 20% of plant species in India are used for their medicinal properties. Hamilton (2003) estimates that India has approximately 44% of medicinal flora. In spite of the fact that it is difficult to estimate the number of medicinal plants present throughout the world, the fact remains that India, with its rich biodiversity, ranks first in terms of the percentage of its flora that contains active compounds (Mandal, 1999). According to Sarasan et al. (2006), over eight thousand plant species have been added to the ICUN (International Union for the Conservation of Nature Resources) and a RET endangered species list since 1996. There has been an increase of over 60% in the number of plants classified as critically endangered during the same period. For the higher plant species to become extinct or nearly extinct by 2050, up to 60,000 of them could become extinct or nearly extinct, according to the International Union for Conservation of Nature (ICUN) and the World Wildlife Fund (WWF) (Phani Kumar et al., 2011; Sharma et al., 2024). The IUCN status of the medicinal plants is given in Tab. 1. In order to preserve and manage these medicinal plant categories and to protect them from extinction, conservation and management plans would be necessary.

Threats to medicinal plants diversity

There are 560 plant species in India listed on the Red List of Threatened Species by the International Union for the Conservation of Nature and Natural Resources (IUCN). About 247 of these plant species are threatened. It has been estimated by the IUCN that about 12.5% of the world's vascular plants, around 34000 species, are under threat (Phartyal et al., 2002). Based on the severity of threats, IUCN

Table 1. Consolidated list of some high valued medicinal plant species of Himalayan Regions.

Botanical name	Family	Plant Part Used	IUCN Status
Aconitum chasmanthum **Stapf ex Holmes**	Ranunculaceae	Rhizome, fruits, leaves	CE
Aconitum ferox **Wall. ex Ser.**	Ranunculaceae	Rhizome, fruits, leaves	CE
Aconitum heterophyllum **Wall. ex Royle**	Ranunculaceae	Rhizome, fruits, leaves	EN
Acorus calamus **L.**	Acoraceae	Leaves, stem and leaves	VU
Swertia chirata **Buch.-Ham. ex Wall.**	Gentianaceae	Leaves, stem	CE
Taxus wallichiana var. chinensis **(Pilg.) Florin**	Taxaceae	Bark, stem	CE
Rauvolfia serpentina **(L.) Benth. ex Kurz**	Apocyanaceae	Flower	EN
Berberis aristata **DC.**	Berberidaceae	Stem, roots and fruits	VU
Asparagus racemosus **Willd.**	Asparagaceae	Dried roots	EN
Nardostachys jatamansi **(D. Don) DC.**	Caprifoliaceae	Rhizomes	CE
Picrorhiza kurroa **Royle ex Benth.**	Scrophulariaceae	Leaves, bark and roots.	EN
Podophyllum hexandrum **Royle**	Podophyllaceae	Leaves, rhizomes, fruits	EN
Aegle marmelos **(L.) Corrêa**	Rutaceae	Leaves, bark, roots, fruits, and seeds	VU
Saussurea costus **(Falc.) Lipsch.**	Asteraceae	Roots	EN
Rhododendron arboreum **Sm.**	Ericaceae	Flowers	EN
Angelica glauca **Edgew.**	Apiaceae	Roots	EN
Arnebia euchroma **(Royle) I.M.Johnst.**	Boraginaceae	Root, leaves	CE
Carum carvi **L.**	Apiaceae	Dry seed	LC
Capparis spinosa **L.**	Capparaceae	Green shoots, fruits	LR
Clematis tibetana **Kuntze**	Ranunculaceae	Flower and soft stem	NT
Dracocephallum heterophyllum **Benth**	Lamiaceae	Whole plant	R
Dactylorhiza hatagirea **(D.Don) Soó**	Orchidaceae	Roots, tubers	CE
Ephedra gerardiana **Wall. ex Stapf**	Ephedraceae	Node, red fruits, stem,root	EN
Geranium wallichianum **D.Don ex Sweet**	Geraniaceae	Roots, leaf and flower	EN
Hippophae rhamnoides **L.**	Elaeagnaceae	Fruits, seed, whole plant	VU
Hyoscyamus niger **L.**	Solanaceae	Flower, fruits	EN
Iris hookeriana **Foster**	Iridaceae	Flower and seeds	CE
Juniperus communis **L.**	Cupressaceae	Leaves, fruits	VU
Justicia adhatoda **L.**	Acanthaceae	Whole plant	VU
Myricaria squamosa **Desv.**	Tamaricaceae	Flowering spikes	EN
Oxytropis lapponica **(Wahlenb.) Gay**	Leguminosae	Flower and leaves	NT
Pedicularis longiflora **Rudolph**	Orobanchaceae	Leaves and flowers	NT
Rosa webbiana **Wall. ex Royle**	Rosaceae	Fruit, flower	NT
Taraxacum officinale **F.H.Wigg**	Compositae	Stems and flowers	LC
Tribulus terrestris **L.**	Zygophyllaceae	Fruits	LC
Waldheimia tomentosa **(Decne.) Regel**	Asteraceae	Flower and fruits	R
Juglans regia **L.**	Juglandaceae	Fruits, bark, leaves, roots	EN

CE: Critical Endangered, EN: Endangered, VU: Vulnerable, LC: Least Concern, LR: Lower Risk, NT: Nearly Threatened, R: Rare.

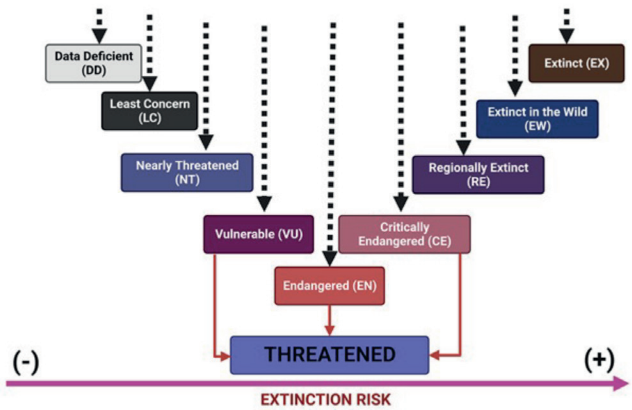

Figure 1. IUCN Red List categories

identifies and categorizes plant species according to Singh et al. (2006): extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, and data deficient. The term rare refers to a species that has a small population that is not endangered or vulnerable but is at risk. They are in danger of extinction in many cases (Fig. 1). Globally, herbal medicine has gained importance over the past few decades, while awareness of dwindling supplies of medicinal plants has been delayed (Bodeker, 2002). Plants used in phytopharmaceutical preparations are primarily obtained from naturally growing areas (Sharma et al., 2020; Thakur et al. 2020). Due to destructive harvesting practices and over-harvesting and exploitation of medicinal plants for the production of medicines without regard for the future, the genetic diversity of medicinal plants is being lost at an alarming rate. There are also numerous factors that threaten their existence, such as habitat destruction, forest degradation, agricultural encroachment, urbanization, pollution etc. (Gupta et al., 1998; Thakur et al., 2021) in Fig. 2.

In view of the vastly increasing world population, increasing anthropogenic activities, rapidly diminishing natural ecosystems, etc. In the midst of climate change, ocean acidification, and a range of other anthropogenic environmental impacts (Rands et al., 2010), native habitats of many herbs and trees are deteriorating, resulting in unsustainable exploitation of Earth's biological diversity. In order to restock the lost biodiversity, huge amounts of

Figure 2. Various threats to medicinal plants of Himalayan regions.

money are spent every year, and there are many conservation protocols available at the present time. There is no substantial improvement in the status of these medicinal plant species in nature, and the number of threatened plant species is gradually increasing (Tripathi, 2008; Mukherjee, 2009). The wide range of medicinal herbs found in the Himalayas are being endangered by several human-caused factors and land use patterns, which can harm the fragile natural equilibrium and threaten indigenous remedies (Sharma et al., 2020). Human activities that are affecting medicinal botanical species and their natural habitats either directly or indirectly constitute the source of these vulnerabilities (Sharma et al., 2021). Due to harvesting, building infrastructure, the agricultural sector, and human settlements, the Himalayan region is experiencing major degradation in land use cover. This is destroying natural habitats for medicinal plants, which is causing population reductions. Due to excessive harvesting and unethical practices for collecting medicinal plants for both traditional and commercial purposes, important medicinal species are being lost, imposing stress on nearby communities. The expansion of agriculture, including cash crops and monoculture farming, leads to the conversion of natural habitats into cultivated lands, soil degradation, biodiversity loss, and increased agrochemical use. In the Himalayan region, rapid infrastructure development and urbanization lead to habitat fragmentation, ecological disruption, plant isolation, and the spread of invasive species, all of which contribute to the decrease of medicinal plants. The Himalayan region's medicinal plant richness, phenology, and distribution are all being impacted by climate change, which puts them under stress and makes them more susceptible to other human-caused potential risks. Integrated conservation methods, including sustainable land use practices and responsible harvesting, involving local populations, policymakers, researchers, and conservation organizations are essential to maintain the rich medicinal plants found in the Himalayan areas (Tali et al., 2018; Ganie et al., 2019; Mishra et al., 2023).

Conservation of medicinal plants diversity

A major focus of the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro was the loss of biodiversity and preserving it (Fig. 3).

So, managing and conserving traditional medicinal plants has become a matter of necessity. Despite the fact that the most effective way to conserve threatened medicinal species is to manage wild populations and their native habitats, some of these plants do not produce seeds or their seeds are too small for soil germination. A plant may be discarded if the

Figure 3. Conservation strategies adopted to conserve medicinal flora

product is of poor quality for commercial distribution due to its heterozygous nature. It shows significant variations in growth, habit, and yield even when grown from seeds. Additionally, there are a number of cultivars that are unsuitable for vegetative propagation through cuttings or grafts, thereby limiting the potential for multiplication of the desired cultivars. As a result, many plants propagated by vegetative means contain systemic bacteria, fungi, and viruses that may adversely affect the appearance and quality of selected plants (Murch et al., 2000). Consequently, it becomes a general problem to multiply disease-free planting materials in large numbers. According to Sarasan et al. (2006) *ex situ* conservation techniques can overcome all these barriers (Negash et al., 2001). Through cultivation and maintenance of plant propagules in plant tissue culture repositories, threatened medicinal plants can be conserved ex-situ using methods outside their natural habitats (Rands et al., 2010). Mass propagation and germplasm conservation have become increasingly useful with *in vitro* techniques due to their superiority over conventional methods of propagation and some distinct advantages over alternative approaches. Among them is (1) collection can be made at any time during the flowering season, provided seed material is not required, (2) clonal material can be produced in situations where it is necessary to maintain elite genotypes by producing clonal material, (3) By using meristem culture, viruses can be eliminated from contaminated tissues, (4) It may be possible to facilitate the germination of seeds and embryos that are difficult to germinate or immature for breeding purposes, (5) In case there is a need for stocks, micropropagation procedures may be used for rapid multiplication at any time, (6) If it comes to germplasm distribution across borders, *invitro* cultures might be a more effective method, in terms of germplasm health status. Storage space requirements are greatly reduced using *in vitro* techniques compared with field storage, another positive aspect. Climate fluctuations,

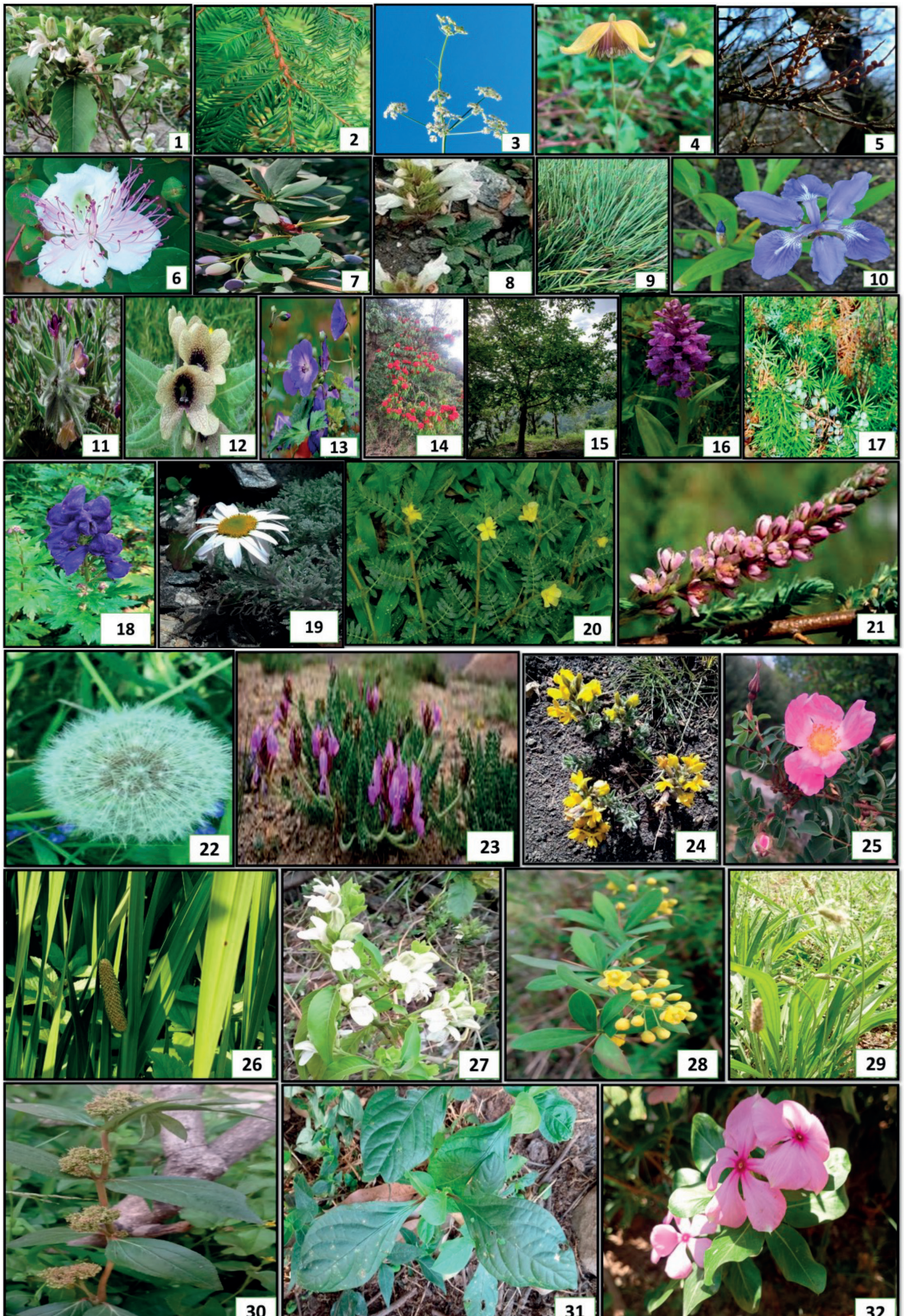

Figure 4. Medicinal flora of Himalayan regions (Adapted from authentic sources: https://identify.plantnet.org/; https://www.flowersofindia.net/

pests, cyclones, insects, and pathogens do not affect cultures in storage facilities (Bhojwani & Dennis, 1999; Shibli et al., 2006). In this regard, micro-propagation has significant potential as a means of achieving true-to-type, rapid and massive multiplication. Also, callus-derived plants exhibit a high genetic diversity that may be exploited to generate superior clones/varieties, particularly in vegetatively propagated species. Medicinally important species that are difficult to regenerate by conventional methods can be multiplied and preserved by tissue culture within short periods of time and within limited space. Cell and tissue culture *in vitro* has become increasingly popular in recent years as a means of conserving plant germplasm for long-term survival, propagating large-scale revegetation in mass quantities, and studying genetic manipulation under precisely controlled conditions. It may be possible to preserve biodiversity of native medicinal plants through a combination of *in vitro* propagation methods and cryopreservation (Singh et al., 2006).

Biotechnological approaches for conservation of medicinal plants

By utilizing scientific developments to address conservation problems and encourage responsible utilization, biotechnological techniques are essential to the conservation of medicinal plants. Micropropagation and tissue culture are both biotechnological methods used to mass-produce plants from small amounts of plant tissue, including embryos, meristems, or stem cells. This method prevents the decline of natural populations while facilitating the quick growth of threatened medicinal plant species (Sharma & Thakur, 2020). Herbal medicine uses cryopreservation methods, like liquid nitrogen cryogenic storage, to maintain the genetic variety of plants. With the use of this technique, plant germplasm such as seeds, embryos, branch tips, or pollen can be preserved for a long time in a dormant state for later use. The synthesis of secondary metabolites, including bioactive substances with therapeutic potential, is stimulated by biotechnological techniques like metabolic engineering, elicitation, as well as plant cell and tissue culture (Sharma et al., 2021). A sustained supply can be ensured by minimizing the demand for wild harvesting by optimizing the production of valuable components in controlled conditions found in bioreactors or culture systems. Pharmacological and functional investigations into medicinal plants are carried out using biotechnological technologies in order to gain insight into their therapeutic characteristics, mode of action, safety, and effectiveness. This information contributes in the creation of quality control guidelines, evidence-based herbal remedies, and legal frameworks for long-term use (Sharma & Thakur, 2020; Sharma et al., 2021; Sharma et al., 2024).

Medicinal flora of Himalayas

The threatened medicinal plants from Indian Himalayan Regions are shown in Fig. 4 (1-32).

***Adhatoda vasica* Nees**
***Taxus baccata* L.**
***Carum carvi* L.**
***Clematis tibetana* Kuntze**
***Hippophae rhamnoides* L.**
***Capparis spinosa* L.**
***Berberis aristata* DC**
***Dracocephalum heterophyllum* Benth.**
***Ephedra gerardiana* Wall. ex Stapf**
***Iris hookeriana* Foster**
***Arnebia euchroma* (Royle) I.M.Johnst.**
***Hyoscyamus niger* L.**
***Geranium wallichianum* D.Don ex Sweet**
***Rhododendron arboreum* Sm.**
***Juglans regia* L.**
***Dactylorhiza hatagirea* (D.Don) Soó**
***Juniperus communis* L.**
***Aconitum napellus* L.**
***Waldheimia tomentosa* (Decne.) Regel**
***Tribulus terrestris* L.**
***Myricaria squamosa* Desv.**
***Taraxacum officinale* Webb**
***Oxytropis acanthacea* Jurtzev**
***Pedicularis longiflora* Rudolph**
***Rosa webbiana* Wall. ex Royle**
***Acorus calamus* L.**
***Justicia adhatoda* L.**
***Berberis lycium* Royle**
***Plantago ovata* Forssk.**
***Euphorbia hirta* L.**
***Achyranthes aspera* L.**
***Catharanthus roseus* (L.) G. Don**

***RHODODENDRON*:** A species of Rhododendron commonly known as Buransh or Laligurans is classified in the Ericaceae family (Iqbal & Negi, 2017). According to Bhattacharyya (2011), India is home to only 12 species of Rhododendrons out of 80 species worldwide. The local population of the Indian Himalayan Region is reported to value most of these species highly. Incense and fuelwood are two of the most widely exploited species. Despite its evergreen nature, it can reach heights of up to 20 meters. Leaf shape is narrow and lanceolate, crowded towards the base. Their length is 10-20 cm and their width is 3-4 cm. On the top of the leaves, the color is glossy green, while on the back, the color is rustic brown. There are several shades of pink to red in the flower (Sekar & Srivastava, 2010). The flowers of buransh trees grow in bright red colors at lower altitudes. Seeds are ellipsoid and the fruit is capsular. It is recommended to collect seeds in the last two to three weeks of October (Singh et al., 2003). For well-flourished growth of *Rhododendron* species, the moist slopes of the eastern Himalayas provide the ideal environment. In Uttarakhand, the *R. arboreum* species are found at altitudes between 1500 m and 3500 m. Among the few plants that can survive such acidic soil are the *R. arboreum* species (Tiwari & Chauhan, 2006). Different parts of the *Rhododendron* tree contain a variety of phytochemicals. Rhododendron leaves are rich in flavonoids, protecting the heart from oxidants. Leaf and flower extracts contain quercetin. The leaves are rich in rutin and sterol. Several compounds can be found in the bark, including betulin, lupeol, and ursolic acid. Compared with synthetic antioxidant drugs, *Rhododendron* extracts exhibit high antioxidant properties (Prakash et al., 2007). Plants native to hilly regions such as *R. arboreum* are used to make jam and jellies. Dysentery and dyspepsia can also be treated with fresh blossoms. Its bark contains bioactive substances that exhibit antifungal properties. An ethanolic extract of the leaves is shown to be antitumor in nature. Several *Rhododendron* species are used for fuel, including *R. barbatum*, *R. falconeri*, and *R. hodgsonii*. Incense sticks are made from leaves and twigs of *R. anthopogon*. A local brew is prepared with fresh corollas from *R. arboreum* and *R. cinnabarium*. Cups, spoons, boxes, and saddles are made from the tender leaves and woods. *R. lepidotum* bark is used for making drinks that have purgative properties. In addition to treating indigestion and lung infection, *R. anthopogon* leaves and flowers can also help with hay fever.

***ACONITUM*:** In the genus Aconitum, there are 250 species of plants that are called aconite, wolfbane, queen's poison, or monkshood. It is a member of the Ranunculaceae family. Many traditional medicines incorporate it into their formulations (Jeelani et al., 2015). Despite the deadly nature of some Aconitum species, they have considerable therapeutic value. The plant's essential compounds must be extracted and formulated in a safe manner in order to overcome its toxic nature (Singhuber et al., 2009). The *Aconitum heterophyllum* is one of the most prevalent species of Aconitum and is often known as Atis, Ativisha, and Indian Atees. An important component of the Ayurvedic system of medicine, it is one of the most valued drugs. Various states of the Himalayan range are threatened by the genus due to the incredibly high demand for raw drugs on their local market (Jabbour & Renner, 2012). Tubers are economically important. These tubers are biennials and are paired. It has a simple stem with numerous branches that are erect and erect. It reaches a height of 15 to 20 centimeters. There are long petioles, glabrous leaves, and heteromorphic leaves. Black pyramidal seeds are embedded in violet-blue sepals. Ativisha grows in the alpine and sub-alpine areas of the Himalayas between 2000 and 5000 meters. It is indigenous to the Western Himalayas (Raina et al., 2011). It is found in abundance in Aconitum species to produce diterpene alkaloids and flavonoids. The plant's medicinal properties are attributed to the alkaloid aconitine in its tubers. As well as tannic acid, starch, fatty acids and their glycerides, carbohydrates, and others, the plant contains various other compounds (Bahuguna et al., 2000). In the case of communicable diseases, such as measles, aconite can prove useful. In addition, it successfully treats diseases such as asthma, diabetes, leukoderma, leprosy, and convulsions. Plant components such as flavonoids and phenols act as antioxidants and antibacterials. Coughs and congestion are effectively treated with it. In addition to being aphrodisiac, it also contains diuretic properties (Beigh et al., 2006). The hot potency of *Aconitum heterophyllum* makes it a popular ingredient in Ayurvedic medicine. Laxative properties are found in the seeds. Anorexia, arthritis, and ascaris are all treated effectively with the roots (Sekar & Srivastava, 2010). There are several names for Aconitum ferox, including Meetha Vish and Monkshood. The sedative nature of this agent also makes it effective in treating severe anxiety issues, fever, heart disease, asthma, and arthritis, as well as diuretic action. Nepal Monkshood, also known as safed bish or Nepal Monkshood, is a tall hairless herb with tuberous roots. It is used for treating acute headaches and rheumatism, as well as for treating boils. The roots of *Aconitum chasmanthum* are the most economically valuable part of the herb.

***BACOPA MONNIERI*:** Brahmi is the common name for *Bacopa monnieri*. It has been used extensively for more than 3000 years by Ayurveda and other ancient Indian medical systems. The plant belongs to the Scrophulariaceae family. There are over 4500 species in this family (Yadav et al., 2013). There is medicinal value to the entire plant. Both normal children and mentally retarded children have shown promising results with this method of improving memory and other psychological abilities. The Brahmi plant stalk grows from 10 to 30 cm in length and is 1-2mm thick (Yadav et al., 2013). Brahmi is an aromatic plant with a tender stem that can reach 10 to 30cm. There are 200 succulent leaves on the main stem and sessile leaves arranged oppositely. The flowering of the plant and the emergence of the fruit occur in the summer. A white to purple-blue flower can be found on it.

Despite the fruit's ovoid shape, its aroma is not discernible. Cooling properties are present in it. Based on Jana's (2006) comparison of the Indian pennywort with *B. monnieri*, the latter turned out to be superior to the former. It is native to the tropics and sub-tropics. The species can be found all over India and Nepal, especially in moist and wet conditions (Sharma et al., 2013). In addition to alkaloids, saponins, glycosides, flavonoids, and stigmasterols, this plant contains several compounds that have therapeutic benefits. Bacosides are the saponins of Brahmi. Flavonoids found in it include luteolin and apigenin. There are two alkaloids that have been isolated from Brahmi, herpes time and brahmine, which if taken at a high dosage may cause extreme headaches (Mitra et al., 2010). With Bacopa, it is possible to treat conditions like eczema, ulcerations, rashes, and rashes. A brain tonic, it is a popular medicine used to treat amnesia and Alzheimer's diseases. By detoxifying the blood, it removes toxins. In addition to preventing hair loss and premature graying, it is also an effective anti-hair loss and anti-hair loss herb. Hair follicles are rejuvenated and baldness is prevented (Bhandari et al., 2006). Anti-diabetic, anti-cancer, and anti-arthritic, it exhibits anti-diabetic and anti-cancer properties. A powerful antioxidant in Brahmi prevents free radical damage to the body (Rakhi & Vashistha, 2011). Aquatic *B. australis* plants are used in aquariums as decorative pieces. The perennial medicinal herb *B. crenata* is also known as moneywort. A creeping plant with an annual stem, *B. repens* can be found in the desert. A commercial use is not available for *B. rotundifolia* plant.

***GLYCYRRHIZA*:** Liquorice is the common name for *Glycyrrhiza glabra*. A variety of diseases can be cured using its ethnopharmacological value throughout the world. It is the roots and tubers of plants that have the greatest medicinal value (Thakur & Raj, 2017). Mulaithi and Yashtimadu are also named for this plant. It is used for flavoring because of its sweet taste. Ayurveda and Siddha use this plant extensively for medicinal purposes. This shrub is perennial in nature and grows to a height of 120 cm. This plant flowers after 2-3 years of planting and is generally pale blue in color. A pod consists of 2 to 5 seeds and measures about 2 cm x 2.5 cm. The roots are cylindrical, which have a diameter of 2 cm and are in a stolen form (Chhetri, 2007). In India, it is common to cultivate glycyrrhiza in areas of the Punjab and the sub-Himalayas. In between the Gangetic plains and the Himalayas, the Sub-Himalayan tract occupies an elevation range of 400 to 1200 meters and above sea level (Dhar et al., 2000). There are a number of compounds that contribute to licorice root's aroma, including anethole, which makes up 3% of the total volatile compounds. Glycyrrhizin gives the fruit its sweet taste, containing 30 to 50 times more sweetness than sugar (Gaur et al., 2010). These plants were used in traditional medicine by the Gujjar community in India. The sugary taste is less immediate than sugar, it lasts a lot longer, and it is tart at first but then it gradually becomes less tart. The root of liquorice contains two isoflavones, glabrene, and glabridin, both of which are phytoestrogens. Aside from saponin triterpenes (glycyrrhetinic acid, liquiric acid, and glycyrrhizin), the roots also contain flavonoids, coumarins, sugars, tannins, starch, choline, amino acids, and phytosterols, which are glycosides. There are many advantages to using Mulethi. It can reduce acid reflux and indigestion and can help to prevent ulcer formation by lowering stomach acid levels. It uses action on action to increase the flow of bile and lower the cholesterol level in the body. In terms of respiratory organ irritation and inflammation, this medicinal plant is useful for soothing and healing those symptoms. By enhancing levels of interferon in the body, Licorice improves the body's resistance power in the face of viruses by competing with them and defending the body from them. Moreover, it can also help with some symptoms of premenstrual syndrome (PMS), such as irritability, breast tenderness, and gassiness, which are commonly experienced during the premenstrual period. As a result of its anti-allergic properties, it has proven to be effective in treating allergic rhinitis, conjunctivitis and bronchial asthma. The ingredient can also be used to treat skin diseases such as eczema, psoriasis, and dermatitis (Thakur & Raj, 2017). In general, *G. asperrima* is commonly known as Chinese Liquorice and is useful for treating acid reflux and other reflux-related problems. The herb *G. glandulifera* contains a high concentration of betulic acid. Liquorice of American origin, *G. lepidota*, has a warm, aromatic quality. Prostate enlargement may be treated with *G. uralensis*, an herbal remedy.

***BERBERIS*:** This plant belongs to the family Berberidaceae and is also known as Indian barberry in English or Daru Haldi in Hindi. Around 500 species of Berberis can be found worldwide. There are a number of Berberis species that have gained significant prominence in traditional medicine. It is a perennial shrub that grows well in temperate climates (Komal et al., 2011). Depending on the variety, it can reach a height of up to 3 meters. Yellow flowers are present in the plant. A variety of yellow to pink seeds are contained in the fruit, which is shaped as an ovoid berry. It has a sweet to acidic taste (Singh & Kakkar, 2009). Among Indian Himalayan plants, Berberis is well known for its herbal properties. It grows in small patches on slopes of hills. It is found in Uttarakhand and Himachal Pradesh at altitudes between 1800 and 3000 meters (middle altitude areas). Wild areas are generally the source of this plant material. A status of endangered has been assigned to this plant by the IUCN. The plant is usually grown for the fruits it produces (Samant et al., 2007). Berberine, a quaternary iso-quinoline alkaloid found mostly in the stems and roots of this herbal plant, is its most important constituent. Roots and stems usually contain it. Quaternary ammonium salts of iso-quinoline alkaloids make up the bark. The leaves and stems of plants can also be used to extract lignans. *Berberis* species in the western Himalayas are rich in fibre, protein, fats and some minerals (Srivastava et al., 2001). Melasma is cured by Berberis extremely effectively. Counteracting stomach disorders is one of its benefits. Liver disorders can be prevented with its use. Due to its ability to prevent water loss, it is used as an occasional mild laxative (Joshi et al., 2011). Jaundice, diabetes, inflammations, and wounds have been treated traditionally with the roots, stems, leaves, and fruits of the plant. (Ray et al., 2011) have confirmed that this plant extracts contain antibacterial, antiviral, antifungal, anticancer, anti-inflammatory, and antidiabetic properties. Inflammatory properties are present in *B. vulgaris*. There are antimicrobial properties in *B. heterophylla*. As a remedy for cholera and serious diarrhea, *B. lyceum* is highly effective (Das et al., 2009). Antimicrobial activity and chronic inflammation are effective characteristics of *B. aetnensis*. Eyes, liver, and heart disease can be alleviated with *B. lyceum*. The antibacterial properties of *B. repens* are well known.

AEGLE MARMELOS**:** As a member of the Rutaceae family, *Aegle marmelos* is widely recognized as the Bael Tree in tropical and subtropical regions, and is a popular vulnerable medicinal plant. Kala et al. (2006) reported that this plant contains a variety of alkaloids, including aegline, marmesin, marmin, and marmelosin. In addition to diarrhea, dysentery, dyspepsia, malaria, fever, jaundice, and skin diseases like ulcers, urticaria, and eczema, nearly all parts of the tree are used to prepare herbal medicines (Sharma & Thokchom, 2014).

7. HEDYCHIUM**:** This plant belongs to the family Zingiberaceae, also known as Kapoor in Hindi, spiked ginger lily in English, and Shati in Ayurveda. Despite its small size, it is a hardy rhizomatous herb (Jugran et al., 2011). There are orange yellow flowers on this perennial herb which grows to 1-2 meters high. Seeding occurs between September and October, with flowering between July and August. There are fragrant leaves on this 1.5-metre-tall plant with a robust stem. The leaves of this plant are lanceolate and 30cm long. Despite its strong odour, the rhizome tastes bitter. The aril has a black color with red markings (Rawat et al., 2011). An endemic herb species of the South-East Asian region is *H. spicatum* and it is found in a variety of countries throughout the region. In addition to being native to the Himalayan region, this species also occupies a wide range of habitats set within subtropical and temperate climate zones in the region. In the Central Himalayan Region of India, the species can be commonly found at altitudes varying between 1200m and 2400 m in moist and rocky habitat near a water body or mixed forest, surrounded by oak trees or oak and pine trees (Rawat et al., 2011). A subtropical zone with sunny weather is the best place for this species. However, a shady area is not a good place for it. A temperature as low as -2°C can be tolerated by this species (Rasool & Maqbool, 2019). It has been reported that *H. spicatum* has antidiabetic properties, tranquillizing actions, pediculicidal effects, antimicrobial properties, antioxidant properties, antimalarial hepatoprotective actions, cytotoxic effects, anti-helminthic effects, stomachic effects, and tonic activities. Its anti-diabetic properties are attributed to 1, 8 cineole, a compound obtained from the rhizome. A study conducted by Chaudhary et al. (2012) demonstrated the anti-microbial properties of the essential oil. *H. acuminatum* is used most commonly for liver problems. Cataracts can be cured with *H. coronarium*. Indigestion is the best application of *H. marginatum* (Devi et al., 2014).

PICRORHIZA KURROA**:** A herb used for medicinal purposes, Kutki or *Picrorhiza Kurroa* grows in the Himalayan region of India and belongs to the Scrophulariaceae family. Katuko, Kuru, Katukarogani, Katuko, Kurri and Katuka are some other names for this plant. Liver disorders have been successfully treated with this herbal medicine. Plant extracts show decent results against liver damage caused by carbon tetrachloride, paracetamol, and alcohol. This plant has been found to have beneficial effects as a laxative, liver stimulant, appetite and stimulant, and febrifuge in Ayurveda. Besides alleviating stomach aches, it is believed to stimulate appetite as well. Asthma and epidemic jaundice are also treated with the plant. In addition to treating disorders related to the chakra system, the herb is also useful for treating billow fever, urinary discharge, hiccups, blood troubles, burning sensations, and leukoderma (Sharma & Thokchom, 2014).

TAXUS WALLICHIANA: Native to the Himalayan region and the surrounding areas, it is also commonly known as the Himalayan yew or the Himalayan Taxus. It is a species of coniferous trees. As a member of the Taxus genus, which contains multiple yew species, *Taxus wallichiana* is a member of the Taxaceae family. This tree is evergreen and can grow up to 25 meters (82 ft) in height, with a conical or columnar crown. The leaves are lanceolate, dark green, and distributed spirally along the branches, with scaly, reddish-brown bark. The Himalayan region, which includes portions of Afghanistan, Pakistan, India, Nepal, Bhutan, and Tibet, is home to this species. At elevations between 1,500 and 4,000 meters (4,900 and 13,100 feet) above sea level, it grows well in mountainous regions. In mixed forests, *Taxus wallichiana* grows best in well-drained soils, frequently in close proximity to other coniferous and broad-leaved trees. Taxol, a substance having anticancer qualities, is produced by *Taxus wallichiana*, which is well known for its therapeutic qualities. The tree's bark, needles, and seeds are the source of taxol, which is used to treat a variety of cancers, such as lung, breast, and ovarian cancers. It is economically significant in the pharmaceutical industries because of its medicinal properties (Sharma & Garg, 2015; Bhuju & Gauchan, 2018).

JUGLANS REGIA: *Juglans regia* is a species of deciduous tree in the Juglandaceae family. It is often referred to as the Persian walnut, English walnut, or common walnut. Native to areas of Central Asia, the Middle East, and the Himalayan region, it is found throughout the region that stretches from the Balkans to China. The enormous *Juglans regia* tree can grow to a height of 25–30 meters (82–98 feet). With 5–9 leaflets per leaflet, its pinnately complex leaves have a broad, spreading crown. In the autumn, the dark green leaves turn yellow. The walnut tree yields round or oval-shaped nuts with a tough, wrinkled shell that is widely recognized as walnuts. For ages, people have been cultivating and appreciating walnuts for their nutritional worth and culinary applications. The nuts are a healthy complement to a variety of foods, salads, baked products, and desserts since they are high in omega-3 fatty acids, antioxidants, vitamins, and minerals. Walnut consumption is linked to a number of health advantages, such as enhanced heart health because of the omega-3 fatty acids it contains, decreased inflammation, lowered cholesterol, and support for cognitive and mental health. They are also a good source of nutritional fiber and protein. *Juglans regia* is a valuable and adaptable species with both economic and ecological significance. It is important for agriculture, forestry, culinary traditions, and human health (Sharma et al., 2022; Sharma et al., 2024).

ANGELICA GLAUCA: It is a perennial herbaceous plant in the Apiaceae family, sometimes referred to as Himalayan angelica or Chuan Xiong in traditional Chinese medicine. It grows naturally in alpine meadows, along forest borders, and on rocky slopes throughout the Himalayan area, which includes portions of India, Nepal, Bhutan, and Tibet. The genus Angelica, which contains a number of flowering plant species prized for their fragrant leaf and therapeutic qualities, is where *Angelica glauca* is categorized. Tall, hollow stems, divided compound leaves with serrated edges, and umbel-shaped clusters of tiny white or greenish-yellow flowers are some of its distinguishing features. It is prized for its medicinal qualities in traditional medical systems like Ayurveda and traditional Tibetan medicine. The plant's most pharmacologically active portions, the roots and rhizomes, are utilized in a variety of herbal treatments. Because of its well-known analgesic and anti-inflammatory qualities, it is helpful in the management of inflammatory diseases, arthritis, and pain. It is frequently used as a natural substitute for non-steroidal anti-inflammatory medicines (NSAIDs). The aromatic leaves and stems are occasionally used as a culinary herb or flavouring in dishes, drinks, and soups. The plant is also a common ingredient in herbal remedies and fragrances due to its aromatic properties (Bisht et al., 2006; Pandey et al., 2011).

SAUSSUREA COSTUS: a perennial herbaceous plant in the Asteraceae family, is also referred to as Costus or Kuth. It is indigenous to the Himalayan region, growing between 2,000 and 4,000 meters (6,500 and 13,100 feet) in height. It is especially prevalent in India, Nepal, Bhutan, and Tibet. The genus *Saussurea*, which includes over 300 species of flowering plants, includes *Saussurea costus*. Strong, meaty rhizomes, long, lanceolate leaves with serrated edges, and tall blooming stems with clusters of tubulars, purple or yellow flowers are some of its distinguishing features. The growth stage and environmental factors affect the plant's appearance. Traditional medical systems, such as Ayurveda, Tibetan medicine, and Unani, have long used *saussurea costus*. The plant's rhizomes are its most pharmacologically active component and are prized for their wide range of therapeutic benefits. The anti-inflammatory, analgesic, digestive, and expectorant properties of costus are well-known. Costus is a widely used element in fragrance and incense manufacture due to its fragrant properties. The rhizomes are useful for creating exotic perfumes because of their characteristic musky odour, which lends depth and richness to fragrances (Nadda et al., 2020; Ali & Venkatesalu, 2022).

RAUVOLFIA SERPENTINA: Native to Southeast Asia and the Indian subcontinent, *Rauvolfia serpentina* is also known as Indian snakeroot or sarpagandha. It is a member of the Apocynaceae family and is well known for its pharmacological qualities and historical application in conventional medicine. The perennial, evergreen shrub or small tree *Rauvolfia serpentina* has a maximum height of 1-2 meters (3-6 feet). Its tall, thin branches are topped with whorls of oval leaves that surround the stem. The shrub bears juicy, red berries with seeds within as well as tiny, white or pink flowers. The most pharmacologically active portion of *Rauvolfia serpentina* is its roots, which have long been utilized in traditional medicine. Bioactive alkaloids such as reserpine, ajmaline, ajmalicine, and serpentine are abundant in it. Due to the diverse medicinal effects of these alkaloids, *Rauvolfia serpentina* is useful in the management of a number of illnesses. The ability of *Rauvolfia serpentina* to reduce blood pressure is one of its most well-known therapeutic qualities. In contemporary medicine, serpine which is derived from the roots is used to treat hypertension, or high blood pressure, by decreasing sympathetic nervous system activity and vasodilation. According to studies, *Rauvolfia serpentina* extracts contain antibacterial qualities that make them effective against fungus and bacteria. Furthermore, the plant's alkaloids have shown antimalarial action, suggesting that it may be used to treat or prevent malaria. Historically, *rauvolfia serpentina* has been used as a sedative and anxiolytic a substance that lessens anxiety. Anxiety, tension, and sleeplessness can be lessened by the plant's alkaloids, which have a relaxing impact on the central nervous system. Because of its medicinal qualities and prospective applications in contemporary healthcare, it is still being studied scientifically and has tremendous pharmaceutical potential (Negi et al., 2014; Roy et al., 2023).

Conclusions

The Himalayas are the highest mountain peak on earth and the largest mountain range in the world. The assessment of the current status and conservation strategies of high-valued medicinal plants from the Himalayan regions underscores the critical importance of preserving these invaluable botanical resources. Several medicinal and aromatic species grow in this area, which plays a major role in herbal pharmaceuticals. As medicinal plants continue to deliver a variety of alternative treatment approaches. Many popular high valued medicinal plants are found in the Himalayas like *Bacopa monnieri, Glycyrrhiza glabra, Picrorhiza kurroa, Aconitum heterophyllum, Swertia chirata, Berberis aristata, Valeriana jatamansi, Juglans regia*. The Himalayan range has a high chance of generating social and economic benefits through the industrial utilization and commercial cultivation of these natural products. In the face of various degrees of threats, these medicinal plant species have been threatened with extinction, so every possible step has been taken to develop biotechnological tools and techniques that can be used to domesticate wild species under threat. It has become imperative that we find out other methods of conserving them. The study attempted to concluded that a number of conservation programmes and strategies that are intended to preserve and manage highly valued medicinal plants in the Himalayan areas in a sustainable manner. These approaches cover a wide range of techniques, such as the creation of protected areas, community-based conservation, sustainable harvesting techniques, habitat restoration, agricultural initiatives, conservation of genetic resources, and biotechnological interventions. The evaluation of highly valuable medicinal plants from the Himalayan areas concludes by emphasizing the necessity of multidisciplinary and integrative approaches to conservation. Prioritizing fair benefit-sharing, sustainable management, and biodiversity conservation can help protect these priceless botanical resources, maintain traditional knowledge, boost local economies, and foster a more resilient and healthy future for people and the environment.

List of Abbreviations

IUCN- International Union for Conservation of Nature
UNCED- United Nations Conference on Environment and Development
WWF- World Wildlife Fund
RET- Rare and Endangered Species

Declarations

Ethics approval and consent to participate

Ethics approval is not required for this study.

Consent for publication

Not required for this study.

Availability of data and materials

Not applicable.

Competing interests

Not applicable.

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Author contribution

Munish Sharma collected the data and involved in draft preparation for the review. Munit Sharma suggested the idea and designed the study. The final editing was done by Munit Sharma and Munish Sharma and approved the final version.

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INSECT POLLINATORS AND PLANT INTERACTIONS: A TAXONOMIC IMPLICATIONS OF POLLEN MORPHOLOGICAL FEATURES IN MELLIFEROUS PLANTS

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Abstract – The current study was the first report on pollen morphology of 17 species belonging to 11 different families that are particularly involved in nectar and pollen collection by honeybees from southern Pakistan using microscopic techniques. The plants were collected after being foraged by honeybees, identified, and studied. Flowering periods, habits and localities of plants were examined. The pollen grains were then passed through the process of acetolysis, measured and both quantitative and qualitative characteristics evaluated. The investigated pollen grains varied in size, shape, colpi/pore and exine sculpturing. The most common type of pollen examined was tricolporate and among the shapes was oblate spheroidal one. Three types of exine sculpturing were observed, psilate, reticulate and echinate. The recorded pollen fertility ranged from 67 to 93 percent, showing that the considered plants are well established in the area. *Brassica campestris* L. *Asphodelus tenuifolius* Cav. and *Astragalus hamosus* L. resulted in the most visited species by honeybees. Our results showed that the pollen morphology of the melliferous plants in the study varies largely. The principal component analysis did not show clusters in relation to specific pollination syndromes and only some strictly phylogenetically related species were close in the graph. For this reason, the pollen grain characters can be considered sufficiently diversified to be used as markers for species identification in melissopalynological analyses. The present study plays a vital role in taxa identification, floral calendar preparation, and production of honey in the study area. This evidence may provide the possibility of evaluating the geographical provenance of honey from melissopalynological analysis at least in southern Pakistan.

Keywords: taxonomic implications; honeybees; melliferous plant; nectars; palynology.

Introduction

Melliferous plants of a region are the species which are visited by honeybees to collect substances used in honey production. Honeybees visit flowers having higher amounts of nectars and pollen grains of better quality (Ahmad & Aslam, 2002). Nectars are the important source to honeybees for their foraging activities, hive formations and honey productions. Seasons have a great impact on melliferous flora; plants blooming in the spring have a comparatively lower nectar yield than in summer (Ion et al., 2018). The melliferous potential of the plants depends also on ecological characteristics of an area. The qualitative and quantitative features of honey depend on the plants from which honeybees collect pollen and nectars. Consequently, the economic activity of beekeepers is directly related to the flowering periods of a particular area. In Southern Pakistan beekeepers arrange their business of honey annually two

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times, one in autumn and another in spring. In melliferous plants pollination occurs through honeybees which increase the annual production of fruits, vegetables, and oils (Gallai et al., 2009). For this reason, an important data is the determination of the flowering periods of different plants. Melissopalynological studies contribute to determining the floral and geographical origin of bees and honey (Agwu & Uwakwe, 19921; Abou-Shaara, 2019).

Pollen grains are fine particles produced in the anthers of a stamen in flowering plants containing the male gametophyte in seed plants. The exine represents the outer and intine is the inner layer of the pollen grain wall. Exine represents various sculpturing patterns, pores, spines and colpi. The field of palynology is not only limited to taxonomy, but it has a lot of applications in other fields such as medicine, forestry, biochemistry, forensics, pharmacy, chemotaxonomy, and systematic botany. Melissopalynology is one of the subcategories of palynology dealing with pollen used in honey production, possible adulterations, foraged of plants by honeybees, its habitat, and the quality of honey (Ashfaq et al., 2018).

Pakistan is considered as a very famous origin of high-quality honey. The investigated area includes both plain and hilly areas. Most common crops of the study area are wheat, gram (chickpea), dates, mango, and maize. The country has a long summer with maximum rainfall occurring in months of July and August. The most represented families of the present study are Asteraceae followed by Poaceae, Fabaceae and Brassicaceae. The climate of the area is semi-arid with hot summer and cool winter seasons (Khan et al., 2020). The most important economic activity of people is agriculture.

Some researchers (Pound et al., 2018; Ahmad et al., 2019) gave basic knowledge of pollen morphology about the melliferous flora of different localities. The aim of the present study was to identify melliferous plants for the first time from southern Pakistan, making its link with plant taxonomic studies, spreading its knowledge among local peoples, describing pollen morphology, its uses in honey formations, and protecting of plants from overgrazing. Such data are important to be able to identify pollen grains in honey samples as the main tool to ascertain the provenance of commercialized honey (Shaheen et al., 2017; Shakeel et al., 2019). Pollen morphological studies aid in the taxonomic and systematic identification of species belonging to the same genera. The melliferous species in the study area were very diverse and important from an evolutionary point of view. Exine thickness, pollen size and shape are the important features useful in taxonomic studies of different plants. Palynology is an important field related to many fields of genetics, pharmacology, evolutionary biology, and plant ecology. The aim of current research work is to identify and explore the bee forage plants for the first time in the study area, making their link with plant taxonomic studies, spreading their knowledge among local people, describing pollen morphology, its uses in honey formations, and conserving the vegetation.

Materials & Method

Field survey

A two-week field trip was arranged during the start of March 2019–April 2020 to the southern districts of Khyber Pakhtunkhwa, Punjab, and Sindh. All such plants were collected and photographed, which are foraged by honeybees. The plants were pressed and dried in newspapers. The specimens were identified with the available species at the Herbarium of (ISL) Quaid-i-Azam University, the flora of Pakistan, and online available literature. During the field trip, interviews were conducted with beekeepers living and working in the study area.

The species collected, identified and analyzed were *Daucus carota* L., *Launaea procumbens* (Roxb.) Ramayya and Rajagopal, *Plantago amplexicaulis* Cav., *Medicago monantha* (C.A. Mey) Trautv., *Cousinia minuta* Boiss., *Convolvulus arvensis* L., *Dactyloctenium aegyptium* (L.) Willd., *Vicia sativa* L., *Fumaria indica* (Hausskn.) Pugsley, *Sisymbrium irio* L., *Malcolmia cabulica* (Boiss.) Hook. f. and Thomson, *Arnebia hispidissima* (Lehm.) A. DC., *Astragalus hamosus* L., *Hypecoum pendulum* L., *Brassica campestris* L., *Asphodelus tenuifolius* Cav. and *Triticum aestivum* L. (Tab. 1). Some of the plants are anemophilous but are visited by bees for pollen collection to feed their young ones due to the unavailability of highly melliferous plants in the study area.

Slides preparation and its microscopic studies

For the microscopical investigation pollen was treated with glycerin jelly for staining purposes. Following the method of (Ashfaq et al., 2018) the percentage of pollen fertility was determined. For different parameter of each species pollen readings were taken. P/E ratios were determined by dividing polar diameter of pollen over equatorial diameter.

For microscopic studies florets were removed from flowers and anthers were separated. The anthers were put on the slide and then treated using acetolysis method (Erdtman et al., 1963). The anthers were crushed so that pollen could come out from the anthers of flowers and debris was removed with the help of camel brush. The slides were then ready for further morphological study using light microscopy.

Table 1. Botanical names, family, collector, locality, and voucher number of collected plants.

S.no	Plant species	Family	Collector	Locality	Voucher no.
1	*Daucus carota* L	Apiaceae	Shabir Ahmad and Shakeel Ahmad	Paniala/ Dera Ismail Khan	SA-QAU-136
2	*Launaea procumbens* (Roxb) Ramayya and Rajagopal	Asteraceae	Shabir Ahmad and Ashfaq Khan	Domail/ Bannu	SA-QAU-125
3	*Plantago amplexicaulis* Cav	Plantaginaceae	Shabir Ahmad and Ashfaq Khan	Darya Khan/ Bhakkar	SA-QAU-178
4	*Medicago monantha* (C.A.Mey) Trautv	Fabaceae	Shabir Ahmad and Shakeel Ahmad	Paniala/ Dera Ismail Khan	SA-QAU-146
5	*Cousinia minuta* Boiss	Asteraceae	Shabir Ahmad and Shakeel Ahmad	Kot Chatta /Dera Ghazi Khan	SA-QAU-154
6	*Convolvolus arvensis* L.	Convolvulaceae	Shabir Ahmad	Gul Imam/ Tank	SA-QAU-143
7	*Dactyloctenium aegyptium* (L.) Willd	Poaceae	Shabir Ahmad and Asif Kamal	Bazar/ Paharpur	SA-QAU-160
8	*Vicia sativa* L	Fabaceae	Shabir Ahmad	Kharmatu/ Kohat	SA-QAU-98
9	*Fumaria indica* (Hausskn.) Pugsley	Fumariaceae	Shabir Ahmad and Muhammad Saqib	Paharpur/ Dera Ismail Khan	SA-QAU-114
10	*Sisymbrium irio* L.	Brassicaceae	Shabir Ahmad and Ashfaq Khan	Kotri/ Jamshoro	SA-QAU-185
11	*Malcolmia cabulica* (Boiss.) Hook. f. and Thomson	Brassicaceae	Shabir Ahmad and Khushdil Khan	Jarma/ Kohat	SA-QAU-137
12	*Arnebia hispidissima* (Lehm.) A. DC.	Boraginaceae	Shabir Ahmad and Asif Kamal	Bahadur Khel/ Karak	SA-QAU-161
13	*Astragalus hamosus* L	Fabaceae	Shabir Ahmad and Abdul Waheed	Abdul Khel/ Lakki Marwat	SA-QAU-155
14	*Hypecoum pendulum* L.	Papaveraceae	Shabir Ahmad and Uzma bibi	Gul Imam/ Tank	SA-QAU-140
15	*Brassica campestris* L	Brassicaceae	Shabir Ahmad and Ashfaq Khan	Shah Alam/ Tank	SA-QAU-138
16	*Asphodelus tenuifolius* Cav	Asphodelaceae	Shabir Ahmad	Nawabshah city/ Nawabshsah	SA-QAU-151
17	*Triticum aestivum* L	Poaceae	Shabir Ahmad and Asif Kamal	Shehbaz Khel/ Lakki Marwat	SA-QAU-122

Statistical analysis

Quantitative characters of pollen comprising polar diameter, equatorial diameter, colpi length, colpi width, spine length, spine width and exine thickness were measured. Consecutive five readings for every parameter of each pollen were noted and their mean, minimum, maximum values and standard deviation of each character were calculated using software SPSS Statistics 20. In tables values are showed as Mean (Min-Max) ± SE. (Tab. 2 and Tab. 3). 15 coded characters (those that showed variation) were used for preparing a matrix used as input for the Principal Component Analysis with the software PAST 3.16 (Hammer et al., 2001).

Results

The pollen morphology of 17 bee forage plants were investigated showing variations in pollen size and shapes. The variation in colpi length and width is represented in Fig. 1, while the variation in exine thickness is shown in Fig. 2. The general aspects of the plants, particularly of the flowers are shown (Fig. 3 and Fig. 4). The quantitative and qualitative features of pollen were described in Tab. 2 and Tab 3, and also shown in Fig. 5 and Fig. 6. Fertility rates of pollen were shown in Tab. 4 showing the stability of species in the study area.

Variations were observed in the bee flora of the study area (Tab. 2). *M. monantha, C. minuta,* showed large size pollen grains. *C. arvensis, F. indica, A. tenuifolius* and *T. aestivum* presented medium size grains, while *D. carota, L. procumbens, P. amplexicaulis, D. aegyptium, V. sativa, S. irio, M. cabulica, A. hispidissima, A. hamosus, H. pendulum* and *B. campestris* showed small sized pollen grains. The shape of the observed pollen grains was oblate-spheroidal, subprolate, prolate-spheroidal and oblate. *D. carota, L. procumbens, V. sativa, M. cabulica, A. hamosus, B. campestris* and *T. aestivum* showed oblate spheroidal shape. *A. tenuifolius, S. irio, D. aegyptium* had prolate-spheroidal shape. *P. amplexicaulis* presented subprolate shape and *A. hispidissima* an oblated one.

A. tenuifolius had the longest colpi length: 44.19 µm, while *P. amplexicaulis* had a minimum of 3.78 µm. *C. arvensis* had a maximum of colpi width: 21.33 µm and *L. procumbens* had the minimal width of 3.00 µm. Colpi noted in species of the study area were tricolporate and triporate. *D. aegyptium*, *T. aestivum* showed pollen grains without colpi but with a pore. *P. amplexicaulis* and *T. aestivum* were the species owning pollen grains with maximal P/E (1.18), while *Arnebia hispidissima* had the lowest P/E (0.71).

The exine thickness reached the highest value of 3.06 µm in *B. campestris* pollen grains, while *A. hamosus* had the minimal value of 0.75 µm. *L. procumbens* was the only species showing pollen grains with spines 1.89 µm long and 1.71 µm wide. The spines of *L. procumbens* pollen grains are an important feature for its identification (Ahmad et al., 2013). *C. minuta* pollen showed no spines on its exine, contrary to what was observed (Zafar et al., 2007). Both exine thickness and spine length are important features for taxa identifications.

Psilate and reticulate exine sculpturing was observed in the investigated species. *L. procumbens* showed an echinated type of pollen. *S. irio*, *M. cabulica, B. campestris* owned reticulate types of exine ornamentations. *A. hamosus* and *T. aestivum* had psilated pollen. The pollen fertility in all species was always over 67%, with *B. campestris* having the highest pollen fertility (93%), while *T. aestivum* had the lowest value (67%).

According to the field trip visits and interviews taken with local people and beekeepers in the study area, most of the honey in the spring season was produced by honeybees

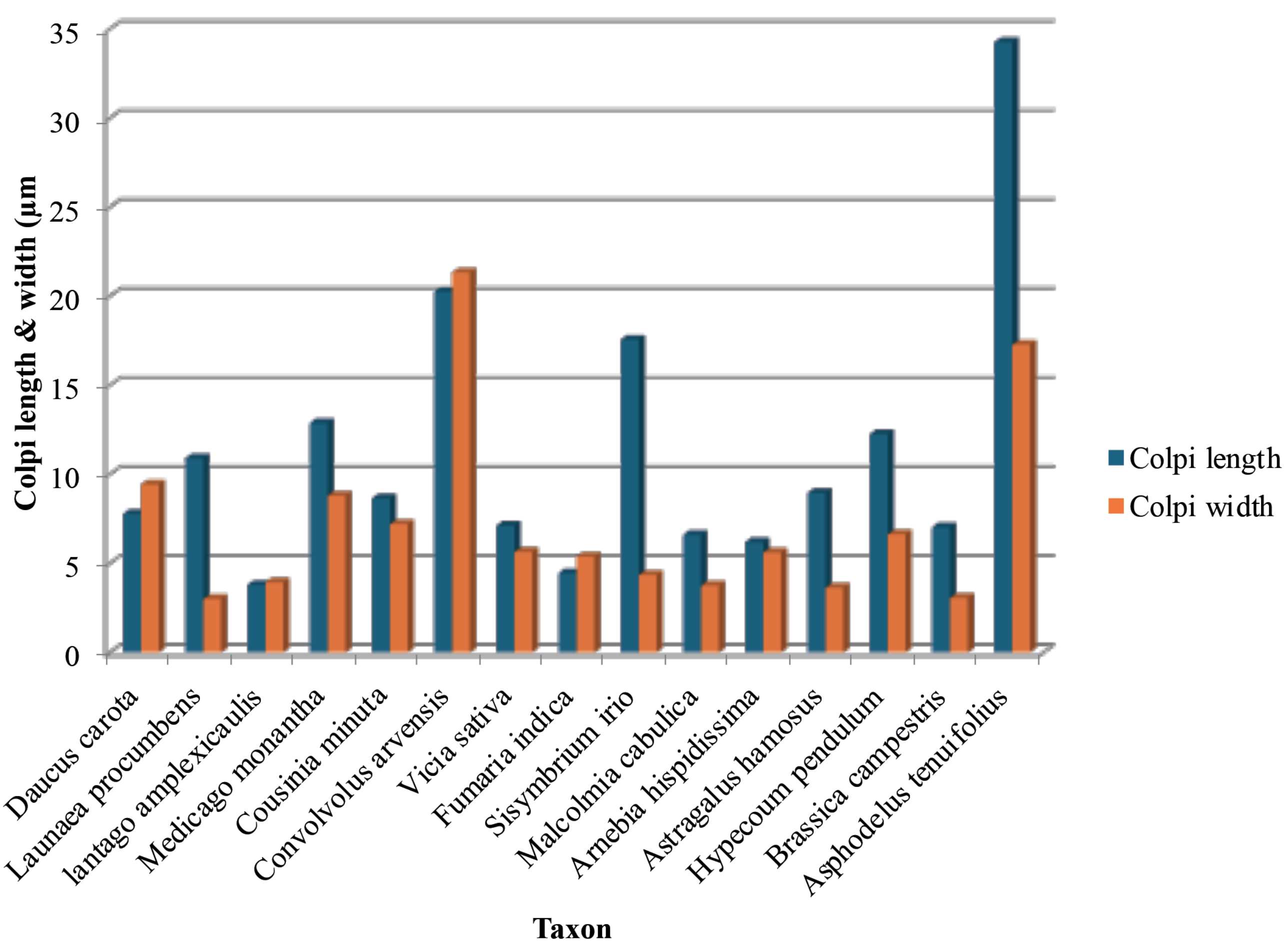

Figure 1. Variations in colpi length and width of melliferous plants from Southern Khyber Pakhtunkhwa, Pakistan.

visiting to *B. campestris*, *A. tenuifolius* and *A. hamosus*. In the field trip, it was observed that honeybees collect mostly pollen and nectar from herbaceous plants including weeds. It was also observed that in the study area honeybees visit white, yellow, purple, and pinkish, light-brown-colored flowers. The most frequently visited species had white and yellow flowers. The pollen grain characters were used to produce a matrix of morphological characters used as input for PAST software. The principal component analysis was shown in Fig. 7. The species was well distributed on the graph with *S. irio* in a particularly isolated position, apparently mainly due to the particularly small colpi length.

Discussion

A total of 17 plants belonging to 11 different families were analyzed through microscopic analysis. Brassicaceae and Fabaceae were the dominant families of the study area during the season of spring, with three species each. Asteraceae and Poaceae (this last family inserted for comparison, not including nectar producing plants but producing pollen) were represented by 2 species, while the rest of the families (Apiaceae, Asphodelaceae, Papaveraceae, Fumariaceae, Boraginaceae, Convolvulaceae and Plantaginaceae) were represented by a single species. Several scientists from different regions of the world reported about local honeybee flora (Bista & Shivakoti, 2001). Ting, (1961) studied Umbelliferae in America. The pollen of Brassicaceae has been studied (Erdtman, 1965). Flowering period of plants in both spring and autumn plays a very important role for honeybees regarding their foraging activities and honey production in southern Pakistan (Latif et al., 2019; Khan et al., 2022).

In the seventeen melliferous plants analyzed from southern Pakistan, a total of five different types of pollen i.e monoporate, monocolpate, tricolpate, tricolporate and pantaporate were reported. The pollen grain shapes examined were oblate-spheroidal, subprolate, prolate-spheroidal and oblate. Apart from pollen types and shapes, it also varies in pores, spines, and sculpturing. Spines found

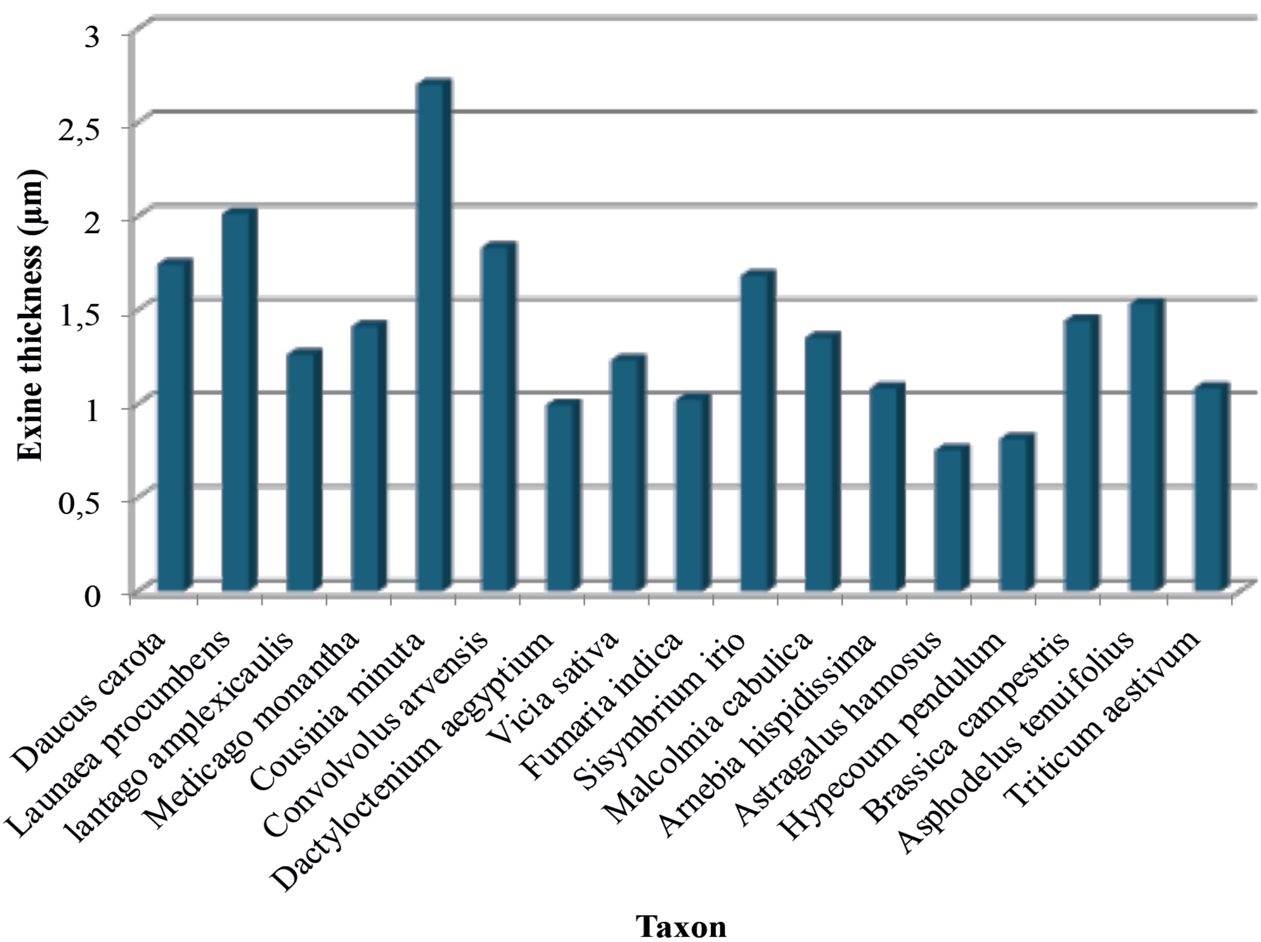

Figure 2. Variations in exine thickness of melliferous plants from Southern Khyber Pakhtunkhwa, Pakistan.

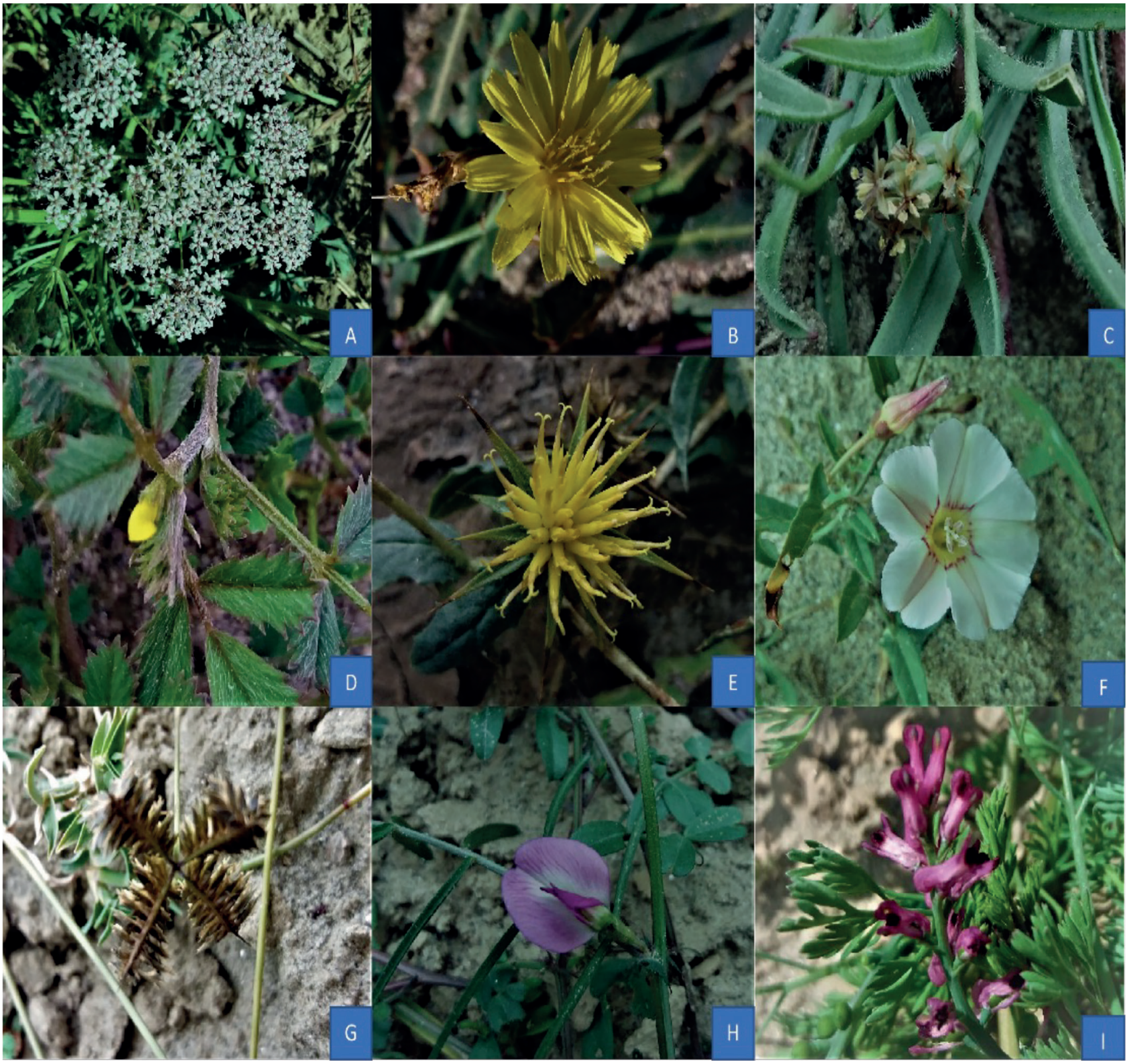

Figure 3. Plants Collected- (A) *Daucus carota* L (B) *Launaea procumbens* (Roxb) Ramayya and Rajagopal (C) *Plantago amplexicaulis* Cav (D) *Medicago monantha* (C.A.Mey) Trautv (E) *Cousinia minuta* Boiss (F) *Convolvulus arvensis* L. (G) *Dactyloctenium aegyptium* (L.) Willd (H) *Vicia sativa* L (I) *Fumaria indica* (Hausskn.) Pugsley

on the surface of pollen were very rare and most pollen studies have found them to be small. The average fertility percentage of the pollen here recorded was 83.03 % with the highest fertility at 89 % and the smallest 67 %. Such data would confirm the predominance of sexual reproduction in the considered species, at least in the geographical region of the study (Johnson, 1975). The high rate of pollen fertility is evidence that the plants are stable and actively reproducing in the study area.

The current study is the first contribution from southern Pakistan about the plants visited by honeybees for pollen and nectar formation. Khan et al., (2012) determined the melliferous potential and medicinal uses of honey in the current study area, but the investigation was only preliminary. The identification of melliferous species helps in developing pollen literature, which is useful for increasing honey production in coming the seasons. Pound et al., (2018) revealed the role of *Brassica* species for honey productions

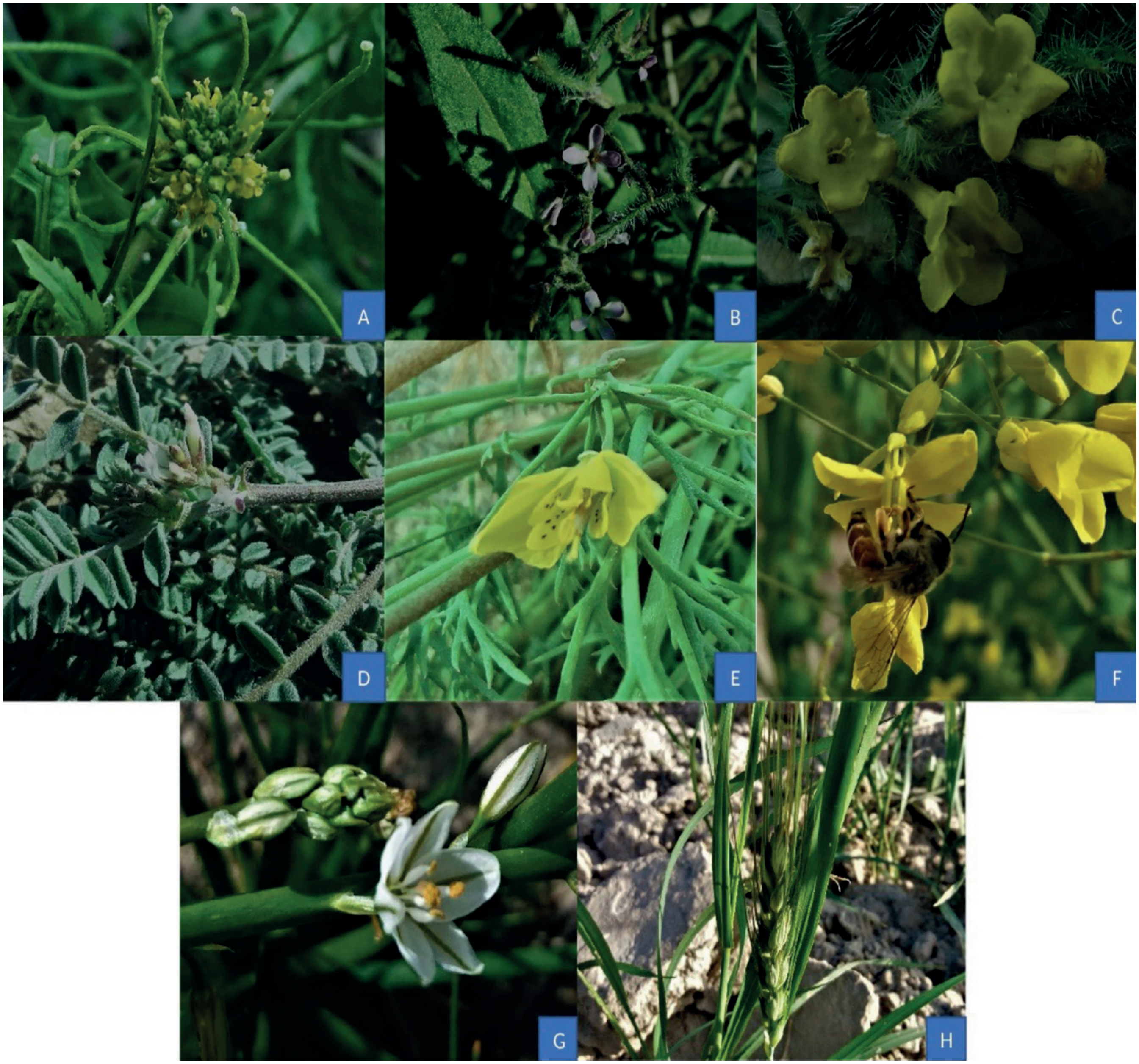

Figure 4. Plants Collected- (A) *Sisymbrium irio* L. (B) *Malcolmia cabulica* (Boiss.) Hook. f. and Thomson (C) *Arnebia hispidissima* (Lehm.) A. DC. (D) *Astragalus hamosus* L (E) *Hypecoum pendulum* L. (F) *Brassica campestris* L (G) *Asphodelus tenuifolius* Cav (H) *Triticum aestivum* L.

in Ponteland, UK. Palynological studies of the study area showed that pollens of the study area are very diverse in nature showing varies of pollen size and shapes which are very helpful for the purpose of identification (Perveen et al., 2004; Yedomonhan et al., 2012).

Different parameters of pollen containing polar diameter, equatorial diameter, colpus length, colpus width, spine length, spine width, number of spines, pores, fertile and sterile pollen are vital features to distinguish one species from another. Pollen examined in current study varies in size and shape. Tricolporate is one of the most common types of pollen (Muller, 1970). *Plantago amplexicaulis* is the only species showing pantaporate pollen.

Pollen data is essential for species identification that are pollinated by honeybees (Yedomonhan et al., 2012). The current microscopic study plays a vital role in the description of pollen morphology (Ashfaq et al., 2018). It has been proved that these results are very helpful for taxonomic identification. The PCA analysis did not show any evident clustering related to the pollination syndrome, since anemophylous plants were quite distantly positioned in the graph. More evident was the clustering of phylogenetically related species, such as *A. hamosus*/*V. sativa* (Fabaceae) or the couple *M. cabulica/B. campestris* (Brassicaceae). For this reason, we can consider the pollen characters useful for melissopalynological identification. Pollen morphological study is very useful in finding out many taxonomic and systematic problems. Some species are known for having other pollinators besides bees and that may influence the general floral structure.

A very wide range of variations were observed using microscopic techniques for selected species from the study area, showing that the field of palynology plays a vital role in the evolutionary studies of plant species. Our light microscopy results show similarity to the previously reported knowledge from different regions of Pakistan (Ahmad et al., 2019; Ashfaq et al., 2018). Pollen size varies from small to large, monocolpate to tricolporate, subprolate to oblate-spheroidal and reticulate to psilate. *B. campestris, M. cabulica, S. irio* have reticulate exine sculpturing, *L. procumbens* have echinated and the rest of the pollen have psilated exine ornamentations. Pollen morphological studies in the present study link the foraging behaviors of honeybees with specific plants. The results of the present study concluded that pollen

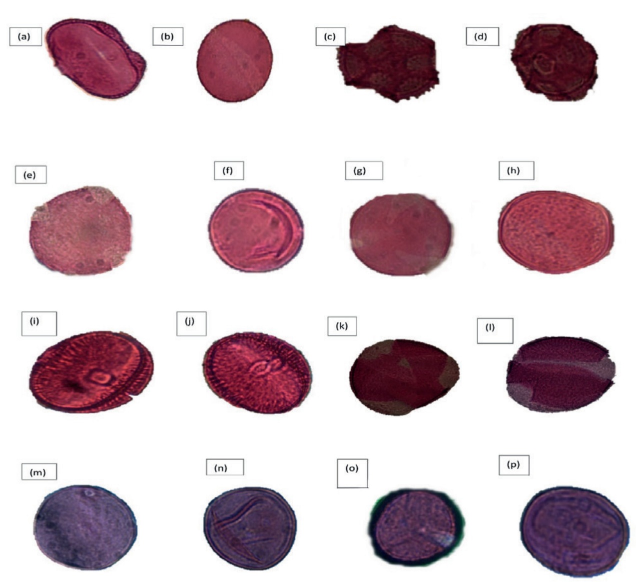

Figure 5. Light microscopy pollen micrographs- *Daucus carota* L (a) Polar view (b) Equatorial view; *Launaea procumbens* (Roxb) Ramayya and Rajagopal (c) Polar view (d) Equatorial view; *Plantago amplexicaulis* Cav (e) Polar view (f) Equatorial view; *Medicago monantha* (C.A.Mey) Trautv (g) Polar view (h) Equatorial view; *Cousinia minuta* Boiss (i) Polar view (j) Equatorial view; *Convolvolus arvensis* L. (k) Polar view (l) Equatorial view; *Dactyloctenium aegyptium* (L.) Willd (m) Polar view (n) Equatorial view; *Vicia sativa* L (o) Polar view (p) Equatorial view.

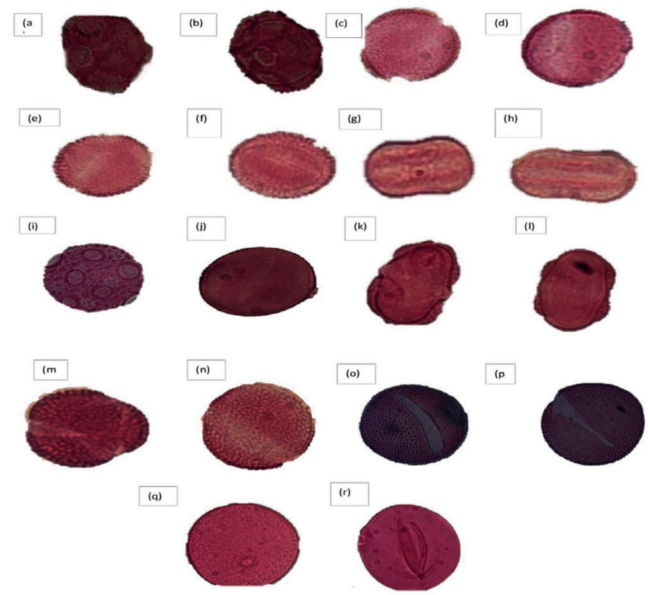

Figure 6. Light microscopy pollen micrographs- *Fumaria indica* (Hausskn.) Pugsley (a) Polar view (b) Equatorial view; *Sisymbrium irio* L. (c) Polar view (d) Equatorial view; *Malcolmia cabulica* (Boiss.) Hook. f. and Thomson (e) Polar view (f) Equatorial view; *Arnebia hispidissima* (Lehm.) A. DC. (g) Polar view (h) Equatorial view; *Astragalus hamosus* L (i) Polar view (j) Equatorial view; *Hypecoum pendulum* L. (k) Polar view (l) Equatorial view; *Brassica campestris* L (m) Polar view (n) Equatorial view; *Asphodelus tenuifolius* Cav (o) Polar view (p) Equatorial view; *Triticum aestivum* L. (q) Polar view (r) Equatorial view.

morphology of melliferous species plays a vital role in plant identification and floral calendar preparation.

D. carota is considered largely pollinated by Diptera (Ahmad & Aslam, 2002). The same can be said about *L. procumbens* (Sajjad et al., 2010). Surprisingly, the observed measures in pollen grains were quite different from those with the polar diameter of 22.44 **µm** in this investigation vs. 34.65 **µm and the width of colpi (3 µm vs. 8.45 µm). We can hence assume that a relatively high degree of intraspecific variation is present, possibly based on the geographical location of the investigated population of the taxa** (Ahmad & Aslam, 2002). Also, the anemophilous *P. amplexicaulis* had pollen measures variable in the current analysis with respect to (Fierascu et al., 2021). Evident differences were observed also in *V. sativa* with respect to the observations of (Khan et al., 2020). *S. irio* was showed in isolated position in the PCA. Our results do not allow us to give a final explanation, but Ara et al., (2019) observed that honeybees feed in this species both on nectar and pollen, and the isolated position may depend on specific adaptation of the pollen grain to this type of nutrition. Also, *A. hispidissima* is an isolated position and this species is known to be used as a source of both nectar and pollen (Abou-Shaara, 2019).

Conclusion

The current study was carried out to identify melliferous plants visited by honeybees using pollen morphology during the seasons of spring and autumn. Variations investigated in the qualitative and quantitative features of pollen showed sufficient changes to be considered useful markers for melissopalynological analysis. This investigation revealed that a complete set of characters is helpful for the identification of species in the field of plant taxonomy and systematics. *C. arvensis* had the largest polar diameter, measuring 51.72 µm and an equatorial diameter 44.43 µm. *M. cabulica* has the smallest polar diameter, 12.69 µm and an equatorial diameter of 13.86 µm. Variations in exine sculpturing have very distinguishing features in plant systematic and biodiversity. The current study was conducted for the first time in the study area to analyze bee foraged species from various localities of the study area, aiming at a better knowledge of melliferous species among local beekeepers and their conservation in the study area. The observed intraspecific variation of pollen grains of the same species from different geographical areas is an indication that these data may also be useful to assess the provenance of honey.

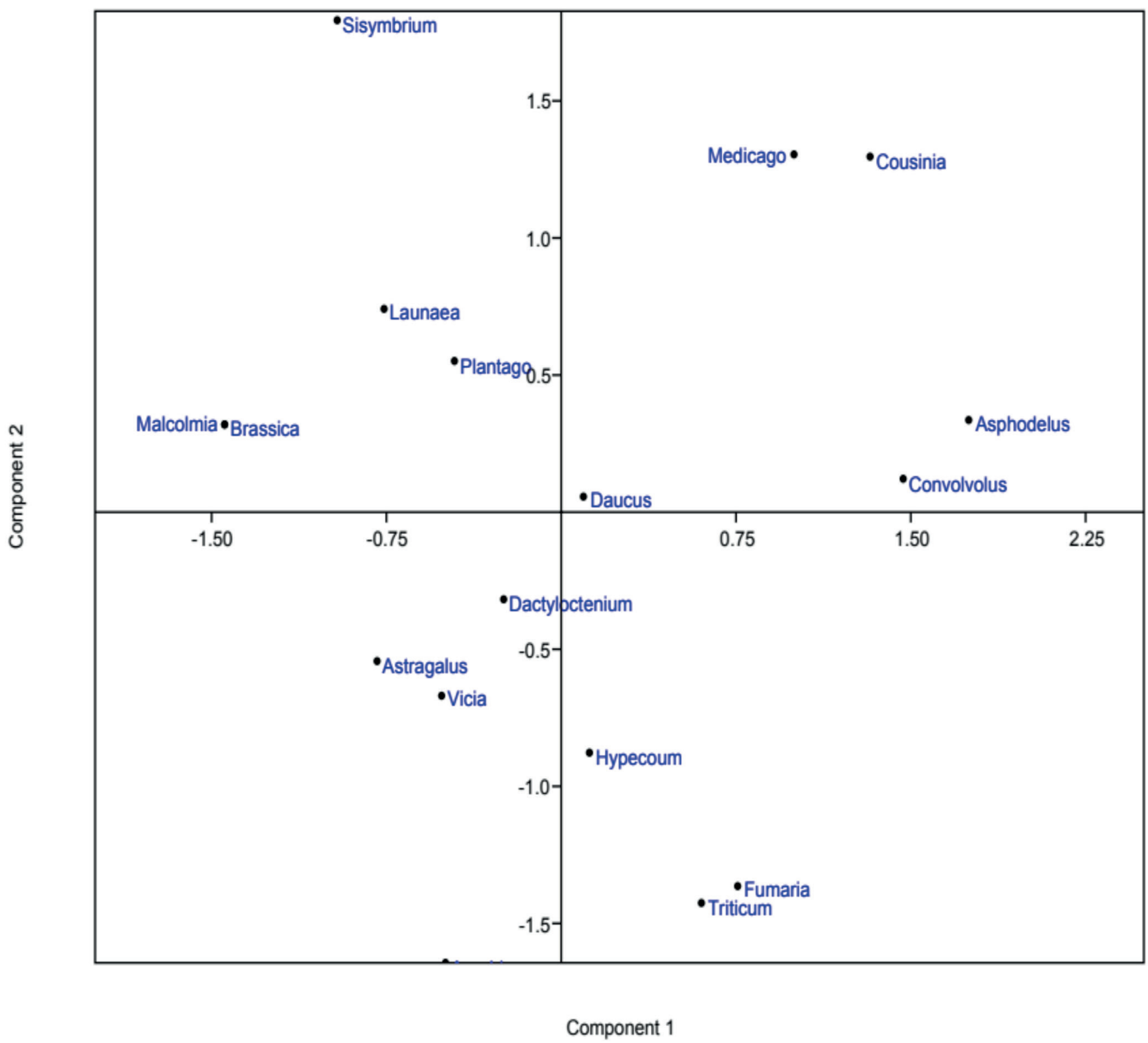

Figure 7. PCA based on the coded pollen grain morphological characters.

Table 2. Micromorphological qualitative features of pollen.

S.no	Species name	Pollen size	Pollen type	Pollen shapes	Colpi/Pore	Spines	Exine sculpturing
1	*Daucus carota* L	Small	Tricolporate	Oblate-Spheroidal	Present	Absent	-
2	*Launaea procumbens* (Roxb) Ramayya and Rajagopal	Small	Tricolporate	Oblate-Spheroidal	Present	Present	Echinate
3	*Plantago amplexicauli*s Cav	Small	Pantaporate	Subprolate	Absent	Absent	Psilate
4	*Medicago monantha* (C.A.Mey) Trautv	Large	Tricolporate	Prolate-Spheroidal	Present	Absent	Psilate
5	*Cousinia minuta* Boiss	Large	Tricolporate	Prolate-Spheroidal	Present	Absent	-
6	*Convolvolus arvensi*s L.	Medium	Tricolporate	Oblate-Spheroidal	Present	Absent	-
7	*Dactyloctenium aegyptium* (L.) Willd	Small	Monoporate	Prolate-Spheroidal	Present	Absent	Psilate
8	*Vicia sativa* L	Small	Tricolporate	Oblate-Spheroidal	Present	Absent	Psilate
9	*Fumaria indic*a (Hausskn.) Pugsley	Medium	Tricolpate	Oblate-Spheroidal	Present	Absent	-
10	*Sisymbrium irio* L.	Small	Tricolpate	Prolate-Spheroidal	Present	Absent	Reticulate
11	*Malcolmia cabulica* (Boiss.) Hook. f. and Thomson	Small	Tricolpate	Oblate-Spheroidal	Present	Absent	Reticulate
12	*Arnebia hispidissima* (Lehm.) A. DC.	Small	Tricolpate	Oblate	Present	Absent	-
13	*Astragalus hamosus* L	Small	Tricolporate	Oblate-Spheroidal	Present	Absent	Psilate
14	*Hypecoum pendulu*m L.	Small	Tricolpate	Oblate-Spheroidal	Present	Absent	-
15	*Brassica campestris* L	Small	Tricolpate	Oblate-Spheroidal	Present	Absent	Reticulate
16	*Asphodelus tenuifolius* Cav	Medium	Monocolpate	Prolate-Spheroidal	Present	Absent	-
17	*Triticum aestivum* L.	Medium	Monoporate	Oblate-Spheroidal	Present	Absent	Psilate

Table 3. Micromorphological quantitative features of pollen.

S.no	Taxon	P/E ratio	Exine thickness Mean (Min-Max) SE (um)	Polar diameter Mean (Min-Max) SE (um)	Equatorial diameter Mean (Min-Max) SE (um)	Length of colpi Mean (Min-Max) SE (um)	Width of colpi Mean (Min-Max) SE (um)	Length of spine Mean (Min-Max) SE (um)	Width of spine Mean (Min-Max) SE (um)
1	*Daucus carota* L	0.96	1.74 (1.35-2.10) ±0.14	19.77 (17.70-21.00) ±0.57	20.55 (17.40-23.70) ±1.25	7.77 (6.30-9.15) ±0.50	9.42 (7.50-11.25) ±0.79	A	A
2	*Launaea procumbens* (Roxb) Ramayya and Rajagopal	0.95	2.01 (0.90-3.30) ±0.41	22.44 (21.00-25.20) ±0.74	23.40 (18.60-25.80) ±1.34	10.89 (9.45-15.00) ±1.05	3.00 (2.25-3.90) ±0.29	1.89 (1.20-2.55) ±0.24	1.71 (1.05-3.15) ±0.37
3	*Plantago amplexicaulis* Cav	1.18	1.26 (0.60-1.95) ±0.27	22.38 (19.50-26.40) ±1.31	18.96 (17.70-20.40) ±0.50	3.78 (2.25-6.45) ±0.73	3.93 (3.00-6.00) ±0.54	A	A
4	*Medicago monantha* (C.A.Mey) Trautv	1.11	1.41 (0.90-1.80) ±0.16	27.42 (26.10-28.95) ±0.49	24.54 (22.50-25.65) ±0.54	12.87 (7.05-18.75) ±1.91	8.79 (6.75-10.20) ±0.72	A	A
5	*Cousinia minuta* Boiss	1.04	2.70 (1.95-3.90) ±0.36	28.62 (27.45-30.30) ±0.50	27.45 (23.70-32.40) ±1.62	8.64 (4.20-15.30) ±2.15	7.20 (3.75-12.75) ±7.20	A	A
6	*Convolvulus arvensis* L.	1.16	1.83 (1.20-3.00) ±0.31	51.72 (44.40-59.25) ±2.95	44.43 (34.05-54.90) ±3.78	20.25 (10.95-36.45) ±4.39	21.33 (14.55-29.10) ±2.40	A	A
7	*Dactyloctenium aegyptium* (L.) Willd	1.07	0.99 (0.60-1.50) ±0.16	22.68 (19.80-22.85) ±1.29	21.06 (19.35-23.40) ±0.87	A	A	A	A
8	*Vicia sativa* L	0.99	1.23 (0.60-1.95) ±1.23	22.68 (16.50-28.50) ±2.10	22.74 (18.45-30.00) ±2.45	7.11 (3.75-10.35) ±1.29	5.64 (2.10-8.70) ±1.32	A	A
9	*Fumaria indica* (Hausskn.) Pugsley	0.89	1.02 (0.45-1.65) ±0.20	31.77 (28.80-35.55) ±1.25	35.64 (24.45-42.45) ±3.13	4.44 (2.85-7.80) ±0.89	5.37 (4.50-6.00) ±0.30	A	A
10	*Sisymbrium irio* L.	1.03	1.68 (1.35-2.10) ±0.13	18.24 (15.75-21.00) ±0.90	17.55 (15.60-18.75) ±0.62	4.05 (2.55-6.45) ±0.67	4.35 (2.70-5.25) ±0.47	A	A
11	*Malcolmia cabulica* (Boiss.) Hook. f. and Thomson	0.91	1.35 (0.90-1.80) ±0.15	12.69 (11.40-13.50) ±0.40	13.86 (12.15-15.30) ±0.68	6.60 (3.45-9.15) ±0.95	3.75 (1.50-7.50) ±1.14	A	A
12	*Arnebia hispidissima* (Lehm.) A. DC.	0.71	1.08 (0.60-1.80) ±0.22	13.65 (12.90-14.70) ±0.30	19.20 (12.30-28.50) ±3.41	6.18 (2.10-10.95) ±1.45	5.61 (1.80-10.35) ±1.44	A	A
13	*Astragalus hamosus* L	0.98	0.75 (0.45-1.05) ±0.11	18.69 (17.70-19.95) ±0.44	18.90 (18.30-19.35) ±18.69	8.94 (6.60-11.40) ±0.88	3.63 (2.10-6.00) ±0.66	A	A
14	*Hypecoum pendulum* L.	0.94	0.81 (0.45-1.35) ±0.15	23.01 (22.20-23.85) ±0.33	24.24 (22.50-25.65) ±0.55	12.24 (8.70-15.30) ±1.29	6.63 (4.35-10.05) ±0.99	A	A
15	*Brassica campestris* L	0.98	1.44 (0.90-1.95) ±0.18	18.93 (17.40-21.00) ±0.67	19.26 (15.00-22.80) ±1.45	7.02 (3.45-11.70) ±1.367	3.06 (1.20-9.00) ±1.49	A	A
16	*Asphodelus tenuifolius* Cav	1.06	1.53 (1.20-1.95) ±0.13	47.01 (44.10-50.25) ±1.08	44.19 (32.85-56.25) ±3.73	34.26 (22.50-43.35) ±3.59	17.28 (6.90-32.10) ±4.60	A	A
17	*Triticum aestivum* L.	1.18	1.08 (0.75-1.50) ±0.13	38.85 (37.20-40.50) ±0.60	32.67 (27.00-38.25) ±2.33	A	A	A	A

Table 4. Pollen fertility percentage for spring melliferous flora from Southern Khyber Pakhtunkhwa, Pakistan.

S.no	Species name	Fertile pollen	Sterile pollen	Fertility (%)
1	*Daucus carota* L	198	25	89
2	*Launaea procumbens* (Roxb) Ramayya and Rajagopal	58	11	84
3	*Plantago amplexicaulis* Cav	36	10	78
4	*Medicago monantha* (C.A.Mey) Trautv	50	11	82
5	*Cousinia minuta* Boiss	85	12	88
6	*Convolvulus arvensis* L.	55	17	76
7	*Dactyloctenium aegyptium* (L.) Willd	52	9	85
8	*Vicia sativa* L	68	16	81
9	*Fumaria indica* (Hausskn.) Pugsley	27	9	75
10	*Sisymbrium irio* L.	176	24	88
11	*Malcolmia cabulica* (Boiss.) Hook. f. and Thomson	113	25	82
12	*Arnebia hispidissima* (Lehm.) A. DC.	63	17	79
13	*Astragalus hamosus* L	244	27	89
14	*Hypecoum pendulum* L.	148	19	89
15	*Brassica campestris* L	478	38	93
16	*Asphodelus tenuifolius* Cav	48	7	87
17	*Triticum aestivum* L.	34	17	67

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Conflict of interest

The authors of this manuscript have no conflicts of interest to declare it.

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A THERMODYNAMIC APPROACH TO INTERPRET THE ECOSYSTEM COMPLEXITY

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Abstract – The authors present a thermodynamic outlook of some significant processes and phenomena in plant evolution and ecology. The same approach is attempted to exhibit the main steps starting from the vegetation science to the ecosystem studies.
Aim is not to write a usual article, but to propose a re-reading of methods and results in the vegetation research field offering a new point of discussion, in which changes in the entropy of systems are displayed in plants such as in human world.
According to the Second Law of Thermodynamics, inanimate matter tends toward a continuous increasing of randomness and the accompanying spreading out of energy. The Living State appears to move in the opposite direction, generating ordered structures with low entropy and high negentropy/ syntropy. At morpho-physiologic level the leaf represents the most specialized organ to capture sun energetic clean source making the photosynthesis the process through which the negentropy trend is recognizable. Syntropic structures and functions are also generated at the community and ecosystem level. Interestingly in studying ecosystem complexity, the ecoindicators application is comparable to a mind process which reduces "entropy" of the traditional vegetation analysis integrating it in a more suitable and efficient-syntropic- way.

Keywords: entropy, synentropy, ecosystem complexity, community, vegetation, soil, humus, ecoindicators

Introduction

From entropy to negentropy and syntropy: the thermodynamic of the living State (LS). A synthetic overview

Many processes or phenomena that take place in Nature are known to occur in a way that minimizes or maximizes entropy production (Arcidiacono & Arcidiacono, 1991; Baldwin, 2009). Entropy is used for describing different phenomena involving both biological and inanimate systems, such as animal locomotion, vegetation, organ size, water basins and social organization too (Bejan & Lorente, 2010).
It is often thought that the structural complexity of living organisms, which seems to increase as the evolutionary tree is ascended, may place Life outside the laws of Physics. According to the Second Law of Thermodynamics, inanimate matter tends toward constantly increasing randomness and the accompanying spreading out of energy: this process implies an entropy increase (Fig.1).

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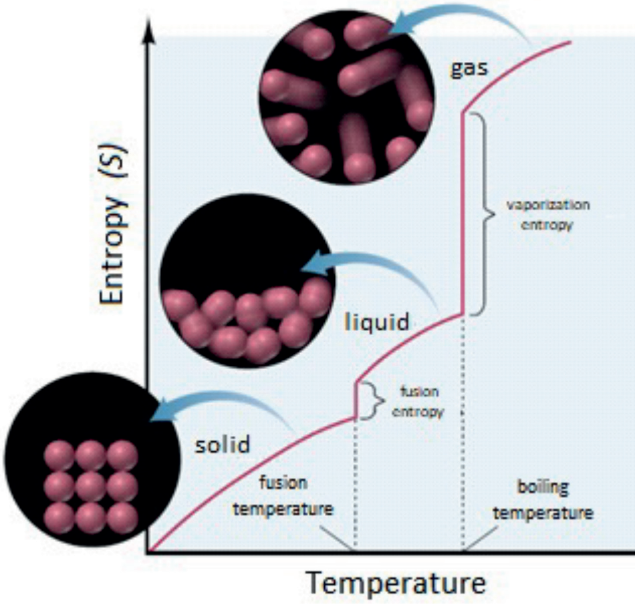

Figure 1. An example of entropy (S) increase in the matter changes: when (a mass) 1Kg of water is heated from < (fusion T°) 0° to > (boiling T°) 100°, a change is observed from a more ordered structure in the ice to a less ordered one of water in the gaseous state.

The Living State appears to move in the opposite direction towards negentropy/syntropy increase. This has been a theme of active debate, which continues, for over 70 years, with no definitive resolution in sight. What is the "vital force" that urgens the Living State (LS) to move toward ever increasing levels of complexity? It is well known that in the physical universe matter and energy are spontaneously degraded into more simple and more random states, as is predicted by the Second Law of Thermodynamics. However, at first sight, this appears not to be the case in the Evolution of the LS as far as order is concerned, is apparently produced from less ordered states. "Order" may be intuitively understood in terms of the complexity of biological structure which modifies (decreases) the degrees of freedom of the molecular and multi-molecular constituents of the system (Arcidiacono, 2005).

A considerable number of academics have developed the general notion, as mentioned above, that the highly ordered nature of the LS represents a violation of the Second Law, or have at least expressed surprise and perplexity in so much as evolution proceeds along a path of increasingly ordered structures (Levins & Lewontin, 1985; Vannini, 2005; Di Corpo & Vannini, 2011; Tooby et al., 2003; Beichler, 2017). "Given the belief that the physical universe is moving toward a static death rather than a thermodynamic balance in which molecular motion continues, it is no surprise that evolutionists believe organic evolution to be the negation of physical evolution." A clear example of this line of thought was expressed by the well-known physiologist Szent-Gyorgyi (1977) who summarized his thoughts on the matter with the rather extremist statement "A major difference between amoebas and humans is the increase of complexity that requires the existence of a mechanism able to counteract the law of entropy". In other words, there must be a force that is able to counter the universal tendency of matter toward chaos and energy toward spreading out. Life always shows a decrease in entropy and an increase in complexity, in direct conflict with the law of entropy. Tooby et al. (2003) write "Thus, to study organisms scientifically it is to be compared with the following questions: Why is it that living things exhibit a miraculously high level of order not found among the non-living? Where does this high level of order come from?", a question posed but not answered.

A line of thought developed, in which the basic studies of the mathematician Fantappiè (1942) played a central role.

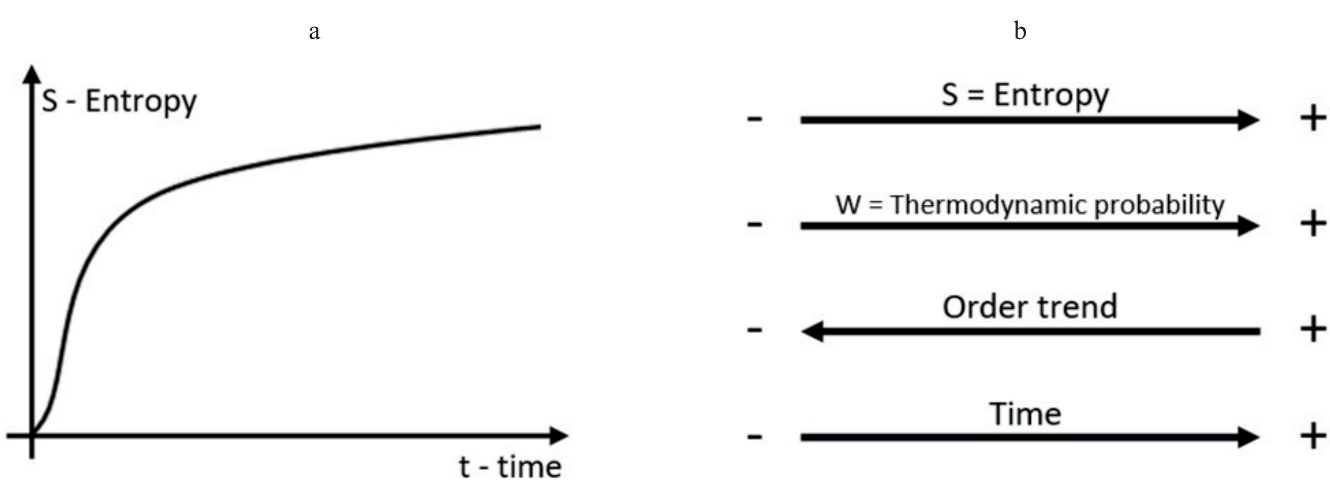

Figure 2. In an isolated system, without exchanges with the outside, entropy (S) increases (a) and, according to Boltzmann (1872), has a favored direction toward disorder (b).

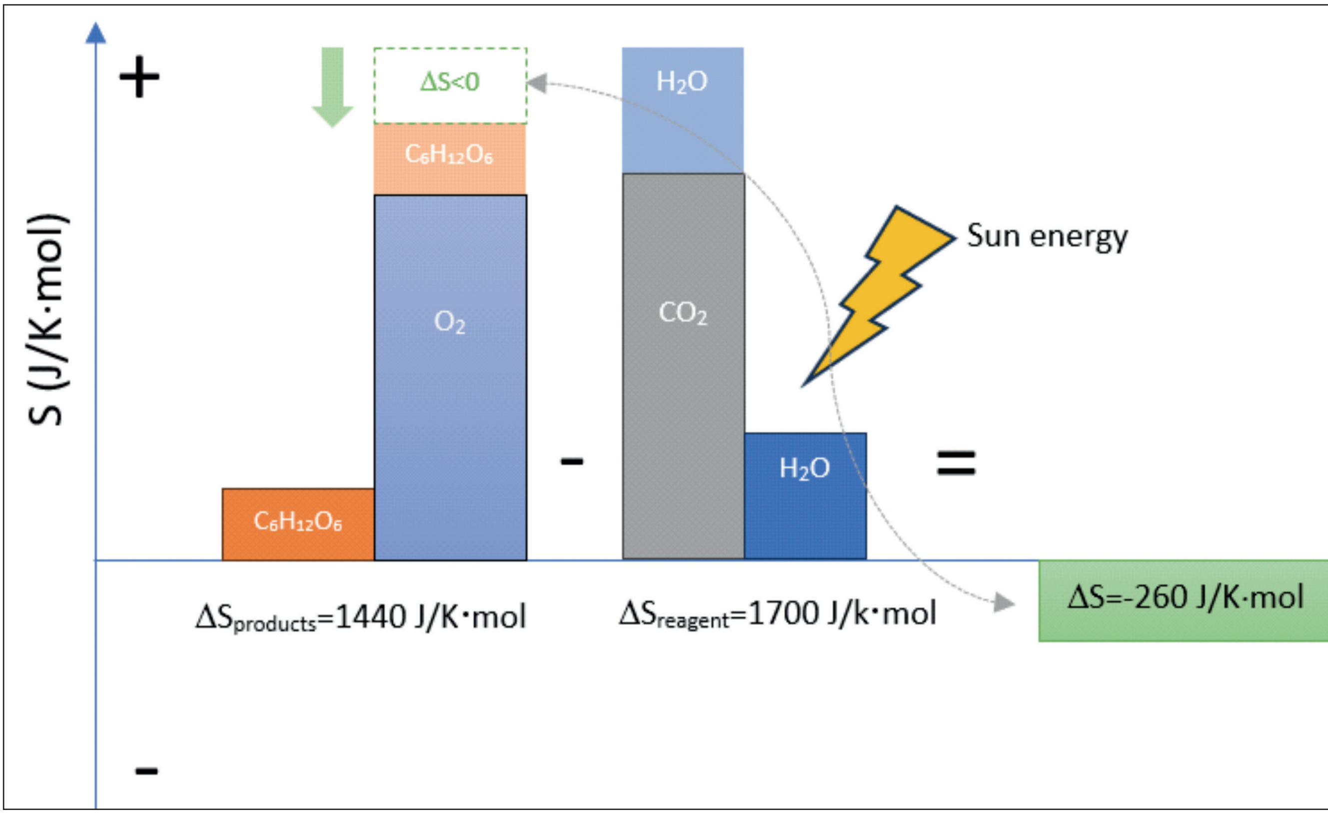

Figure 3. Entropy difference, expressed by J/K ·mol, between photosynthesis reagents and products is < 0.

During his study on the positive and negative time energy, he discovered that the positive time energy is subjected to entropy law (Figs. 2 a, b), while the negative time energy is governed by a complementary law which he called "syntropy", combining the Greek words syn (convergency) with tropos (tendency).

In approximate terms, syntropy is considered to represent the spontaneous creation of order, much as the negentropy of Schrödinger (1963) and is supposed that life phenomena are governed by a principle which is symmetrical to entropy.

Syntropy in plant evolution: structures, functions, relationships as indicators of syntropy

Although the photosynthesis is recognized as the only mechanism which supports the life on the Earth, and that plants are the only living organisms able to capture solar energy, botanical scientist has not investigated enough the changes of energy status following a thermodynamic point of view. Instead, the entropy aspects of the photosynthesis more intrigued the physical scientists, especially regarding the Schrödinger assumption: "there must be a continuous influx of enough negentropy to at least balance the increase of entropy that inevitably accompanies the irreversible processes inside the organisms which rapidly would decay into the lifeless state of maximum entropy" (Yourgrau & Merwe, 1968). Green plants acquire negative entropy along with energy during photosynthesis from the sunlight incident on their leaves (Yourgrau & Merwe, 1968). Nearly the 30% of the incident photon energy gets converted into electron energy that powers the sugar production; the solid glucose is more ordered and complex molecule than gaseous carbon dioxide adsorbed by leaves as reagent (Fig. 3).

In the photosynthesis process there are five entropic components to consider:

1. The declining entropy in the glucose formation
2. The increasing entropy of glucose breakdown
3. The increasing entropy of the environment heat-receiving
4. The declining entropy of biological matter being assembled
5. The declining entropy of energy concentrating in new matter

It should be emphasized that in more complex and ordered structures and functions (points 1,4,5), entropy decreases. The leaf, absorbing solar energy, plays a central role in the mechanisms above mentioned (Tsukaya, 2018).

The possibility of development of leaves with syntropic adaptations seems to be prevalent (and perhaps limited) in the most recent groups in the evolution of Angiosperms.

Plants evolution in the terrestrial environment, which occurred during the last geological periods, has led to deep modifications, both in the structures for the photosynthetic function (leaf characters) and in those for reproduction (flower development): in the latter, the development of a striking phenomenon of co-evolution with insects has been the most general driving factor for evolutionary processes, both in the morphological field and in the physiology of reproduction.

Scheme – Development of vegetative adaptations to obtain greater evolutionary efficiency

Dissemination	
anemophilous	entomophilous
Growing	
horizontal	vertical
Low diversity	high diversity
Poor organization	complex organization

At morpho-physiologic level the leaf represents the most specialized organ to capture sun energetic clean source, reaching the leaves after discharging entropy increase into other celestial bodies, to produce, through photosynthesis, organic matter that also turns out to be clean. This organic matter is concentrated in different organs of a tree, – leaves, branches, trunk, roots – to carry out specific functions with maximum efficiency.
Following the process of growing, woody species with notable vegetative development, develop eco-physiological conditions showing a clear difference between leaves directly exposed to sun radiation and leaves in the shade. In plants living in the shade, transpiration decreases, allowing an important water saving. The organic matter coming from plants is deposited and then concentrated in the upper layers of the soil, the humus, where consumer organisms live: bacteria and mycorrhiza. These differences are further marked in woody perennial plants, in which the living conditions in the shaded parts are clearly different from those exposed to direct sunlight. These conditions are already evident, in general, in shrub plants, but reach their maximum effect in plants with arboreal structure, in which the tree crown, as specialized photosynthesis site, is directly exposed to sun, and has a maximum of laminar development in the leaves. The lower parts are reduced just to the trunk, a cylindrical structure corresponding to the condition of minimum external surface, allowing the development of adaptations for saving water in the physiological transpiration processes: external insulating layer (cortex), no photosynthetic activity, the exclusive function of transferring liquids and dissolved substances.
In summary plants, during a very long period of evolution (much longer than the terrestrial animals evolution), have developed a life cycle allowing them to use a "clean" energy source and to generate ordered structures in relationship with the photosynthesis process.
More generally, according to Capra (2021) and Von Bertalanffy (in Davidson, 1983), evolution of biological systems represents the product of their ability (individuals, species) to enact processes of self-organization such that new biological systems emerge.

Syntropy at community level

The whole process of colonization of the earth's surface by plant species occurs because of the action of sun energy, which, in turn, favors plants organization into plurispecific communities: phytocoenoses. Colonization process followed by selection occurs in all the environments of the earth. Lastly, phytocoenoses with peculiar characteristics and specific species composition settle down in a site. Summarizing, the phenomenon here described as **primary succession** or "development of polyphytic phytocoenoses" occurs through selection of stages among present species with different morphologic characters (herbaceous, shrubby, arboreal) and different ecological requirements (heliophilous/shady species, xerophilous/hygrophilous species).
In the phytocoenoses or communities, the presence of species is not regulated by random factors, but appears to be the result of a well-defined choice of "cyclical organization" which produces vegetation types differentiated according to the general environmental conditions. This cyclical organization of the system could be interpreted as a selection process, which distributes the species into distinct groups,

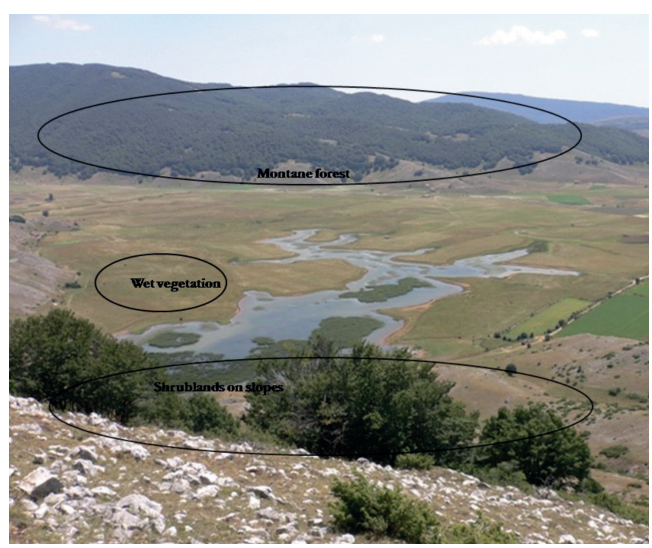

Figure 4. An example of distinct species groups (communities), in relationship with their adaptations to environmental factors.

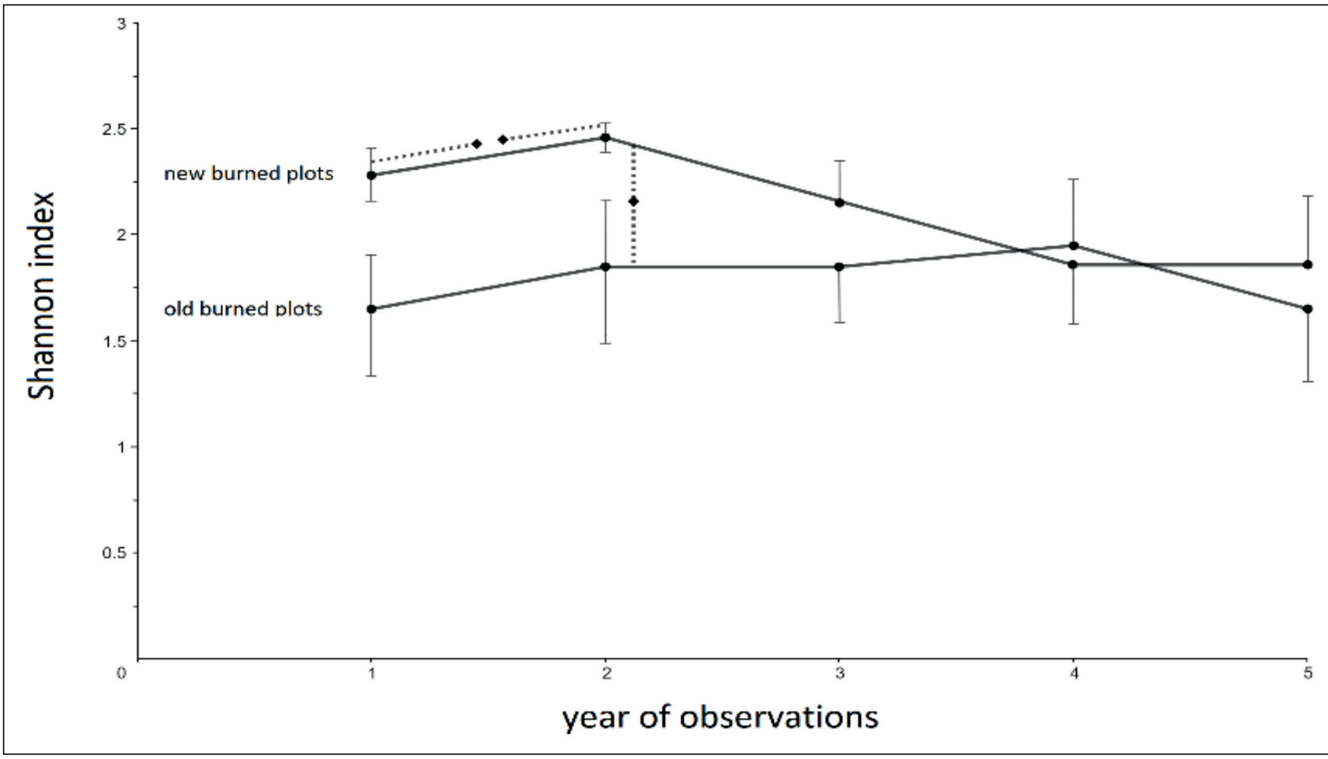

Figure 5. Shannon diversity values calculated in old and new burned plots: after fire species entropy increases and declines in the next years when the pre-fire community recovers.

based on their adaptations to physical-chemical factors, clustering plant species with similar ecological requirements into distinct units (Fig. 4).

All the process generates order increase in the system, entirely consistent with the general theory of the second law of thermodynamics, according to the formulation of Maxwell and Boltzmann (see Rowlinson, 2005). So, phytocoenoses formations can be considered as an entropy lowering in the system which can only occurs because of a high energy input, and which falls within the concept of syntropy (Arcidiacono & Arcidiacono, 1991).

In a **secondary succession** we observe a similar trend toward a syntropy *state*. After a natural or anthropogenic disturbance, *f.i.*, the community is subjected to species disassembly: new species move from the surrounding areas to colonize the soil provoking an entropy increase well described by Shannon diversity index (Shannon & Weaver, 1949), as measurement of the entropy (Fig. 5: De Lillis and Testi, 1992).

Syntropy at ecosystem level

The whole ecosystem, considering the network of linkages with animals and abiotic components, shows a chaotic condition with entropy increasing after fire (Dickman, 2021). In the last stages, instead, a new species composition and, in many cases, the recovery of pre-fire community is established. Entropy decreases and its symmetrical negentropy/syntropy still characterize the ecosystem with ordered structures, functions, and relationships (Guelpa & Verda, 2017).

We can consider another ecosystem example: plant-climate-soil as a model of complex and ordered system (Fig. 6), whose component interactions produce the major humus forms that we know as Mor, Moder, Mull and Amphi (Wilson et al., 2001; Ponge, 2003; 2013; De Nicola et al., 2014).

The picture can be an example of ecosystem complexity where a network of relationships and correspondences among soil-humus-vegetation has been identified by the study on humus and vegetation in Castelporziano Presidential Estate (Rome). The woodland is represented by *Fraxinus oxycarpa* humid subcoastal forest (FRO) and Mesomull humus form was found in correspondence of this forest type. Water fluctuations explain the natural disturbance recognizable in the undergrowth in Fig. 6.

Plant evolution would proceed generating many subsystems, each within another and all connected among them: *f.i.*, humus forms represent a subsystem into the soil system, in spatial and temporal contiguity with vegetation system. Bacteria and mychorrhiza with the plant roots create another subsystem into the soil. Each subsystem has syntropic structures and functions, according to the theory of entropy decrease in relationship with complexity and order increase (Di Corpo & Vannini,

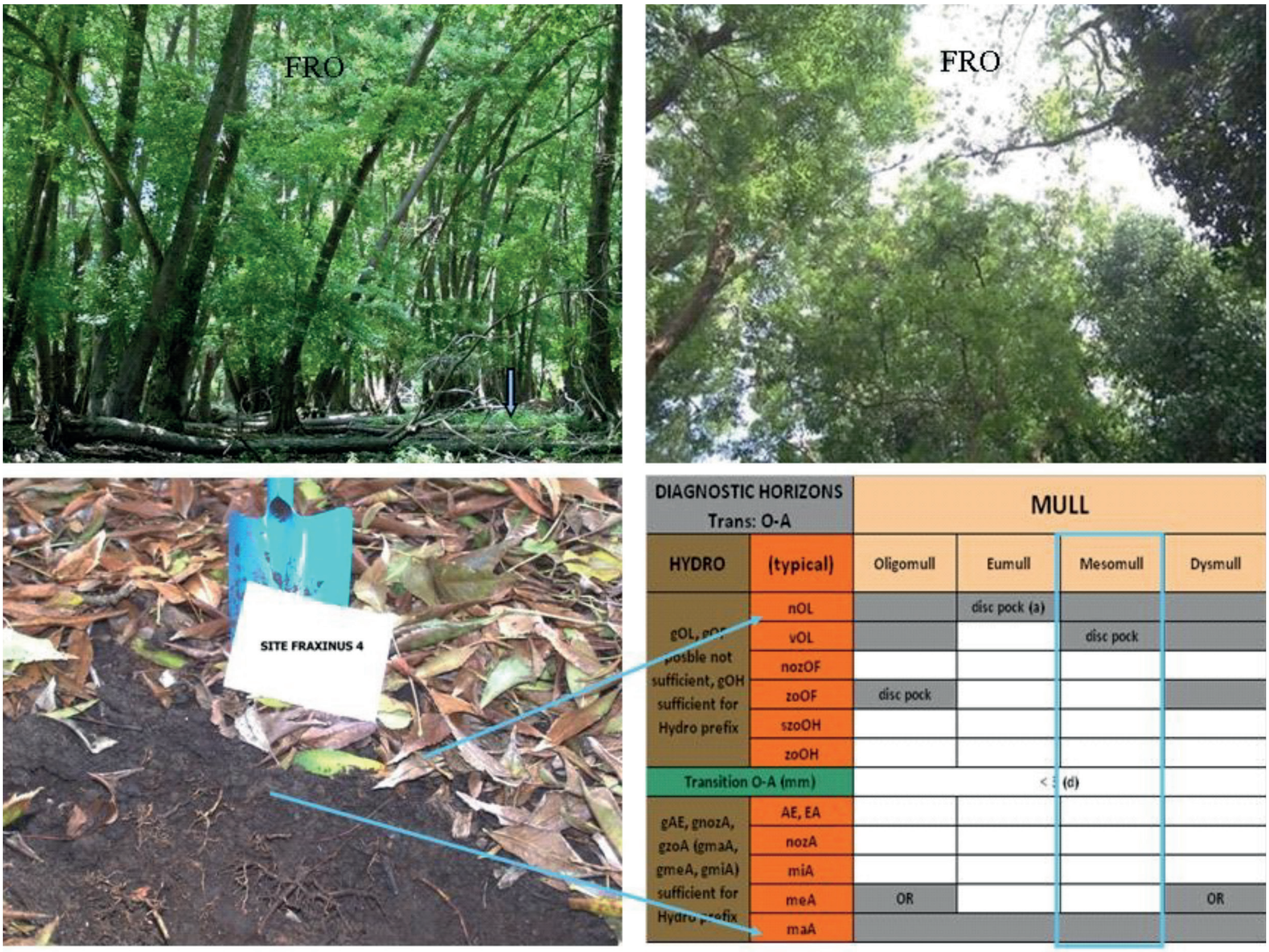

Figure 6. The woodland is represented by *Fraxinus oxycarpa* humid subcoastal forest (FRO) and Mesomull humus form was found in correspondence of this forest type. This humus form is characterized by a continuous OL – Organic Litter horizon and absence of OL and OF- organic Fragmentation horizons. Water fluctuations explain the natural disturbance recognizable in the undergrowth. (The arrows combine the horizons recognizable in the photo with those reported in the table (key of classification of the humus forms of the Mull humus system). The small arrow in the forest shows the location of the profile in the area (De Nicola et al., 2018).

2011). Also, the carbon storage is an expression of order and entropy decrease. The carbon stock stored in the humus and soil layer shows a wide range among the forest types and in general a high concentration; however, the tendency is to increment with the increase of water availability, as in the humid *Fraxinus oxycarpa* forest (Cicuzza et al., 2015).

When natural (water in the case of Castelporziano), or anthropogenic (boars or fire *f.i.*) disturbance affects a community or ecosystem, the tendency toward order and syntropy it reverses pushing the ecosystem in a new chaotic condition of entropy increase. The process of order recovery starts again restoring the same species composition or, if disturbance time is too long and the impact too much strong, generating another species assemblage and consequently a different plant association.

In fact, every disturbance event, whether natural or anthropogenic, generates within a community or ecosystem, a chaotic, but time-limited, disarranging in the composition and distribution of species. By measuring these changes, for example, through Boltzmann's entropy equation* (1872), taken up by Shannon's diversity index (1949), an increase in values is observed: this means that entropy, the quantitative expression of species disorder, increased affecting vegetation structure (Guelpa & Verda, 2017).

*[S= k log W shows the relationship between entropy and the number of ways the atoms molecules of a certain kind thermodynamic system can be arranged]

According to Boltzmann and Shannon, after a fire event, *f.i.*, or soil inundation due to rain regime changes, entropy increases in relation to the highest probability of finding in burned or

flooded areas, many species from surrounding environments that are no longer specific to the previous community: high Shannon diversity-entropy values are, in fact, recorded (Fig. 5). In physical transformations this corresponds to the equilibrium *state* characterized by maximum entropy, but in the LS the tendency toward restoration of order returns, also when the former community is replaced by another more suited to the new environmental conditions generated by repeated and intense disturbance events (Potter et al., 2003). Successions represent, indeed, good examples of the entropy variations: it increases in the pioneer stages when a chaotic species movement occurs and decreases in the last ones when the community reaches a stability in balance with environmental factors. The temporarily lost of order pushes ecosystem towards a new process of self-organization and order generation.
We can assert that entropy variations follow two ways: at general level it increases also in the LS, like in the inanimate matter, but locally it decreases when we consider living organisms as self-organizing systems creating "order out of randomness", always far from thermal equilibrium.
As some researchers stressed, "self-organization is a property of dissipative nonlinear processes that are governed by a global driving force and a local positive feedback mechanism, which creates regular geometric and/or temporal patterns, and decreases the entropy locally, in contrast to random processes" (Aschwanden et al., 2018; Capra, 2021).

Entropy and Syntropy at cultural/scientific level

According to the second thermodynamic law, the Universe and Life evolution is the story of the progressive establishment of increasingly energy-intensive and entropic dissipative structures, where the human societies show the highest values (Fig. 7).
It is very interesting to note that man occupies the final position of an exponential curve representing the energy spreading out along the time arrow (Roddier, 2021).
From another point of view, instead, we must consider that "whatever the human mind finds itself having to understand, order is an indispensable condition"(Arnheim, 2001) and that "in each science, depending on the purpose and topic, man tells himself" (Spengler, 1925).
This assumption is intended to be the basis on which to develop the question of entropy also in the way in which man approaches the study of any level and topic.
In recent times some scientists in vegetation science and plant ecology asked themselves the following question: "May ecoindicators be a way to reduce in the vegetation analysis long time effort with consequent energy spreading out and consequent entropy increase"?

Traditional approach in vegetation science

The best naturalists of the last century had a knowledge of the nature at 360°; a list of plant species sampled and classified, already at that time, was not a simple list of names but rather a scan of an area with information on climate, soil….Even if the concept of "species as indicators" was not yet developed and codified, that kind of scientist already had a holistic outlook (Montelucci, 1953-54; 1976-77; 1980; Pignatti, 1995; 2011). Traditionally, from the floristic composition of a site, Raunkiaer's forms spectrum or the percentage of different geographical distribution of chorotypes were well established in botanical literature, as simple ecological indicators derived (Fanelli et al., 2006).
The development of the vegetation science produced a lot of important results concerning the community classification and consequent realization of an European database (European Vegetation Archive; Chytrý et al., 2016), very useful for the more recent research on habitat classification and its quality evaluation; the limit of this approach is due to analysis exclusively based on a floristic assemblage, which may lead to redundancy in syntaxonomical framework emphasizing small floristic variation that may only be due to local conditions (Crosti et al., 2010).

Application of ecoindicators system

Pignatti (1988) build up the topic of bioindication on the beginning of phytosociology, when Braun-Blanquet

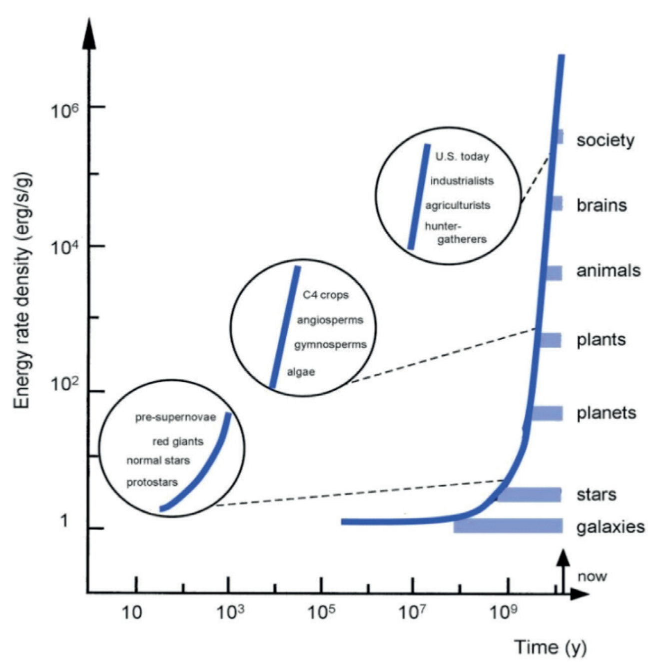

Figure 7. Dissipated energy by unit mass in watt/Kg of different systems as a function of time.

(1926) and Ellenberg (1963) foresaw the development of a multi-methodological analysis overcoming the approach exclusively based on floristic assemblages, advancing the use of ecoindicators. So, following Pignatti (1988; 1995; Pignatti et al., 2001), the life forms as well as the chorological types firstly represent good bioindicators of climate and geographical plant distribution. Ellenberg (1974-1979) marks a shift from a qualitative to numerical approach with his consolidated bioindication model. So, when the Ellenberg ecoindicators for climate (L-Light, T-Temperature, K-Continentality) and soil (pH-Reaction, F-Moisture, N-Nutrients), *f.i.*, started to be applied in different European countries (Zarzycky, 1984; Hill et al., 2000; Borhidi, 1995; Pignatti et al., 2005) and in many ecological studies (Schaffers & Sýkora, 2000; Pignatti et al., 2001, Testi et al., 2006; Fanelli et al., 2007), shortcutting the long-time measurement of chemical and physical parameters, many important results were obtained and new criteria for habitat evaluation and environmental monitoring were given to scientific community (Ellenberg et al., 1992; Körner, 1994), such as to territory managers. The demand of monitoring of species, communities and landscapes becomes ever more pressing at different scale levels: Ellenberg Indicator Values-EIV and ecological maps are a powerful tool in this respect. Ecomaps allow to translate the results of punctual observations into a dynamical and spatial model that can be used as a powerful tool to monitor the coenological shift of species and communities and identify the main ecological factor responsible for the changes (Testi et al., 2006).
The traditional approach changed: mapping EIV instead species or communities is a sort of quantum jumping which would show the states of ecological factors of the species or plant communities in each time (Testi et al., 2021).

A dip from vegetation to soil through soil/humus ecoindicators

To reduce the long-term effort of soil/humus and vegetation sampling and analysis, such as measurements of physical-chemical soil parameters and species/communities' classification, two composite indicators coming from EIV as proposed by Rogister (1978): RxN (Reaction x Nutrient) and R/N (ratio between Reaction and Nutrient) were applied with success to vegetation forestry in Italy. Forest associations and humus forms resulted in agreement each other, described by only two indices (Testi et al., 2021). "So, reducing redundancy to few ecoindicators, two mains in this case, we provided a measure of complexity detecting syntropic structures and functions of the living organisms, through processes of self-organization, dynamic of network and complexity increase, always keeping away from thermodynamic equilibrium (Pignatti, 2003; Varela et al., 1974; Aschwanden et al., 2018). In this way we think to have taken up the initial challenge of Ellenberg and Pignatti and develop their pioneer thought" (Testi et al., 2021). Furthermore, since the two humus indexes resulted highly predictive, we can also overturn the steps of vegetation and humus sampling and analysis: basing on the knowledge and classification of the humus forms in a site, we can predict the vegetation that is expected in correspondence, shortcutting the long-time traditional soil and vegetation analysis.

Conclusion

The processes affecting the order with consequent syntropy decrease, occur at global such as local scale, but are time-limited, since the LS always tends, locally, toward a state of syntropy. More specifically dynamics of populations, communities, ecosystems generate changes in the state of order namely of syntropy of a system or subsystem; structures and functions may be considered as "ordered islands in a physical-chemical matrix", which is subjected to the transformations proper of an isolated system going toward entropy increase.
According to Arcidiacono & Arcidiacono (1991),

- In Nature, entropic and syntropic phenomena are closely overlapping and intertwined
- Syntropic phonemena are anti-dissipative
- In syntropic phenomena, we observe a decrease in entropy with the course of time, because the degree of mixing and uniformity of the system decreases with consequent differentiation processes and complexity increase
- In syntropic phenomena a continuous exchange of matter and energy occurs.

A following synthetic scheme summarizes the main characters and differences between isolated and open systems:
Entropy > in isolated systems with dissipation of energy not reusable; increasing of freedom degrees.
Entropy < in open systems (LS) with high available energy; decreasing of freedom degrees.
Structures and functions supporting the inverse entropy direction toward syntropy:

- Leaf shape – laminar surface to optimize the capture of radiation
- Stomata distribution and dimension optimizing O_2 and CO_2 conductance
- Efficiency in water and nutrients absorption by roots
- Transpiration
- Hydraulic mechanism in the trunk

According to the second thermodynamic law, at general scale, Universe such as living organisms spread out energy causing the entropy increase (Fig. 7), but at local scale we observe biological evolution as the story of a progressive establishment of syntropic-ordered structures and functions characterized by complexity.
As concerns the cultural-scientific point of view, we can dare a similar trend of syntropy increase or entropy decrease when we shift from traditional methods in vegetation analysis into methods relying on ecoindicators, resulted more performing by many studies. So, we can give an answer to the initial question about ecoindicators and complexity: in studying ecosystem complexity the ecoindicators approach and application are comparable to a mind process which reduces "entropy" of the traditional vegetation analysis integrating it in a more suitable and efficient – syntropic- way.

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SANDRO PIGNATTI'S COLLECTION IN THE LIBRARY OF THE DEPARTMENT OF ENVIRONMENTAL BIOLOGY

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Abstract – Library sciences study the specific value of Personal Collections preserved in libraries. It is crucial to valorise them and to make them accessible. Here we present the collection which was donated by Professor S. Pignatti to the library of the Department of Environmental Biology. It contains rare reference materials, mainly concerning his extensive research in the fields of flora, vegetation, phytogeography and ecology, through which it is possible to follow one century of scientific history of what Pignatti has sometimes referred to as "the green belt of the planet".

Keywords: personal collections; authorial libraries; library and archival collections; phytogeography; vegetation; ecology; sandro pignatti.

"Guardando la biblioteca che fu di un grande studioso, ci assilla la domanda che cosa da quelle migliaia di volumi sia filtrato nella personalità intellettuale di lui e abbia contribuito a formarla. È disperante solo tentare di mettersi alla ricerca delle fonti dei pensieri umani: le influenze più profonde sono forse le meno evidenti. A ogni modo quei volumi racchiudono il segreto di un colloquio appassionato durato una vita intera: anche questo ce li fa degni di rispetto" (Barberi F. citato in Guerrini, 2017, p. 94)[1].

For several years, the field of library sciences has been studying the specific value of Personal Collections preserved in our libraries; not only the actual archival collections – for example the importance for twentieth-century culture of the writers' archives preserved at the University of Pavia thanks to Maria Corti – are study material, but now the libraries themselves become tools to study the personalities and working methods of authors and scholars who donated their collections to public institutions.

It is therefore crucial, in the first place, to make these materials accessible by cataloguing them in a way that attests their presence in each specific library, and facilitates their search through the National Library Service (SBN) catalogue. Today, however, it also seems necessary to promote these personal libraries as a tool for exploring and analysing esteemed personalities in science and culture. In this regard we can follow the example of the National Central Library of Rome with the Falqui Hall, where the bookstock of the literary critic Enrico Falqui is preserved and can be viewed; and now with the Calvino Hall – which was established to celebrate the 100th anniversary of his birth – which contains the writer's bookstock and furniture.

Sapienza University is also moving in this direction. The

[1] "Looking at the library that was a great scholar's, the question nags at us what from those thousands of volumes filtered into his intellectual personality and helped shape it. It is despairing just to try to set out in search of the sources of human thoughts: the deepest influences are perhaps the least obvious. In any case, those volumes hold the secret of a lifelong passionate conversation: that, too, makes them worthy of our respect". [trad. dell'a.]

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Enrico Barone Library at the Economy Department has completed cataloguing the Alexandre Marc Collection and the Library of the Environmental Biology Department has completed cataloguing the De Notaris Collection. For both collections Documentary Exhibitions are present on-line.

Sandro Pignatti's bookstock contains his rich personal collection of monographs, periodicals, abstracts, pamphlets, phytosociological tables (also unpublished), charts, correspondences with eminent scholars of Plant Science such as Tüxen, Braun Blanquet and other notable personalities. The collection was donated by Pignatti himself to the library of the Department of Environmental Biology between April 2016 and June 2020.

Looking at single documents and at the collection as a whole, the prolific intellectual activity of Pignatti, his network of relationships, the multiplicity of his interests, and the historical-cultural context in which the Professor operated, emerged. Sandro Pignatti held his long career in University teaching, from 1955, at the Universities of Pavia, Padova, Trieste and lastly at Sapienza University of Rome where he was full professor in Ecology from November 1st 1988 and where he is currently Professor Emeritus. In 1999 he became a member of the National Academy of Lincei.

The rich library and documentary heritage we briefly want to illustrate, originates and develops from his teaching experience as well as from the extensive research carried out in the fields of flora, vegetation, phytogeography, ecology, with particular reference to Mediterranean and Alpine ecosystems.

The main topics of the publications of his Collection are the studies of the flora of Europe and other continents and countries, especially Japan and Australia.

Many works also include environmental issues: conservation of threatened and endangered habitats and species, consequences of antropogenic impact, national parks, origin and development of the environmentalist movement, especially in Germany; other works focus on forest vegetation, nature conservation, vegetation evolution in different European areas – Germany, Austria, Balkans, Poland, Czechoslovakia, Eastern Europe, Iberian Peninsula, France, Mediterranean and Asiatic Regions.

In addition, the works concerning the evolutionary taxonomy are the focal point of the two editions of his monumental work "Flora d'Italia" – the latter divided in 4 volumes (Bologna, Edagricole, 2017-2019) – in which the Author made significant changes in the systematic classification of the botanic families described, using the modern approach based on DNA techniques for the identification of phylogenetic affinities between taxa, instead of using the approach based uniquely on morphology.

Pignatti's Collection testifies to the polyedric nature of his personality, the multiplicity of his interests, and his vast scientific production in the fields of Botany and Ecology. All of this has an impact on his bookstock representing a complex and original reality where documents often blur the line between the bibliotheconomical and archival types.

For example documents containing original handwritten notes are recurrent: books with dedication and signature of the donor which mostly concide with the author of the book, ownership notes or reading notes with the signature of the Professor, comments and original elaborations; most of the documents are marked with a stamp of ownership. During the catalogation process we registered the presence of any autographic interventions which serve as a sort of roadmap of cultural and human exchanges and mark milestones in the professional and personal life of Sandro Pignatti.

The books preserved almost all of the paratextual elements such as covers, original dust jackets and wraparound bands. Between the pages bookmarks, working notes and newspaper clippings are frequently present.

The majority of the works are exclusive of this personal collection which represents a priceless, diverse heritage, through which it is possible to follow a century of scientific history of the world sometimes called by Pignatti "la fascia verde del pianeta" (lit. "the green belt of the planet").

After acquiring the Collection, it was immediately clear that specialists with diverse competences would be required to interpret the collection: from the librarian and the archivist to the researchers in the different fields.

The arrangement of the Collection was also a matter of consideration: the choice was whether to maintain the original disposition of the documents or rather to adjust them to the new location, preserving at least a memory of the original arrangement.

After several meetings with the Professor and analysing the material, we realized that there was not always a systematic or deliberate order, as the Professor collected and kept his own documentation not only on the basis of specific and contingent needs, but sometimes, probably, also in relation to events he casually experienced; indeed we often found within the collection traces of different arrangements which oriented us to identify and consider the various changes and their motives.

The first analysis showed that part of the material was arranged by subject and geographical area, while another part was organized in folders arranged according to sociological syntaxa[2].

For the accomodation of the collection, which occupied a hundred linear metres in the library and another hundred in the warehouse, and to preserve its organicity, it was

[2] Sociological *taxa* are the units into which the Phytosociological Science classifies vegetation of all environments on the planet. *Taxa* or rather *syntaxa* are organized in a hierarchical system of classification that includes sets (of Phanerogams) ordered from the largest to the smallest: class, order, alliance, association, referring to the simplest scheme devised by Braun-Blanquet "the "Father of Phytosociology" (cfr. Braun-Blanquet Josias & Huns Jenny, 1926. Vegetations-Entwicklung und Bodenbildung in der Alpinen Stufe der Zentralalpen. – Schweiz. Naturforsch. Gesell. Bd. LXIII- Abh. 2).

necessary to undertake a series of adjustments of the space and the furnishings of one of the halls in the library in order to guarantee that the materials could be consulted in accordance to the most advanced criteria of library science, to provide the user of the library with the information required.

When the materials were acquired and inventorised, they were placed on shelving selected and purchased following the rules and criteria of the Technical Office of Sapienza University, with a dedicated plaque.

The Collection has been marked with its own location section in order to certify and preserve over time its origin and that of the individual documents, and to guarantee formal identity in the electronic catalogue and shelf material unity as requested by the Professor himself at the time of donation. In addition, he also specifically asked that all the materials on shelf should be available for consultation and loan, in compliance with the library's rules and Regulation; except for the pamphlets and periodicals for which the library, in agreement with the donor, asks for specific and justified requests for the use of the document.

Due to their considerable number and the variety of topics they cover, the monographs were apropriately arranged following the Dewey Decimal Classification, which is particularly useful for books placed on open shelves, in order to be directly accessible to the users. This would allow them to consistenly navigate the collection, which was recently added to the library of Department of Environmental Biology. The pamphlets were organized in appropriate containers trying as much as possible to maintain the original arrangement; they are numbered per box and, within the box, in numerical sequential order.

Regarding the periodicals, not all of them had significant consistency; many were single issues or gifts sent for a brief period. These also reveal the diverse interests of Sandro Pignatti. At the end of 2020 we started the stocktaking job and the insertion in the catalogue of the National Library Service (SBN). As of today, we have inserted 1800 items.

The topic of the author libraries is one of the most appealing for a librarian: the privilege of receiving a donation, but above all, spreading knowledge of the material, which is not merely stored but also utilized, is one of the most thrilling activities, in addition to being the primary means of heritage preservation. The conservation and the study of such collections are even more crucial and necessary in the University sphere, as stated by Fiammetta Sabba:

"Un nuovo ambito di analisi per le biblioteche d'autore potrebbe aprirsi partendo dunque da qui, dalle Università e dai loro fondi librari e archivistici, come luoghi eletti per le attività di didattica, sperimentazione e laboratorio, e soprattutto per la ricerca, che distingue un paese come il nostro erede di una tradizione letteraria, culturale e storica di grande valore e prestigio" (Sabba, 2016, p. 432)[3].

For completeness, we would like to provide some data regarding the Environmental Biology library. This important specialized library in Botany in central Italy serves as a focal point for professors, students, and experts on the subject. It is located on the first floor of the Botany building, which was designed by Giuseppe Capponi between 1933 and 1935, within the University campus of Sapienza, commissioned by Marcello Piacentini.

One notable characteristic of the project is the allocation of around 500 square metres of space dedicated to the library. It is structured into specific and well-defined areas: the botanical library, the periodicals reading room, and an office.

The library owns a collection of around 70000 documents including monographs, periodicals and pamphlets. For years it has been guaranteeing 50 hours of access per week, Monday to Friday, from 9:00 am to 7:00 pm. It provides to the users support on national and international catalographic research and assistance in database search and in the main electronical resources of Sapienza University.

In addition to consulting the entire bibliographic heritage through a totally automated catalogue, containing all the bibliographic informations, the library offers local and inter-library lending, and search and supply of articles not available in library service.

The library organises periodical introductory courses in the use of the resources and the informative and documentary services which take place within the library itself, or in collaboration with other libraries in similar fields. The library owns an important, antique and rare bibliographic heritage consisting of Collections from various origins which hold scientific, artistic and historical value as they represent the meeting point between aesthetic beauty and the enduring importance of botanical research and its ongoing developments.

The library owns 1237 volumes dating back to the period between 1535 and 1837, many of which are illustrated with exceptionally beautiful engravings depicting plants and flowers. These are pictorical works enriched with explanatory texts, serving the educational purpose of distinguishing between different species.

Many of these volumes also serve as a valuable source of information for understanding the role of Botany in the advancement of science. Additionaly, they are rarely found in other Italian libraries, as indicated by the national automated SBN catalogue (National Library Service).

The donation from Pignatti significantly enriched the library's book collection.

[3] "A new field of analysis for author libraries could open up, therefore, starting from here, from universities and their libraries and archival collections, as elected places for teaching, experimentation and laboratory activities, and above all for research, which distinguishes a country like ours, with a literary, cultural and historical tradition of great value and prestige". [trad. dell'a.]

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RELEVANCE OF PUBLIC ACTIVE PARTICIPATION TO ADDRESS THE COMPLEXITY OF SCIENCE-BASED ISSUES, SUCH AS BIODIVERSITY LOSS

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Abstract – In the light of the increasingly prominent role played by science in public life, the definition of a space for public participation is a key issue in the current relationship between science and society. This is especially true when science-based issues, which are often ethically sensitive and controversial, are at stake, such as environmental issues. The paper suggests the usefulness of an active role of the public together with researchers and policymakers in both the decision-making process and the activities to be carried out to address the complexity of current science-based issues, with a focus on the anthropogenic loss of biodiversity and the demand for its conservation and restoration.

Keywords: science-society relationship, science-based issues, citizen science, anthropogenic loss of biodiversity, environmental ethics.

Introduction

Science-based issues, which are often ethically sensitive and controversial, such as environmental issues, require a broader assessment than either researchers or politicians alone can provide. Indeed, these issues usually entail accepting a trade-off between different perspectives and values, a choice which has an increasingly strong impact on the life of everyone – including, in some cases, living organisms other than humans. Hence, the necessity to foster a discussion involving a plurality of experts as well as the public in general and the directly affected population.

As David Tilman, a professor in ecology, highlighted concerning the current anthropogenic loss of biodiversity, which is one of the key environmental issues of the 21st century and a topic of growing importance in the public debate[1], "The loss of biodiversity will diminish the capacity of ecosystems to provide society with a stable and sustainable supply of essential goods and services, but many of the very actions that harm biodiversity simultaneously provide valuable societal benefits" (2000, 210). In addition, note that decisions on which biodiversity conservation or restoration strategy to pursue imply making choices on which values – instrumental or intrinsic – and interests – economic, scientific, aesthetic, philosophical, cultural – to prioritise, as well as which entities to protect or foster – such as humans (poor or wealthy people), non-human animals, plants, species and ecosystems (Jamieson, 2008). In this regard, it is worth underlining the close connection between biodiversity

[1] There is a growing scientific and ethical concern about the current loss of biodiversity given the value of biodiversity for the well-being and survival of life forms (Baard, 2022), and the peculiarities of its decline: the unprecedented speed and scale with which this phenomenon has been occurring over the last fifty years, and the type of causes. Precisely, five anthropogenic causes: habitat fragmentation; overexploitation of resources; climate change; invasive exotic species; pollution (Wilson, 1992; IPBES, 2019). The current loss of biodiversity is considered by many scholars to be the sixth mass extinction and an outcome of the Anthropocene epoch (McNeill & Engelke, 2014; Pellegrino & Di Paola, 2018).

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and the territory in which we and other living beings live: the type of biodiversity found in each territory shapes the territory itself, defining the landscapes inhabitants can enjoy and the resources available to them. Human activities, such as massive deforestation or intensive urbanisation, transform the biodiversity found in a territory, but also change landscapes and natural resources. These transformations are due to human decisions and have an impact on all the life forms, even more so in the current era of the Anthropocene[2] (Pellegrino & Di Paola, 2018). Whether this impact should be considered good or wrong depends on the different points of view and values or interests that are favoured. Thus, it is crucial to have reasons that are not only good but also shared by all stakeholders for making claims for the conservation or restoration of biodiversity, as decisions in this direction raise conflicts among several aspects that we consider important (Sarkar, 2005).

In the light of this scenario, decisions concerning biodiversity conservation and restoration cannot be made by researchers or politicians alone, but ought to be the result of a broad decision-making process shared by all the stakeholders involved and based not only on scientific data but also on awareness of the underling values and interests.

Moreover, this broad decision-making process seems to be especially relevant in the increasingly frequent conditions of *post-normal science* in which researchers find themselves operating. These conditions are characterised by disputed and uncertain scientific evidence, plurality of values at stake and urgency of political decisions – such as the case of the challenges posed by the recent Covid-19 pandemic (Funtowicz & Ravetz 1993/2020; Tallacchini, 2020; L'Astorina & Mangia, 2022). As the philosopher Georg Toepfer, whose research interests include the philosophy of biology, pointed out, the concept of biodiversity is a paradigmatic example of this new scientific context given the dimension of uncertainty and the different perspectives of value involved. "First, the investigation of biodiversity has to cope with uncertainties on the factual as well as the axiological or ethical level. We simply do not know enough about the amount and function of biological diversity; we do not know, for example, whether there are currently three or 100 million species of animals on earth, and we do not know how they contribute to the stability of our ecosystems. Second, we do not know how we should evaluate biodiversity: instrumentally or intrinsically. Third, stakes are high because we are currently facing a loss of biological species probably on the level of one of the five mass extinction events in earth history. Finally, decisions are urgent because this is an irreversible loss and we do not know whether there will be a tipping point when things get worse at an increased speed and scale" (2019, 343).

Based on this framework, this paper highlights the usefulness of an active role of the public together with researchers and policymakers in both the decision-making process and the activities to be carried out to address the complexity of issues related to the anthropogenic loss of biodiversity and the demand for its conservation and restoration, as well as other ongoing science-based issues. This suggestion will be illustrated starting with some considerations concerning the idea of informed social consent, which was raised in bioethical debates, and the proposal of involving citizen advisory groups in the research activities that have such an important impact on public life. Then, the innovative way of producing scientific knowledge called citizen science will be focused on. In particular, the co-creation procedure within citizen science will be highlighted by reporting some benefits which emerged in the Italian environmental epidemiology study "Aria di Ricerca in Valle del Serchio"[3].

A space for public participation

During his years[4] as chairman of the Italian National Bioethics Committee, Giovanni Berlinguer – a physician and teacher in social medicine with an interest in bioethical issues – worked to highlight all the different positions that emerged from time to time when examining the various controversial issues on which the Committee gave an opinion. A democratic way of proceeding centred on giving voice to all positions rather than that of the majority alone. Moreover, this focus on giving visibility to the Committee's internal plurality was accompanied by an openness towards dialogue with the various external viewpoints through the promotion of public debates on the issues raised due to developments in biology and medicine.

In this regard, in the 2001 document *Orientamenti per i Comitati Etici in Italia,* Berlinguer suggested that the internal structures of individual states should set up appropriate democratic instruments. They are necessary, he emphasised, both so that citizens are informed about problems and so

[2] To date, the term 'Anthropocene', i.e. the age of the human, has not yet been formally recognised as designating a new geological epoch. This, however, does not diminish the relevance of the observation that we now live in an epoch characterised by the ability of us humans to profoundly transform the Earth system.

[3] The authors of the present article participated in the research activities of the scientific project "Aria di Ricerca in Valle del Serchio", which is a part of the larger European project *Cities-Health*, as reported later in this text. https://www.ariadiricerca.it/ last accessed April 23, 2024

[4] Giovanni Berlinguer was appointed chairman of the Italian National Bioethics Committee in 1999 and remained in office until 31 December 2001.

that forms of debate can develop that are relevant to public decisions and implementation measures. Furthermore, Berlinguer put forward the idea of informed social consent, in line with the procedure adopted for medical issues. “It may perhaps be said that the principle of ‘informed consent’, a principle that is universally recognised but which is often emptied of that dialogical content that should characterise the relationship between the doctor and the patient, may also apply, on a broader scale, to the relationship between scientific research and citizens. The creation of an ‘informed social consent’ regarding the priorities of biomedical research, its methods and its applications can help guarantee and promote science, at a time when the scientific advances that are taking place almost on a daily basis raise growing hopes and concerns”[5] (Comitato nazionale per la bioetica, 2001, 6).
This social consensus, we can say, is becoming increasingly urgent in the context of contemporary science, which is characterised by uncertainty regarding the information that is acquired and by the pervasive impact of advances in knowledge and technological innovations on the lives of human beings, and more in general on living beings and the environment. In the light of the increasing relevance of science for the public good, the definition of a space for public participation is a key issue in the current historical phase. Indeed, the problems we are currently facing – from the ecological crisis to the extensive use of artificial intelligence – cannot be delegated to, let alone solved by, politicians and scientists alone, as they are issues that affect the life of every individual (Rufo, 2023).
The idea of informed social consent can already be seen in the *International Declaration of Human Rights* of 1948, which in Article 27 states “Everyone has the right freely to participate in the cultural life of the community, to enjoy the arts and to share in scientific advancement and its benefits” and later in 1966 within the *International Convention on Economic, Social and Cultural Rights* approved by the General Assembly of the United States, in which Article 15 recognises “the right of everyone to enjoy the benefits of scientific progress and its applications”. However, ever since the formulation of such a right, as the physician and bioethicist Carlo Flamigni points out in an article dedicated to informed social consent, the question has arisen as to how participation should be considered: whether only as taking part in the benefits of scientific advancements or as real participation in scientific life. In this regard, Flamigni concludes by arguing that “in the literature devoted to this topic, there is convergence on the fact that this right goes far beyond the mere enjoyment of the benefits of scientific progress and certainly includes access to the decision-making room, where so-called ‘scientific creation’ is conceived”[6] (Flamigni, 2017, 203).
On this point, the reflection that the philosopher Philip Kitcher articulates in the volume *Science in a Democratic Society* (2011) is particularly pertinent. The author, moving from the conception of science as a social institution that serves to solve people’s problems and that has a role in public knowledge, explicitly proposes the objective of achieving an integration of scientific competence with democratic values. Kitcher elaborates a concept of science that is influenced by the philosophical pragmatism regarding the reformulation of a non-positivistic conception of science. The author shows how, due to the complexity of much scientific research, value judgements are deeply rooted in its practice. The central issue, therefore, does not lie in the presence of values in science but in the nature of the value judgements made, the value patterns adopted and the way they are applied, as well as the transparent reporting of all these aspects. Specifically, regarding the relationship between science and society, in light of the increasingly prominent role of science in public life, Kitcher argues that the research agenda should be shaped by the informed ideas of a wider public. In his argumentation in this regard, he advances the thesis of the involvement of citizen advisory groups who represent a wide variety of human viewpoints, who are aware of the situation in particular research areas, and who act as intermediaries between the research community and the public in order to reproduce, as far as possible, a conversation that proceeds through mutual engagement with all potential stakeholders. In addition, the author also promotes informed citizen engagement in matters concerning certification. That is, the stage where new findings are accepted or rejected as part of public knowledge.

Active engagement in citizen science practices

In recent years, the idea of taking non-scientists behind the scenes of scientific research is gradually being realised through innovative forms of public participation in scientific

[5] Italian original text: “Si può forse dire che il principio del ‘consenso informato’, un principio che è universalmente riconosciuto, ma che spesso viene svuotato di quel contenuto dialogico che dovrebbe caratterizzare il rapporto tra il medico e il malato, può valere anche, su scala più ampia, per il rapporto tra la ricerca scientifica e i cittadini. Il creare un ‘consenso sociale informato’ alle priorità delle ricerche biomediche, ai loro metodi e alle loro applicazioni può svolgere una funzione di garanzia e di promozione della scienza, in un periodo nel quale i suoi quotidiani progressi suscitano speranze e preoccupazioni crescenti”.

[6] Italian original text: “nella letteratura dedicata a questo tema c’è convergenza sul fatto che questo diritto va molto più in là della semplice fruizione dei vantaggi derivanti dal progresso scientifico e include certamente l’accesso alla stanza delle decisioni, là dove la cosiddetta ‘creazione scientifica’ viene ideata”.

research. One example of this, for instance, is the so-called citizen science: a new way of producing scientific knowledge that actively involves citizens and which aims to achieve shared social goals.

In 2014, the expression citizen science was introduced in the *Oxford English Dictionary* with the following definition: "Scientific work undertaken by members of the general public, often in collaboration with or under the direction of professional scientists and scientific institutions". This definition was subsequently used by the European Commission (2016), which promotes this type of activity in order to increase the capacity of the research agenda to be aligned with the interests of the public and at the same time strengthen public trust in researchers and research institutions (European Commission, 2020). In 2015, key principles were developed to carry out scientifically and ethically sound citizen science projects, respecting the stakeholders involved and taking into account the new demands placed on them (ECSA, 2015).

The concept of citizen science is constantly evolving, and many definitions have been given (Cavalier & Kennedy, 2016; Eitzel et al., 2017). A main feature is the active engagement of the public, which can take place at various levels: from mere data collection to maximum involvement, i.e., active participation in all stages of the research activity. In the case of a research study, from the identification of the questions to be investigated, the outlining of the study protocol and its implementation, to the interpretation of the results and their dissemination. Note how the highest level of involvement is desirable for democratic participation. When researchers and members of the public collaborate in all phases of a study or research activity, these citizen science studies/activities are defined in the literature as co-created (Bonney et al., 2009; Froeling et al., 2021).

The following section will report some benefits that emerged from the co-creation dimension within the Italian participatory environmental epidemiology study "Aria di Ricerca in Valle del Serchio". The aim is to provide some points for reflection in the direction of considering the co-creation modality as a useful tool to be employed in addressing the complexity of issues related to biodiversity loss as well as ongoing science-based issues.

Benefits of the co-creation approach

"Aria di Ricerca in Valle del Serchio" is an environmental epidemiology and citizen science study, whose objective is to measure the frequency of kidney failure in an area affected by potentially polluting production activities (De Marchi et al., 2022). It was part of the larger European project *Citizen Science for Urban Environment and Health* (acronym *CitieS-Health*) which ran from 2019 to June 2022 in five European countries – Italy, Lithuania, the Netherlands, Slovenia, and Spain[7]. Each research group adopted the co-creation procedure to conduct a pilot study on environmental pollution and health.

Since this was an environmental epidemiology context, the right to health regarded public health in general. In particular, the study carried out in Italy (in Valle del Serchio, Tuscany) concerned the right to public health of a community living in a place where potentially polluting production activities are present. All the phases of the study were carried out with the active involvement of the local population, which resulted in certain benefits that do not always emerge in traditional research contexts (Ficorilli, 2022).

A first benefit is having identified and implemented a participatory approach between researchers and citizenship, in which the following points were identified in an inclusive and shared manner: what good needs to be pursued, how it should be done, what possible consequences can be considered to be morally and socially acceptable. For example, in the first phase, the citizenship participated through public meetings and public events in outlining the research objective. In this way, the investigation focused on a public health objective considered relevant by the local community – and not only interesting from a scientific point of view.

Another benefit concerned the application of the principles of research ethics. In fact, the research group, which included researchers and members of public, was particularly scrupulous about the specific characteristics of the research context. We can cite, as an example, the phase of results interpretation in which the citizens, including some local administrators, showed particular care in assessing the impact that the results would have on the community in which they themselves live. They were, on the one hand, particularly open towards fostering the community's awareness of the results obtained, which show some criticality even though they are not yet solid results. On the other hand, they were also cautious in disseminating the results so as not to cause unjustified alarm.

Finally, there was also a benefit concerning transparency in what was being done and would be done. In this regard, the possible uncertainty regarding the results that would be obtained from the survey was clearly explained from the outset. This uncertainty was due to the small numerical size of the population involved and the weakness and/or lack of information on the population's exposure to risk factors for kidney disease. At the same time, the importance

[7] https://cordis.europa.eu/project/id/824484; https://citieshealth.eu/ last accessed April 23, 2024.

of conducting a scientific investigation aimed at public health prevention was emphasised. Another example refers to the phase of outlining together with citizens the contents of the information sheet concerning participation in the study. In that document, particular attention was paid to making explicit reference to the value of storing and using the personal data and biological samples that would be collected in coded form. On the one hand, this represents a value for researchers considering the difficulty in re-contacting participants to collect further information necessary for the study. On the other hand, there is value also for the participants who, thanks to this codification, have a guarantee that their biological samples and data will be stored and used anonymously, and at the same time have the possibility of exercising a series of rights, including the right to request the return of individual results. This clarity is not always enforced in traditional research contexts, where time-consuming and technical language is often used.

Concluding remarks with a focus on biodiversity loss

In the light of the benefits outlined above, it seems plausible to argue that citizen science practices, especially in the sense of co-created practices, constitute particularly appropriate spaces for public participation. Indeed, this type of active engagement fosters an informed and transparent conversation, and shared choices among all potential stakeholders on ongoing science-based issues, which are often ethically sensitive and controversial. Such issues require a broader assessment than either researchers or politicians alone can provide. A debate involving a plurality of experts, as well as the public in general and the directly affected population in particular, is therefore necessary. This is also true in matters pertaining to the current anthropogenic loss of biodiversity and the demand for its conservation and restoration.

Consider, for instance, the different positions and controversies that the creation of large wilderness areas that are free of significant human disturbance may raise to protect many endangered species. The pursuit of such a conservation objective may conflict with the interest that local communities may have in accessing and using the resources in those areas for their own benefit. This interest becomes particularly relevant when the community depends heavily for its survival on the biological resources that it is intended to preserve. In some cases, local communities may be subject to forced displacement to establish and maintain intact natural habitats. As the philosopher of biology Sahotra Sarkar underlines, "Since these strategies are necessarily at least in part coercive, we should have strong ethical justifications for our actions" (2005, 46). In addition, this "human cost often generates political opposition, which can be inimical to biodiversity conservation" (ibid., 44). On the other hand, the management of such protected areas may require the downsizing of a predator species to prevent it from driving a predated species to extinction, thus conflicting the survival interest of the species with the survival interest of individual non-human animals (Jamieson, 2008; Pollo, 2011).

A different situation in which conflicts may arise regards the implementation of wind farms to produce renewable energy. Wind turbines compete with many animals, plants, ecosystems and natural landscapes. One has only to imagine of the extent of the territory occupied by the blade bases, the impact of the blades themselves on the flight of birds, the changes to the territory required to ensure access to the installations – for example road construction and the felling of forests or other types of plants. Wind energy, in short, almost always comes at a price in terms of diminishing biodiversity and radical changing landscapes. However, one objection to all this, supported by many, is that alternative energies are still a necessary and indispensable improvement on the road to ecological conversion or transition (Pellegrino, 2021).

Another example concerns the composite framework when assessing biodiversity regarding food, and how best to preserve and foster it. In this regard, the philosopher Andrea Borghini addressed the question concerning the nature of the criteria for inclusion in conservation effort by focusing on the portion of the living realm that he calls "the edible environment", that is "not simply those plants and animals […] that were domesticated for human consumption, but also the thousands of species that are regularly consumed by some human population and that are regarded to some degree as wild" (2019, 417). Among other things, Borghini argued that "the diversity of the edible environment is deeply entrenched with human cultures, so that the criteria for biodiversity measurement must reflect human perspectives within different societies, embedded in the conceptions of plants, animals, and dieting" (2019, 431). Moreover, he pointed out that preferences for certain forms of life over others are based on multiple values – e.g., food sovereignty, food security, gastronomic pleasure, and intrinsic value. Therefore, in addressing issues of biodiversity conservation and promotion in this regard "It is important to explore how such values differ across societies and whether convergence over a few selected values is a desirable goal, or if lack of convergence is actually more fruitful for the purpose of the biodiversity of the edible environment" (ibid., 431).

Faced with such complexity embedded in issues related to biodiversity conservation and restoration, we suggest that their identification, understanding, evaluation and management may be facilitated by adopting a participatory

approach in which the public is an active subject together with researchers and policymakers in both the decision-making process and the activities to be carried out. Indeed, the active role of the public fosters the inclusion of the different points of view which are involved; transparency regarding the values, interests and priorities at stake; the identification of shared objectives and values; and the enhancement of the public's scientific and ethical knowledge and skills. All these aspects are relevant to balancing, in an ethically and socially approvable manner, losses and gains, which any choice in the conservation and restoration of biodiversity inevitably entails; as well as evaluating how to fairly distribute the impact of such choices among present and future individuals (Kitcher, 2011).
As zoologist and wildlife conservation policy and practice expert John G. Robinson points out, in order to have successful biodiversity conservation programmes, it is essential that the design of such programmes takes the local social context into consideration. Indeed, Robinson declares "If opposition to a given conservation approach is sustained, if local stakeholders are disenfranchised, and if the benefits of conservation do not outweigh the costs over the long term, then that particular conservation approach will ultimately fail (Robinson, 2011, 963).

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ALIEN FLORA IN FRESHWATER ECOSYSTEMS: BASIC KNOWLEDGE FOR MITIGATING THREATS TO NATIVE BIODIVERSITY IN LAZIO REGION (CENTRAL ITALY)

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Abstract – Freshwater ecosystems are among the most biologically diverse on Earth providing essential ecosystem services for nature and society. Human impacts on lakes, rivers, streams, wetlands, and associated riparian habitats are dramatically reducing biodiversity and robbing critical natural resources and services. Degradation of freshwater ecosystems is more rapid than that of terrestrial ones. Main anthropogenic threats include pollution, land-use change, and biological invasions. Regarding biological invasions, one of the main drivers of biodiversity loss at the global level, the listing of floristic diversity represents a fundamental step to help manage non-native species.

Here we present the list of aliens occurring in freshwater ecosystems of Lazio region (central Italy) and their characterization. The list includes 118 taxa (11.9% of the alien regional flora), belonging to 89 genera and 49 families. Richest families are Asteraceae (18 taxa; 15.2%) and Poaceae (10 taxa; 8.5%); richest genera are *Amaranthus*, *Cyperus*, *Euphorbia*, *Oenothera*, *Symphyotrichum*. Eleven taxa are listed among the worst European alien species. Therophytes, which usually suggest xeric conditions, are highly represented (30 taxa; 28.8%), highlighting their capability of adapting to diverse environmental conditions. The high proportion of taxa with a wide distribution (28 taxa; 23.7%) reveals the occurrence of r-selected species; 38 taxa (33.2%), native to the Americas, confirm the high migration and commercial flows between New and Old Worlds. Several taxa occur in more than one habitat (aquatic, riparian, humid), with aquatic habitats including the highest percentage of invasive taxa (27.3%). Most invasive species are: *Alternanthera philoxeroides*, *Lemna minuta*, *Ludwigia peploides*, *Pontederia crassipes* (free-floating macrophytes) and *Arundo donax* and *Robinia pseudoacacia* (terrestrial species). The number of invasive species decreases with inundation rates, whereas casual aliens increase. The analysis of Hydroecological regions shows a high percentage (33.3%) of invasive taxa in HER13 ("Appennino Centrale"), probably due to the occurrence of industrial sites in the Sacco river valley (southern Lazio).

Keywords: biodiversity, biological form, chorotype element, hydroecoregions, invasive species, status of naturalization.

Introduction

Plant invasion is a global phenomenon associated with human-mediated transportation of non-native plants beyond their native distribution ranges (Seebens et al., 2021; Gloria et al., 2023). During the last few centuries, the number of alien species increased in many taxonomic groups, mainly due to trade, transport, and land-use change (Hulme, 2009; Pauchard & Alaback, 2004). Biological invasions are considered the fifth main driver of biodiversity loss at the global level, especially due to naturalized and invasive aliens, which respectively include about 14000 and 2500 taxa at the global scale (Bhatta et al., 2023). These aliens are one of the most significant causes of ecosystem disruption (the so-called ecological impact) but are also responsible for economic (e.g. reduction of agricultural productivity; Matzrafi et al., 2023) and social impacts (e.g. allergenicity; Pecoraro et al., 2024).

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Freshwater ecosystems (embracing streams, rivers, lakes, riparian areas, and other wetlands) represent key hotspots for biodiversity and, at the same time, are one of the most impacted natural systems worldwide due to human activities and climate change (Reid et al., 2019; Bolpagni, 2021; Polce et al., 2023). Especially, vulnerability to human activities is related to the behaviour of inland waters as filters and acceptors for effluents (Severini et al., 2020), which in turn facilitates physico-chemical perturbations, generated and exerted on the catchment scale, and spread of alien species (Bolpagni, 2021).

In this context, many countries have established laws and regulations to safeguard freshwater ecosystems, including the designation of protected areas, setting of water quality standards, and implementing measures to control pollution and habitat destruction. At the international level, protection tools include the UN *Ramsar Convention on Wetlands* (1971) and, in the European Union, the *Habitats Directive* (92/43/EEC), the *Birds Directive* (79/409/EEC), and the *Water Framework Directive* (2000/60/EC, focused on ensuring good qualitative and quantitative health, i.e. on reducing and removing pollution and on ensuring that there is enough water to support wildlife and human needs).

As part of the ongoing studies on the flora of Italy, with a special focus on Lazio region (central Italy; e.g., Celesti-Grapow et al., 2013; Iberite & Pelliccioni, 2010; Iberite & Iamonico, 2015; Iberite et al., 2015, 2017; Iamonico et al., 2014, 2020, 2022a; Di Pietro et al., 2022; Iamonico, 2022; Sciuto et al., 2023), we present the list of the alien species occurring in freshwater ecosystems, providing an overview from various point of views (status of naturalization, biological forms, and chorotypes).

Material and methods

The floristic list of alien taxa occurring in freshwater ecosystems of Lazio region was first extracted from a comprehensive work on the vascular flora (Anzalone et al., 2010) and from the regional atlas of the alien flora (Lucchese, 2017). In turn, these sources are based on all previously published literature along with field collections made by the authors themselves. For more updated information, a further check of published literature from 2017 to 2024 was carried out and additional floristic records were added, i.e. from Iamonico (2015, 2021, 2022), Stinca et al. (2021), Iamonico et al. (2022b), Iamonico & Nicolella (2023, 2024). As regards the flora by Anzalone et al. (2010), we considered all the aliens occurring at least in one of the following habitats (the name originally adopted by the authors is in brackets): marshland ("acquitr." = acquitrini), tributary ("affl." = affluente), clayey ("argill." = argilloso), river ("F." = fiume), canal ("F.so" = fosso), stream ("torr." = torrente), banks ("sponde"), humid environments ("amb. umidi" = ambienti umidi), humid forests ("boschi umidi"), pebbly riverbeds ("greti"), humid uncultivated lands ("incolti umidi"), and riverbanks ("rive"). As regard the flora by Lucchese (2017), since no habitat was clearly specified, we referred to the localities in which the species were recorded [e.g., *Ipomoea purpurea* (L.) Roth was considered as a riparian species since it was indicated along the banks of Tiber River]. All these habitats were therefore arranged into five zones, according to dominant fluvial dynamic processes as proposed by Dufour & Rodríguez-González (2019), and thus into three main macro-habitats, i.e. aquatic, riparian, and humid (Tab. 1).

Table 1. Macro-habitats of occurrence adopted in the present study (last row of the table) based on the zones proposed by Dufour & Rodríguez-González (2019) and respective ecological/biological features.

	Zone 5	Zone 4	Zone 3	Zone 2	Zone 1
Water occurrence	Permanently inundated	Frequently inundated	Regularly inundated	Occasionally inundated	Rarely inundated; inundation absent
Sediments	High sediment dynamics	High sediment dynamics	Significant sediment deposition	No significant sediment dynamics	No sediment dynamics
Plants ecology	Aquatic plants tolerant of permanent inundation and burial	Emergent aquatic and riparian plants tolerant of frequent inundation and burial	Riparian plants tolerant of regular inundation and moderate sedimentation	Riparian plants with vary inundation tolerance	Plants tolerant to soil moisture or alluvial groundwater regime
Biological form	Hydrophytes	Helophytes	Phanerophytes	Phanerophytes	Geophytes, Therophytes
Macro-habitat type	Aquatic	Aquatic	Riparian	Riparian	Humid

Suddenly, each taxon (the nomenclature follows the *Portal of the Flora of Italy*; PFI 2024) was associated with a biological form and chorotype according to Pignatti et al. [2017-2019; chorotypes where reclassified according to Iamonico (2022)], and with the status of naturalization in the region according to Anzalone et al. (2010) and Lucchese (2017).
Concerning the habitat, we firstly considered the proposal by Dufour & Rodríguez-González (2019) who classified plants along rivers into five zones based on the dominant fluvial dynamic processes. Based on these five zones, we classified three habitats, i.e. aquatic, riparian, and humid (Tab. 1).
Finally, we analyzed the occurrence of alien species with respect to the hydroecoregions (HERs), i.e. geographic areas with a narrow variability in chemical, physical and biological features of water courses and adopted by the European Member States for the implementation of the *Water Framework Directive* (Wasson et al., 2006). A GIS approach [overlapping of layers of HERs and Province boundaries of Lazio region (Wasson et al., 2006)] was used.

Results and discussion

The list of alien plants occurring in freshwater ecosystems of Lazio region includes 118 taxa [corresponding to 11.9% of the total alien regional flora (539 taxa according to Galasso et al., 2024)], belonging to 89 genera and 49 families. Four taxa belong to Monilophyta [*Azolla filiculoides* Lam., *Cyrtomium falcatum* (L.f.) C.Presl, *Nephrolepis cordifolia* (L.) C.Presl, and *Salvinia molesta* D.S.Mitch.], whereas the remaining 114 are Angiosperms (Gymnosperms are lacking). The richest families are Asteraceae Bercht. & J.Presl (18 taxa, corresponding to 15.2% of the flora) and Poaceae Barnhart (10; 8.5%); 24 families comprise between 2 and 5 taxa, whereas 23 families include one taxon each. The richest genera are *Amaranthus* L., *Cyperus* L., *Euphorbia* L., *Oenothera* L., and *Symphyotrichum* Nees (3 taxa each); 18 genera comprise 2 taxa each, whereas 66 genera are monospecific. To be noted that 11 taxa (marked with an asterisk in the following list) are included in the list of the worst alien species in Europe (Nentwig et al., 2018).

MONILOPHYTA
DRYOPTERIDACEAE
Cyrtomium falcatum (L.f.) C.Presl, H ros, East Asian, CAS

NEPHROLEPIDACEAE
Nephrolepis cordifolia (L.) C.Presl, G rhiz, Tropical, CAS

*SALVINIACEAE
Azolla filiculoides Lam., I nat, Tropical, NAT
Salvinia molesta D.S.Mitch., I nat, Wide distribution, CAS

ANGIOSPERMAE
ACANTHACEAE
Ruellia simplex C.Wright, NP, American, CAS

AMARANTHACEAE
**Alternanthera philoxeroides* (Mart.) Griseb., I rad, South American, NAT
Amaranthus blitoides S.Watson, T scap, North American, INV
Amaranthus hybridus L. subsp. *hypochondriacus* (L.) Thell., T scap, U.S.A. and Mexico, NAT
Amaranthus retroflexus L., T scap, North American, INV
Beta vulgaris L. subsp. *vulgaris*, H scap, Eurimediterranean, CAS
Dysphania ambrosioides (L.) Mosyakin & Clemants, H scap, Tropical, INV

APIACEAE
Apium graveolens L., H scap, Mediterranean, NAT
Coriandrum sativum L., T scap, South-Western Mediterranean, CAS

APOCYNACEAE
Nerium oleander L. subsp. *oleander*, P caesp-P scap, Stenomediterranean, CAS
Trachelospermum jasminoides (Lindl.) Lem., P lian, Asian, CAS

ARACEAE
Colocasia esculenta (L.) Schott, G rhiz, Asian, NAT
Lemna minuta Kunth, I nat, Tropical, INV
Lemna valdiviana Phil., I nat, American, CAS
Wolffia arrhiza (L.) Horkel ex Wimm., I nat, Tropical, CAS
Zantedeschia aethiopica (L.) Spreng., G rhiz, African, NAT

ARALIACEAE
**Hydrocotyle ranunculoides* L.f., I nat, Tropical, NAT

ASPARAGACEAE
Asparagus officinalis L. subsp. *officinalis*, G rhiz, Eurimediterranean, CAS
Chlorophytum comosum (Thumbs.) Jacques, H scap, African, CAS

ASTERACEAE
Artemisia annua L., T scap , Eurasian, NAT
Artemisia verlotiorum Lamotte, G rhiz, East Asian, INV
Bidens aurea (Aiton) Sherff, H scap, American, CAS

Bidens frondosa L., T scap, North American, INV
Cotula coronopifolia L., T scap, African, NAT
Delairea odorata Lem., Ch frut, South African, CAS
Eclipta prostrata (L.) L, T scap, Tropical, CAS
Erigeron annuus (L.) Desf. subsp. *annuus*, T scap, North American, NAT
Erigeron karvinskianus DC., H scap, Tropical, NAT
Guizotia abyssinica (L.f.) Cass., T scap, African, CAS
Helianthus tuberosus L., G bulb, North American, INV
Inula helenium L., H scap, Orophile-South-East-European, CAS
Senecio inaequidens DC., T scap, African, INV
Symphyotrichum lanceolatum (Willd.) G.L.Nesom, H scap, North American, CAS
Symphyotrichum novi-belgii (L.) G.L.Nesom, H scap, North American, CAS
Symphyotrichum squamatum (Spreng.) G.L.Nesom, H scap, Tropical, INV
Tripleurospermum inodorum (L.) Sch.Bip., H bienn, European, CAS
Xanthium orientale L., T scap, American, INV

BALSAMINACEAE
Impatiens balfourii Hook.f., H scap, Asian, CAS
Impatiens parviflora DC., T scap, East Asian, NAT

BRASSICACEAE
Armoracia rusticana G.Gaertn., B.Mey. & Scherb., G rhiz, Est European, CAS
Brassica nigra (L.) W.D.J.Koch, T scap, Eurimediterranean, CAS
Rorippa austriaca (Crantz) Besser, H scap, Pontic, NAT

CANNABACEAE
Cannabis sativa L., T scap, Asian, CAS

CANNACEAE
Canna indica L., G rhiz, Tropical, CAS

CAPRIOFOLIACEAE
Lonicera japonica Thunb., P lian, East Asian, NAT

CLEOMACEAE
Polanisia dodecandra (L.) DC. subsp. *trachysperma* (Torr. & A.Gray) Iltis, T scap, North American, CAS

CONVOLVULACEAE
Ipomoea indica (Burm.) Merr., G rhiz, Tropical, NAT
Ipomoea purpurea (L.) Roth, T scap, Tropical, CAS

CUCURBITACEAE
Cucurbita maxima Duchesne subsp. *maxima,* T scap, American, CAS
Sicyos angulatus L., T scap, North American, CAS

CYPERACEAE
Cyperus alternifolius L. subsp. *flabelliformis* Kük., H caesp, Tropical, CAS
Cyperus eragrostis Lam., G rhiz , Tropical, CAS
Cyperus esculentus L., He, Tropical, NAT

EBENACEAE
Diospyros kaki Thunb., P scap, Western Asian, CAS
Diospyros lotus L. , P scap, Asian, CAS

EUPHORBIACEAE
Euphorbia humifusa Willd., T rept, Asian, NAT
Euphorbia nutans Lag., T scap, North American, NAT
Euphorbia pulcherrima Willd. ex Klotzsch, NP, Central American, CAS

FABACEAE
Acacia dealbata Link, P scap, Australian, NAT
Amorpha fruticosa L., P caesp, North American, NAT
Robinia pseudoacacia L., P scap-P caesp, North American, INV

HALORAGACEAE
Myriophyllum aquaticum (Vell.) Verdc., I rad, South American, NAT
HYDRANGEACEAE
Hydrangea macrophylla (Thunb.) Ser., NP, East Asian, NAT

HYDROCHARITACEAE
Elodea canadensis Michx., I rad, North American, INV

JUGLANDACEAE
Carya illinoinensis (Wangenh.) K.Koch, P scap, North American and Mexican, CAS

LAMIACEAE
Melissa officinalis L. subsp. *officinalis*, H scap, West Asian, NAT

LINDERNIACEAE
Lindernia dubia (L.) Pennell, T scap, North American, NAT

LYTHRACEAE
Punica granatum L., P scap, West Asian, NAT

MALVACEAE
Abutilon theophrasti Medik., T scap, Pontic, NAT
Hibiscus moscheutos L. subsp. *moscheutos*, H scap, Circumboreal, CAS

Hibiscus trionum L., T scap, Tropical, CAS

MORACEAE
Morus alba L., P scap, East Asian, CAS
Morus nigra L., P scap, West Asian, CAS

MYRTACEAE
**Eucalyptus camaldulensis* Dehnh. subsp. *camaldulensis*, P scap, Australian, CAS
**Eucalyptus globulus* Labill. subsp. *globulus*, P scap, Australian, CAS
Melaleuca williamsii Craven subsp. *synoriensis* Craven, P caesp, Australian, CAS

NELUMBONACEAE
Nelumbo nucifera Gaertn., I rad, Paleotropical, NAT

ONAGRACEAE
**Ludwigia peploides* (Kunth) P.H.Raven subsp. *montevidensis* (Spreng.) P.H.Raven, H caesp, American, NAT
Oenothera glazioviana Micheli, H bienn, European, NAT
Oenothera rosea L'Hér. ex Aiton, H bienn, South American, CAS
Oenothera stricta Ledeb. ex Link subsp. *stricta,* H bienn, South American, CAS

PHYTOLACCACEAE
Phytolacca americana L., G rhiz, North American, INV
PLATANACEAE
Platanus hispanica Mill. ex Münchh., P scap, Eurimediterranean, NAT
Platanus orientalis L., P scap, Sud-Est European, NAT

POACEAE
**Arundo donax* L., G rhiz, Sub Wide distribution, INV
Echinochloa colona (L.) Link subsp. *colona*, T scap, Tropical, CAS
Dactyloctenium aegyptium (L.) Willd., T scap, Tropical, NAT
Panicum capillare L., T scap, North American, CAS
Panicum dichotomiflorum Michx., T scap, North American, CAS
Paspalum distichum L., G rhiz, Wide distribution, INV
Phyllostachys reticulata (Rupr.) K.Koch, P scap, West Asian, CAS
Setaria adhaerens (Forssk.) Chiov., T scap, Tropical, CAS
Setaria parviflora (Poir.) Kerguélen, H caesp, South American, NAT
Zea mays L. subsp. *mays*, T scap, Tropical, NAT

POLYGONACEAE
Fallopia baldschuanica (Regel) Holub, P lian, Eurasian, CAS

PONTEDERIACEAE
**Pontederia crassipes* Mart., I nat, Tropical, INV

ROSACEAE
Prunus cerasifera Ehrh., P scap-P caesp, Pontic, NAT
Prunus persica (L.) Batsch, P scap-P caesp, East Asian, CAS

SALICACEAE
Populus ×canadensis Moench, P scap, North American, NAT
Salix babylonica L., P scap, Tropical, CAS
Salix ×fragilis L., P scap-P caesp, Eurosiberian, CAS

SAPINDACEAE
Acer negundo L., P scap, North American, NAT

SIMAROUBACEAE
Ailanthus altissima (Mill.) Swingle, P scap, Asian, INV

SOLANACEAE
Datura stramonium L., T scap, Wide distribution, INV
Nicotiana tabacum L., H scap, North American, CAS
Physalis angulata L., T scap, American, CAS
Solanum chenopodioides Lam., T scap, South American, NAT
Solanum lycopersicum L., T scap, South American, CAS

ULMACEAE
Ulmus laevis Pall., P scap, Middle European, CAS
Ulmus pumila L., P scap-P caesp, East Asian, CAS

URTICACEAE
Boehmeria nivea (L.) Gaudich., H caesp, Asian, CAS

VERBENACEAE
**Lantana camara* L., P caesp, Tropical, CAS

VITACEAE
Vitis ×instabilis Ardenghi, Galasso, Banfi & Lastrucci, P lian, European, NAT
Vitis riparia Michx., P lian, North American, INV

Prevalent life forms (Fig. 1) resulted to be therophytes and phanerophytes (30 taxa each, corresponding to 28.8% of the flora). Therophytes are annual species, which usually suggest xeric conditions in habitats such as pastures, uncultivated lands, or synanthropic environments (see e.g., Iamonico 2022). The high percentage of therophytes in freshwater ecosystems can be explained since the recorded taxa are able to colonize places characterized by ecological conditions not strictly related to the presence of water [e.g. *Euphorbia nutans* Lag., *Nicotiana tabacum* L., *Prunus persica* (L.) Batsch, *Solanum chenopodioides* Lam.].

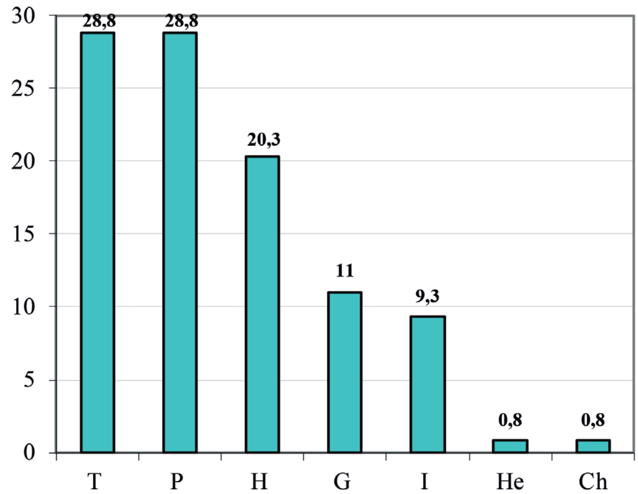

Figure 1. Percentage (axis y) of plant life form spectra of the vascular alien flora of aquatic, riparian, and humid habitats in Lazio region. T: therophytes; P: phanerophytes; H: hemicryptophytes; I: hydrophytes; G: geophytes; He: helophytes; Ch: chamaephytes.

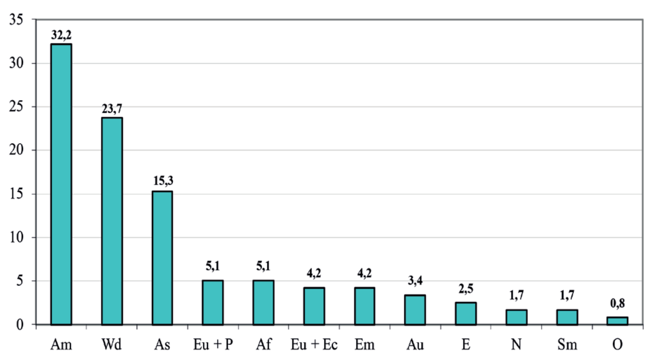

Figure 2. Chorological spectrum of the vascular alien flora of aquatic, riparian, and humid habitats in Lazio region (percentages along axis y). Am: Americas; Wd: Wide distribution; As: Asia; Eu + P: Eurasian + Paleotemperate; Af: South Africa; Eu + Ec: European + Euro-Caucasian; Em: Eurimediterranean; Au: Australia; E: Eastern; N: Nordic; Sm: Stenomediterranean; O: Orophytes-South-East-European.

The chorological spectrum (Fig. 2) shows a high percentage of wide distributed species (Cosmopolitan, Subcosmopolitan, and Tropical; 28 taxa, 23.7%), revealing the occurrence and spread of r-selected species in regional freshwater ecosystems. Most of the aliens are native to the Americas (38 taxa; 33,2%), confirming the high migration flow between the New and Old Worlds (see also Lucchese, 2017).

The distribution of taxa per habitat shows that some taxa occur in more than one habitat. Aliens that exclusively grow in riparian habitats are 19 (16.1%), while 56 taxa (corresponding to 47.5%) also occur in other places (uncultivated lands, walls, sidewalks, etc.). Humid habitats host 23 taxa (19.5%), while 11 taxa (9.3%) are exclusive to aquatic habitat. Finally, there are 8 taxa (6.8%) occurring in both aquatic and riparian habitats. Although non-native species of aquatic habitats are few, they include the higher percentage of invasive (27.3%). The number of invasive species decreases based on inundation rates of the habitat (see Tab.1), whereas the casual increase (Fig. 3).

The relationship between habitats and biological forms (Fig. 4) shows that: 1) aquatic habitats include mostly hydrophytes, 2) phanerophytes represent the most common biological form in riparian habitats, 3) the most represented biological forms in humid habitats are hemicryptophytes (39.1%) and therophytes (34.8%), and 4) chamaephytes and helophytes are overall rare.

The regional territory is intercepted by four hydroecoregions (HER11 "Toscana", HER13 "Appennino Centrale", HER14 "Roma-Viterbese", and HER15 "Basso Lazio"; Fig. 5). HER14 (the wider hydroecoregion in the region and embracing the Metropolitan City of Rome Capital) hosts 95 taxa, followed by HER15 (63 taxa), HER13 (43 taxa), and HER11 (21 taxa). The status of naturalization (Fig. 6) shows a high percentage of invasives in HER13 (33.3%), probably due to the occurrence of industrial sites in the Sacco river valley (Frosinone Province).

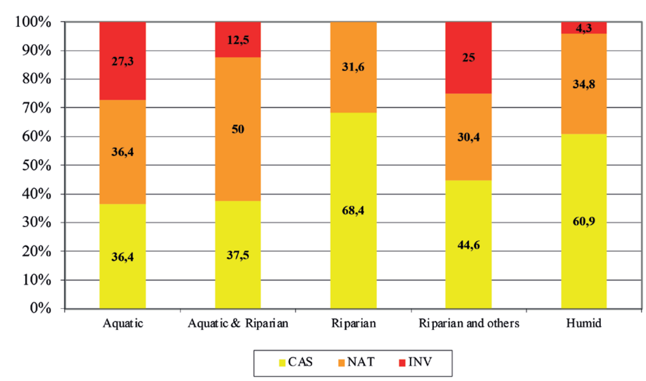

Figure 3. Percentage of casual, naturalized, and invasive taxa (axis y) per habitat (axis x).

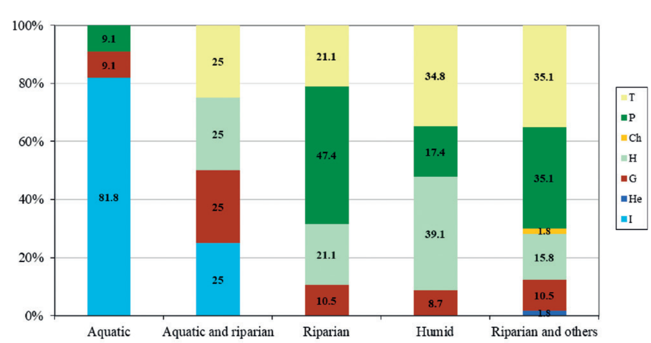

Figure 4. Percentage of biological forms (axis y) per habitat (axis x). T: therophytes; P: phanerophytes; ; Ch: chamaephytes; H: hemicryptophytes; G: geophytes; He: helophytes; I: hydrophytes.

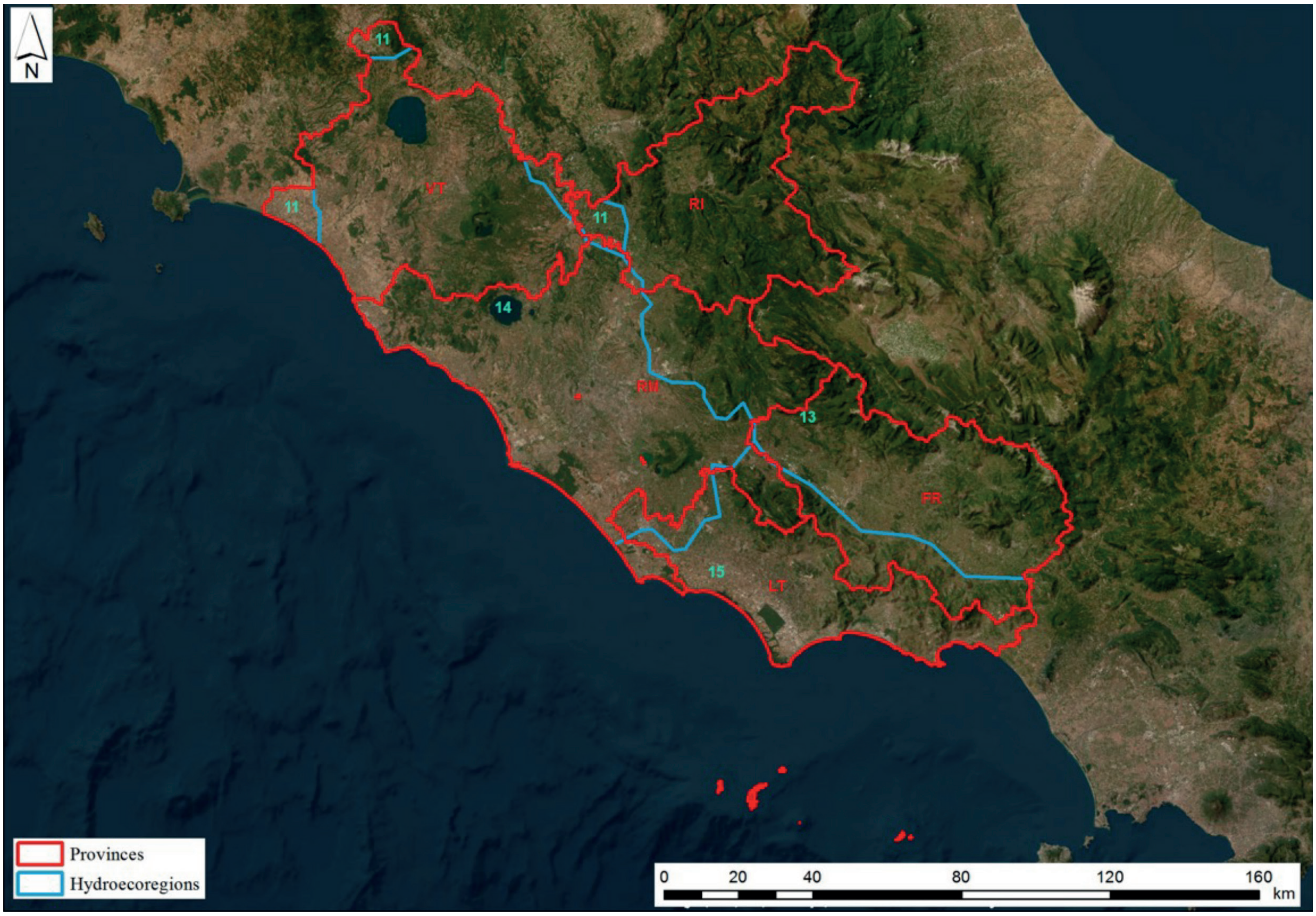

Figure 5. Hydroecoregions in Lazio region. Numbers 11, 13, 14 e 15 refer hydroecoregions codes "Toscana", "Appennino Centrale", "Roma_Viterbese", and "Basso Lazio" respectively. Codes for administrative provinces: VT = Viterbo; RI = Rieti; RM = Rome; FR = Frosinone; LT = Latina.

Concluding remarks

Freshwater ecosystems are very affected by biological invasions throughout Europe, with a high rate of recorded IAS (Invasive Alien Species) (European Environment Agency 2020; Polce et al., 2023). These ecosystems are also highly vulnerable to other different stressors (such as water eutrophication, land-use change, increasing temperatures), which may trigger irreversible shifts upon which biodiversity and ecosystem services may be lost (Angeler et al., 2014). In this context, non-native species amplify the whole vulnerability of freshwater ecosystems to environmental changes (Angeler et al., 2014) so that they necessarily must be controlled and managed, starting from floristic listing and characterization (Haber, 1997; Wu et al., 2008; Langmayer & Lapin, 2020; Balogianni et al., 2022; Wagensommer, 2023).

The assessment performed for compiling the Red List of Ecosystems of Italy confirmed such a co-occurrence of threats (Blasi et al., 2023; Capotorti et al., 2023), especially for the following ecosystem types that occur in the Tyrrhenian ecoregion (approximately coincident with HER14 plus HER15 in Lazio region):

– the peninsular riparian forest ecosystems with *Salix alba* L., *S. purpurea* L.subsp. *purpurea, S. brutia* Brullo & Spamp., *Populus alba* L., *P. nigra* L. subsp. *nigra*, *Alnus glutinosa* (L.) Gaertn., *Fraxinus angustifolia* Vahl subsp. *oxycarpa* (M.Bieb. ex Willd) Franco & Rocha Afonso, *Hypericum hircinum* L. subsp. *Majus* (Aiton) N.Robson, assessed as endangered because of a forecasted increasing trend in biological invasion combined with a declining distribution and negative effects from intensive agriculture;

– the peninsular riparian hygrophilous freshwater ecosystems with *Phragmites australis* (Cav.) Trin. ex Steud., *Typha* sp. pl., *Carex riparia* Curtis, *C. acuta* L., *Agrostis stolonifera* L. subsp. *stolonifera, Ranunculus flammula* L., *Scirpoides holoschoenus* (L.) Soják, *Paspalum* sp. pl., *Scrophularia canina* L., *Helichrysum*

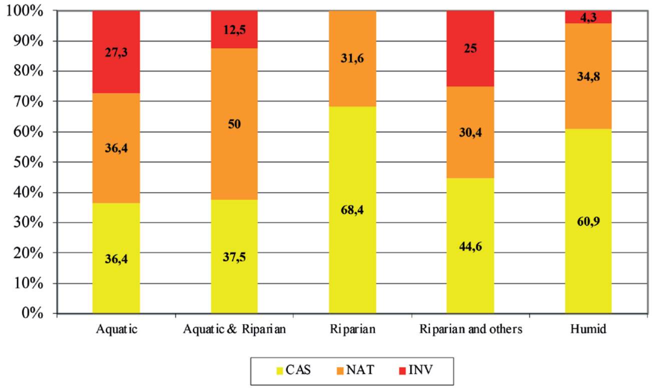

Figure 6. Percentage of casual, naturalized, and invasive taxa (axis y) per hydroecoregions (axis x). HER11: "Toscana"; HER13: "Appennino Centrale"; HER14: "Roma_Viterbese"; HER15: "Basso Lazio".

italicum (Roth) G.Don subsp. *italicum,* assessed as vulnerable because of current and forecasted trend in biological invasion combined with soil sealing and negative effects from intensive agriculture;

– the peninsular running freshwater hydrophytic ecosystems with *Ranunculus trichophyllus* Chaix*, Helosciadium nodiflorum* (L.) W.D.J.Koch subsp. *nodiflorum*, *H. inundatum* (L.) W.D.J.Koch, *Glyceria fluitans* (L.) R.Br., *Baldellia ranunculoides* (L.) Parl., *Nasturtium officinale* W.T.Aiton, assessed as vulnerable because of forecasted trend in biological invasion combined with a declining distribution, soil sealing, poor biological quality of waters and negative effects from intensive agriculture.

With an increased detail, the analyses here presented highlighted that most impacted natural freshwater ecosystems in Lazio region are related to aquatic and riparian habitats. Aquatic habitats host the highest proportion of IAS (27.3% on the total non-native species therein occurring), whereas riparian habitats host the highest absolute number of IAS (14). Aliens in aquatic habitats, particularly dangerous to biodiversity due to the ability to compete for space and natural resources (light, nutrients, etc.), include the following free-floating macrophytes that can be considered especially detrimental: *Alternanthera philoxeroides* (see e.g., Iamonico & Sánchez-Del Pino 2016), *Lemna minuta* (Ceschin et al., 2016), *Ludwigia peploides* (Gori et al., 2023), and *Pontederia crassipes* (Brundu et al., 2013) (Fig. 7). These species are characterized by vigorous vegetative reproduction and tend to form highly dense mats which not only positively compete which the autochthonous flora (especially for light; Lind et al., 2022) but also change the physico-chemical features of the water body (by reducing light penetration that, in turn, causes a variation of nutrient cycling and a reduction of ecosystem resistance to the invasion).

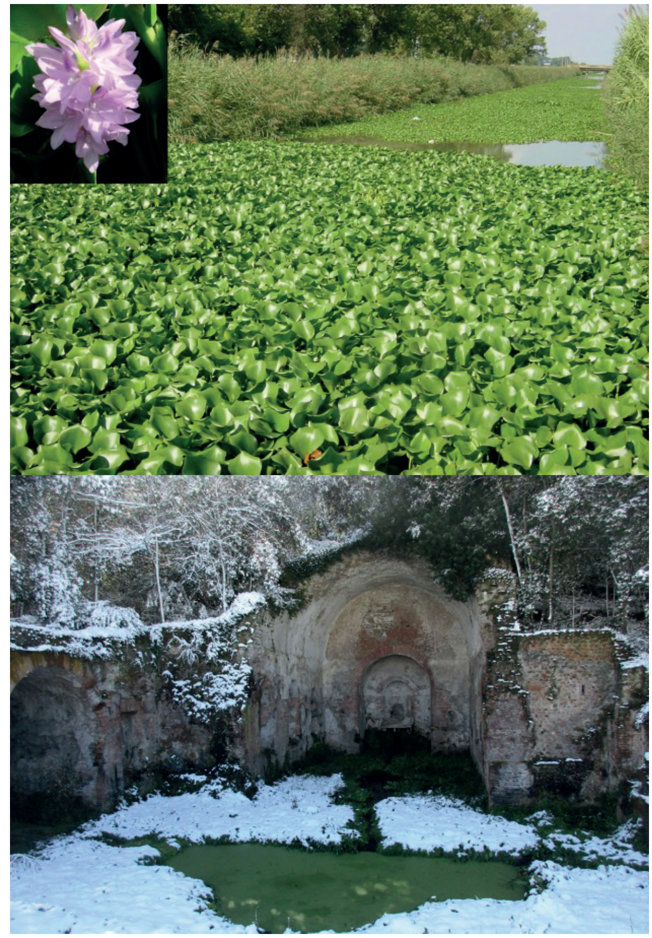

Figure 7. *Pontederia crassipes* (top image; photo by M. Iberite) at località "Canale della Botte" (Borgo Ermada, Latina city) and *Lemna minuta* (bottom image; photo by D. Iamonico) in aquatic habitat of Egeria nymphaeum during snowfall in February 2018 (Caffarella valley, Regional Park of Appia Antica, Rome).

Figure 8. *Arundo donax* along Almone river (Rome city) (photo by D. Iamonico).

Concerning the areas regularly or occasionally inundated (riparian habitats), the species that causes major threat seem to be *Arundo donax* (Fanelli, 2002) and *Robinia pseudoacacia* (Lucchese, 2017), which commonly form monospecific populations (Fig. 8). Specifically, *R. pseudoacacia* (reproducing both from seeds and by sprouting from the roots) sometimes even completely replace native riparian vegetation dominated by *Populus* sp. pl. and *Salix* sp. pl. and competes for pollinating bees (Branquart et al., 2015).

Due to the important role of riparian and humid habitats, in both ecological terms (Geist, 2011) and ecosystem services capacity (Vári et al., 2022), we argue for a continue monitoring of alien species growing in these environments with the final aim to carry out actions for control/reduction and, when possible, complete eradication of most threatening populations.

Aknowledgements

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FACTORS INFLUENCING THE GERMINATION CAPACITY OF *PRIMULA PALINURI*, CLIFF-DWELLING ENDEMIC SPECIES OF SOUTHERN ITALY

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Abstract – *Primula palinuri* Petagna is a narrow-endemic plant species native to southern Italy, thriving along the Tyrrhenian coast. In this study, we compared various aspects of two populations of *P. palinuri* growing in different sites: one by the sea in the SAC Capo Palinuro (IT8050008) and the other about two kilometres inland in 'La Vaccuta,' SAC Fascia Interna di Costa degli Infreschi e della Masseta (IT8050011). Seed abundance, size, germination capability, and salt response were analysed. Seed counts and measurements were conducted using image analysis software. Germination responses were assessed at three at three alternating temperatures (6-15°C, 10-20°C, and 15-25°C) in the first experiment. Subsequently, seeds from both sites were subjected to increasing NaCl concentrations (0mM, 50mM, 100mM, 200mM, and 300mM) at 6-15°C and 10-20°C. All germination experiments took place in Petri dishes containing filter paper and distilled water. No discernible differences in capsule and seed sizes were observed between the two sampling sites. However, the study revealed that higher temperatures and elevated salt concentrations negatively affected the germination rate in both populations, particularly in seeds from the innermost site. This suggests a preference for cooler regimes and higher tolerance to saline conditions in the sea-exposed population for optimal germination. This research delved into the physiological and ecological adaptations of *P. palinuri* to its current environmental envelope, investigating how seed properties, temperature, and salinity stress influence seed germination potential. The findings provide promising insights that could significantly enhance conservation and management strategies for this species. The study aimed to compare the germination characteristics of two populations of *Primula palinuri* along the Tyrrhenian coast of southern Italy.

Keywords - vulnerable populations, cliff area, endangered and rare species, germination, adaptation.

Introduction

With approximately 6,700 species, 1371 of which are endemic (Peruzzi et al., 2014), Italy is among one of the richest countries in vascular plant species in the Mediterranean biogeographical region, a well-known global biodiversity hotspot (Myers et al., 2000; Thompson, 2005). Various factors have contributed to the country's high rate of endemism: (a) geographical position, (b) diverse bioclimatic conditions, (c) complex palaeogeographical and (d) paleoclimatic history (Blondel & Aronson, 1999; Thompson, 2001; Comes, 2004; Blasi, 2010; Casazza et al., 2016).

Primula palinuri Petagna is an endemic plant species listed since 1997 as endangered in the Red Book of Plant Species of the IUCN Red List of Threatened Plants and recognised under Annexes II/IV of the Habitats Directive (92/43/EEC) and the Bern Convention (1979). In the Campania region (Italy), *P. palinuri* is safeguarded by Regional Law No. 40 of 1994.

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P. palinuri is the only species of the *Primula* genus with a Mediterranean ecology. The other taxa are mainly found in mountainous regions on Upper Triassic/Lower Jurassic limestone and dolomite substrates (Aronne et al., 2014; Aronne et al., 2018). Geological changes and coastal retreat have played a pivotal role in delineating the current range of *P. palinuri*'s populations, with the existing stations representing remnants of larger populations that existed in the past (Ricciardi, 1973). Today, the species counts only restricted populations growing on north-facing sea cliffs along the Southern-Tyrrhenian coast of Italy (Silvestro et al., 2020; Pignatti et al., 2017). Genetic and demographic monitoring insights indicate a scarcity of young individuals over the entire areal, highlighting a lack of regeneration and establishment. This poses significant concerns for the species's long-term survival in its current habitat (De Micco & Aronne, 2012a, De Micco & Aronne, 2012b).

Vertical cliffs provide sensational opportunity to mitigate inter-specific competition, predation, fire, shrub encroachment, human activities, and effects of climatic changes in the surrounding landscape (Larson et al., 2000; De Micco & Aronne, 2012). Gathering on cliff habitats is a common strategy of rare chasmophytes with narrow ecological niches and low competition capacity (De Micco & Aronne, 2012). Such environments shape peculiar microhabitats where both early-stage and mature *P. palinuri*'s plants find the proper conditions to complete their biological cycles. Physical factors of cliff spots, such as light, temperature, water availability, and salinity, differ significantly from nearby flat areas (Aronne et al., 2018) and often play a pivotal role in determining species recruitment (Silvestro et al., 2020), representing the bottleneck for the species fitness (Del Vecchio et al., 2020). This is common in species that inhabit tightly defined habitats because of co-adaptation processes (Navarro & Guitian, 2003).

To cope with such ecological selection processes, *P. palinuri* developed specific adaptive strategies, including morphoanatomical modifications to regulate water relations, protect against high radiation levels, and defend against biotic factors (Aronne & De Micco, 2004; Stachowicz, 2001; Padilla & Pugnaire, 2007). These adaptations allowed the species to colonise and thrive in such a narrow environmental envelope, shaping community structure and dynamics (Baraloto et al., 2005). The research conducted by Strumia et al. (2020) highlights that *P. palinuri* cannot resume germination with increasing saline concentrations, and seawater completely inhibits the germination of its seeds. These findings add salinity to other environmental factors previously identified as significant constraints for the germination of *P. palinuri* seeds, such as exposure to direct light and soil type (Aronne et al., 2018). This evidence underscores the importance of considering a range of environmental factors, including salinity, in fully understanding the germination of *P. palinuri* seeds, and provides further insights into the biology and ecology of this threatened species. In this study, we examined whether different expositions to temperature and salt concentrations, reflecting the proximity/distance to the sea of two populations of *Primula palinuri*, introduced different germination patterns in the species as a consequence of site-specific adaptations. Although *P. palinuri* develops highly viable seeds, with generally 85% germinating success (De Micco & Aronne, 2012), little is known about the various environmental factors and mechanisms influencing seed germination, seedling survival, and early plant development.

Methods

Field sampling

P. palinuri seeds were collected in July 2022 from two sites within Parco Nazionale del Cilento Vallo di Diano e Alburni: Porto Palinuro and Vaccuta, Campania region, Italy. Porto Palinuro is located near the sea in the Special Area of Conservation (SAC) 'Capo Palinuro (CP) - IT8050008', while Vaccuta (VAC) is approximately 2 km innermost, in the SAC 'Fascia Interna di Costa degli Infreschi e della Masseta - IT8050011' (Fig. 1).

The two study areas are characterised by a Mediterranean climate (Daget, 1977) but with a different average annual rainfall regime of 763 mm in CP and 838 mm in VC, respectively (BIO12 Wordclim). The precipitation regime is concentrated mainly in autumn and winter, while a dry period persists from May to September (Aronne et al., 2015). The temperature pattern is also different at the two sites, with about 12.5 °C and 9 °C, respectively (BIO11 Worldclim). This climatic scheme influences water availability, moisture conditions and soil salinity in the topsoil, with significant implications for plant species' germination and survival, including *P. palinuri.*

Seed sampling for the germination test was carried out according to the protocols of Bacchetta et al. (2006). In each site, fruits (capsules) from visible healthy individuals with similar size and exposure were sampled at a distance among them of at least 50-100 m to ensure genetic variability. Individuals were sampled in July, complying with the end of the ripening period of the species (Pignatti et al., 2017). A total of eight individuals were sampled to avoid detrimental effects on *P. palinuri* populations (Bacchetta et al., 2006).

According to Bacchetta et al. (2006), samples underwent a post-maturation process of 15 days before laboratory trial to ensure uniform seed maturity levels.

Capsule and Seed Morphometric Traits

In the field, we counted the number of capsules of eight individuals per sampling site. These capsules were then delivered to the Germplasm Bank of the Botanical Garden of Rome.
The capsules were then opened to count the number of seeds contained within each capsule. Seeds were divided in two separate containers, one for each sampling site. From each container, 50 seeds were randomly selected, and their major (mm) and minor axes (mm) were measured. This process allowed us to gather data on the size of the seeds from each sampling site, providing information on seed morphology and potential variations between populations. The morphometric traits were measured using NIS-Element Br. 2.10 with images from a digital camera Nikon Digital Sight DS-U1, mounted on a stereo microscope Carl Zeiss.

Seed germination in response to different ecological factors

The germination potential of sampled seeds was tested by conducting different treatments according to the protocols of Bacchetta et al. (2006) and ISTA (2006). Before conducting any experiments, seeds were sterilised using a 5% hypochlorite bleach solution for 5 minutes according to the protocol of Magrini et al. (2019).
We conducted two germination trials to investigate: (a) the germination response of *P. palinuri* seeds to various temperature conditions and (b) the effect of different salt concentrations on seed germination with two fixed temperature range. Analyses were performed exclusively on seeds from ripe capsules.
To assess the germination capability of *P. palinuri* seeds at

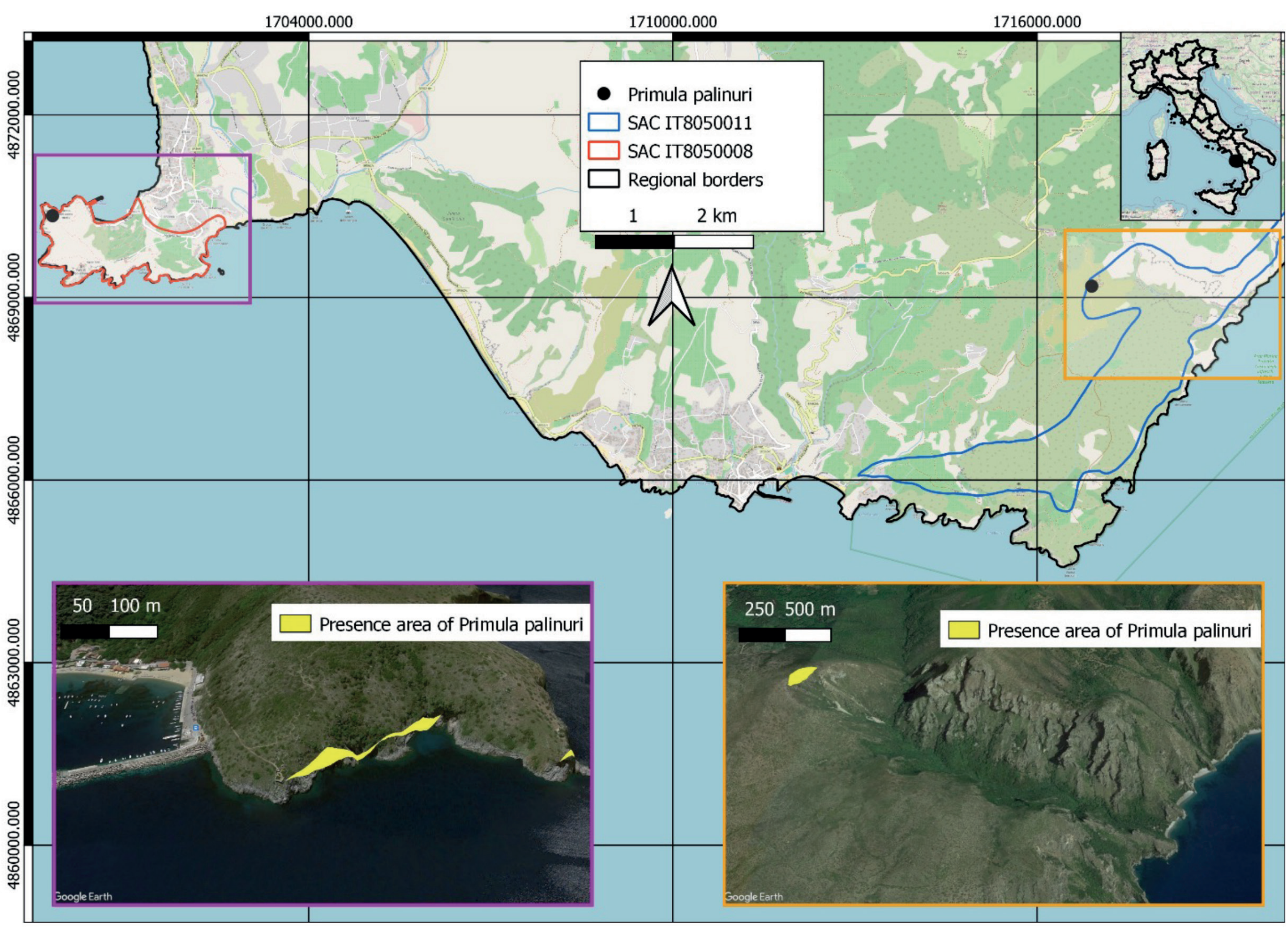

Figure 1. Distribution of P. palinuri sampling site (Capo Palinuro and Vaccuta) within Parco Nazionale del Cilento Vallo di Diano e Alburni. The points in red represent the sampling points, the lines in red represent the boundaries of the area in the Natura 2000 network. The boundaries in black are the regional boundaries.

differenttemperatures,weconductedexperimentsingermination cabinets at three temperature levels: 6-15°C (minimum), 10-20°C (average), and 15-25°C (maximum). Cabinets were programmed to alternate between 12 hours of darkness and 12 hours of light, simulating natural day-night cycles. We adjusted the cabinets so that the 12 hours of light coincided with the highest temperature level, replicating natural conditions. To simulate the effects of light on seeds, Petri dishes containing the seeds were incubated with cold white fluorescent tubes, which provided a photon flux density of ensuring an adequate light source for the germination process.

Afterwards, we conducted experiments to assess the germination capability of *P. palinuri* seeds under different salt concentrations (NaCl solution). Five fixed salt concentrations were used: 0 mM (control), 50 mM, 100 mM, 200 mM, and 300 mM, following the protocol of Ghazi N. Al-Karaki (2001). The germination cabinets were set up with the same alternating 12-hour light-dark cycle applied in the previous experiment. The Petri dishes containing the seeds were placed in the cabinets and exposed to the cold white, fluorescent tubes, providing a photon flux density of during the light period. The experiment was conducted at 10-20°C, the average temperature in which the two populations differed, and at 6-15°C, the temperature where the behaviour of the two populations is similar.

For all the germination experiments, 4 replicates of 25 seeds were set up for each site per treatment. Each replicate was placed in Petri dishes with a layer of filter paper as a substrate and distilled water. Germination was assessed daily for each sample for a total test duration of 30 days. Seeds are determined to be germinated when they have produced a visible rootlet with a length greater than 1 mm (Bacchetta et al., 2006). At the end of the germination tests, we assessed missing viability tests (cut test).

Data analysis

Poisson Generalized Linear Models (GLM) with logarithm as a link function were run to investigate potential variations in the number of seeds per capsule among the different sampled populations. To compare the size of the minor (mm) and major (mm) axes of the seeds between the two sampling sites, we estimated a Gamma GLM, with a logarithmic link function.

GLM Beta Regression Models, with a logit link function, were employed to compare the maximum germination response of seeds placed under different treatments (temperature, salt). We used a log-logistic time-to-event model to analyse germination times, focusing on cumulative proportions rather than individual times. Traits like flowering or germination, measured at intervals, are considered interval-censored due to varying observation times, making precise event timing uncertain (Onofri et al., 2018). The two-parameter log-logistic function is given by the equation:

$$f(x) = \frac{1}{1 + \exp^{(b(\log(x) - \log(e))}}$$

In the equation, the parameters 'b' and 'e' represent the slope at the inflexion point of the curve and the germination percentage at the inflexion point, respectively. The parameter 'e' then expresses the day on which 50% of all germinated seeds per treatment (T50) is reached.

All analyses were carried out using R software version 4.3.1, *drcSeedGerm* package (Onofri et al. 2018), *drc* (Ritz et al., 2016) and *betareg* (Cribari-Neto & Zeileis, 2010).

RESULTS

The estimated Poisson GLMs revealed distinctions between the two sampling sites, indicating a greater number of capsules produced per individual at the VAC sampling site, the innermost one (Fig. 2a). Conversely, the count of seeds per capsule did not exhibit variance between the two sites (p-value > 0.05; Fig. 2b).

The estimated Gamma GLMs highlighted overlapping between the confidence intervals of the major and minor axes of seeds sampled in CP and VAC (Fig. 3), indicating no significant difference between the seed morphometries of the populations (Tab.1).

The GLM Beta regression model estimated to explore maximum germination variability across the sampling sites indicates variations under the different treatments (Fig. 4).

For the minimum temperature trials, VAC and CP exhibited similarly high germination percentages (estimated at 92% and 91%). The overlap between the confidence intervals of both populations suggests no significant difference between them (Fig. 4). The 10-20°C treatment induced a higher germination percentage in CP (CI 2.5% 86% and CI 97.5% 95) compared to VAC (CI 2.5% 58% and CI 97.5% 90%) but with more pronounced confidence intervals. No estimation models were applied for the maximum temperature treatment as the seeds of both sampling sites did not germinate at all.

The time-to-event model showed that when treated at the minimum temperatures, seeds from CP sampling site take a shorter time to germinate than VAC (Tab. 2). The estimated time to reach 50% germination (parameter 'e') is 12,01 and 13,83 days, respectively. At the intermediate temperature ranges, the germination speed of CP's seeds is even more pronounced than for those from VAC with 13,24 and 17,74 days, respectively, and already in the initial stages (parameter 'b')

The Beta regression model was also estimated to explore the germination variability of target *P. palinuri's* populations

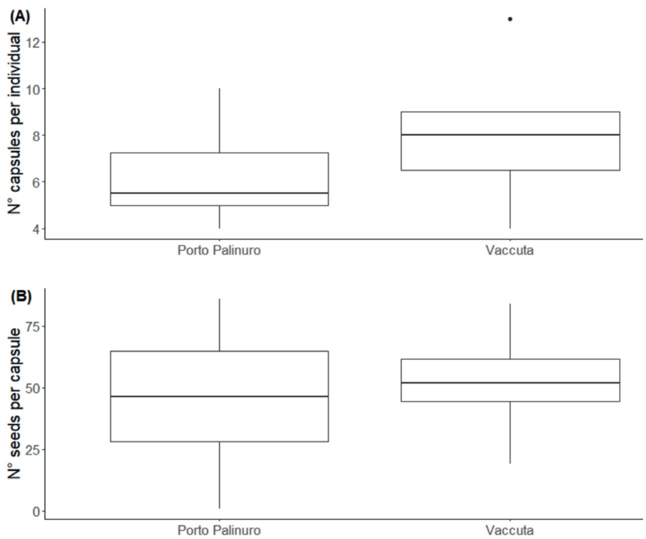

Figure 2. Boxplot of number of capsules per individual **(a)** and number of seeds per capsule **(b)** of the two-sampling site.

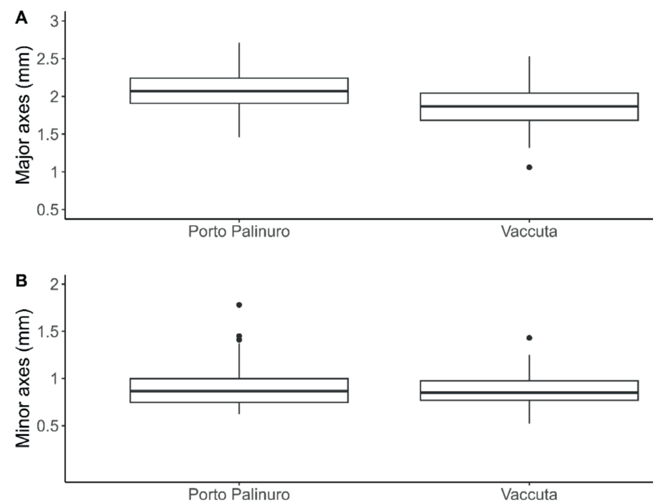

Figure 3. Boxplot of major (a) and minor axis (b) of seeds and width (c) and length (d) of the two populations of Primula palinuri

treated with different salt concentrations at 20-10°C. At 0 mM salt concentrations, seeds from both sampling sites achieved very high maximum germination (90% and 84%) (Fig. 5A).
The germination percentages began to shrink with the increase of the salt concentrations (50- and 100- mM NaCl). However, CP's seed responds better than VAC, maintaining rather high germination performances (87% or 71%), while in VAC, the percentages drastically decrease at 65% and 36% when seeds are treated with 50 mM and 100 mM, respectively. No model was estimated for 200 and 300 salt solutions because the germination was practically neil.
The time-to-event model confirmed the negative impact of salt exposure on *P. palinuri* seeds. Germination time does not consistently change when seeds from both sites are treated at 0 mM NaCl (control) but progressively slows down at increasing salt exposition (Tab. 3).
The results of the Beta regression model estimated for salt treatment at 6-15°C showed that seeds from both sampling sites exhibited notably high germination rates at 0 mM salt concentrations (95% and 94% respectively) (Fig. 5B). However, as salt concentrations increased, germination percentages gradually declined, although they remained relatively elevated compared to results obtained at temperatures of 20-10°C with salt treatment. Notably, germination was still observed at salt concentrations of 200 mM and 300 mM.
Furthermore, CP seeds demonstrated a more favourable response compared to VAC, maintaining relatively high germination rates even at salt concentrations of 200 mM and 300 mM (65% or 31%). In contrast, VAC seeds experienced a significant decrease in germination percentages at higher salt concentrations, dropping to 19% and 36% at 200 mM and 300 mM in both sampling site.
The time-to-event model confirmed the adverse effect of salt exposure at 5-16°C on *P. palinuri* seeds. Germination time remained relatively consistent when seeds from both sites were treated with 0 mM NaCl (control), but it progressively slowed down with increasing salt exposure (Fig.6).

Discussion

Our results demonstrated that variations in temperature and salt tolerances among physically separated populations are

Table 1. Estimates (fourth column) of GLM for capsule and seed sizes (first column) for each sampling site (second column) and confidence intervals (2.5 third column, 97.5 fifth column).

Parameters	Site	CI (2.5%) mm	Estimate (mm^3)	CI (97.5%) mm
Seed Major axis	Palinuro	2.00	2.08	2.17
Seed Major axis	Vaccuta	1.70	1.86	2.05
Seed Minor axis	Palinuro	0.85	0.91	0.96
Seed Minor axis	Vaccuta	0.77	0.88	1.03

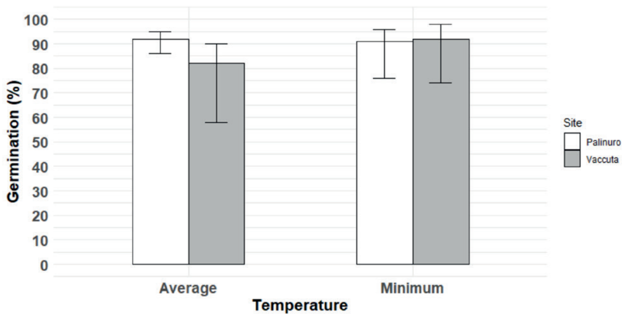

Figure 4. Estimates of maximum germination in percentage of Beta regression model for temperature regimes for each sampling site and confidence intervals. the center rapresent The model estimates, lines represent the 2.5% and 97.5% confidence intervals.

pivotal in determining *Primula palinuri*'s seed germination, rates and speed. Although the occurrence of chasmophytes depends on a complex interaction of different ecological factors, which are difficult to disentangle, our results indicate that cliff-related features are fully involved in the germination patterns and distribution of *P. palinuri*.

Lower temperatures and limited salt concentrations generated high and faster germination rates in the seeds of both studied sites. Conversely, higher temperatures and excessive salt regimes had a strong inhibitory effect, with no germination observed at the warmest (15-25 °C) and saltiest experimental conditions when temperatures were set at 10-20°C (≥ 200 mM NaCl) and a significant reduction of germination capacity and speed already at the intermediate T and salt ranges (10-20 °C; 50- and 100- mM NaCl) for VAC's seeds. CP's seeds showed higher germination rates and shorter times to reach 50% germination than those from VAC, suggesting a greater resilience to such environmental drivers related to the distance from the sea of the two sites.

Inland cliffs like VAC are likely to have cooler temperatures and lower soil salt concentrations due to the gradual decrease in marine influence and other site-level proxies, including wind intensity, altitude, topography, light radiation, and moisture, as one moves away from the coast (Del Vecchio et al., 2020). Seeds originating from the PC coastal cliff area appear to carry peculiar adaptations to tolerate greater temperature and salt conditions. *Primula palinuri* might also follow a germination pattern dependent on avoiding high temperatures, allowing germination to occur primarily during autumn (Barbi, 2008). Although these pieces of evidence are based on laboratory tests, they indicate the existence of optimal requirements for these cliffs' environmental proxies that should be further investigated to approach the species' germination niche (and recreate maximum germination performances) and mitigate both global and local disturbance effects (e.g. climate change, habitat loss) (Larson et al., 2000; Harley et al., 2006; Somero, 2010).

The two sites also differ in fruit productivity, with CP delivering significantly fewer capsules but an equivalent number of seeds. No relevant variations in seed size (major and minor axes length) were detected between the two sites' populations. Although temperature is implied in influencing morphological patterns in capsules and seeds (Mamo et al., 2006) further variables should be taken into account (e.g. altitude, sun exposure) in addition to those related to the distance to the sea to explain fruit structure modification. Comparison of *Primula palinuri* with non-Mediterranean taxa, such as species from mountain regions, should also be relevant to verify the persistence of patterns and local adaptations.

Conclusions

In this study, we investigated the germination capability of *Primula palinuri* Petagna orthodox seeds under various treatments reflecting site-specific conditions. For the first time, it was examined whether and how the distance from the sea may introduce changes in the seeds' ecophysiological responses of

Table 2. Estimates (fifth column) of time-to-event model for temperature (second column) for each sampling site (third column) and confidence intervals (2.5 fourth column, 97.5 sixth column).

Parameter	Temperature	Site	CI (2.5%)	Estimates	CI (97.5%)
B	Average	Palinuro	-9.91	-8.43	-6.95
B	Minimum	Palinuro	-9.87	-8.35	-6.83
B	Average	Vaccuta	-5.17	-4.38	-3.59
B	Minimum	Vaccuta	-11.443	-9.78	-8.12
E	Average	Palinuro	12.72	13.24	13.76
E	Minimum	Palinuro	11.55	12.01	12.48
E	Average	Vaccuta	16.35	17.74	19.13
E	Minimum	Vaccuta	13.33	13.83	14.35

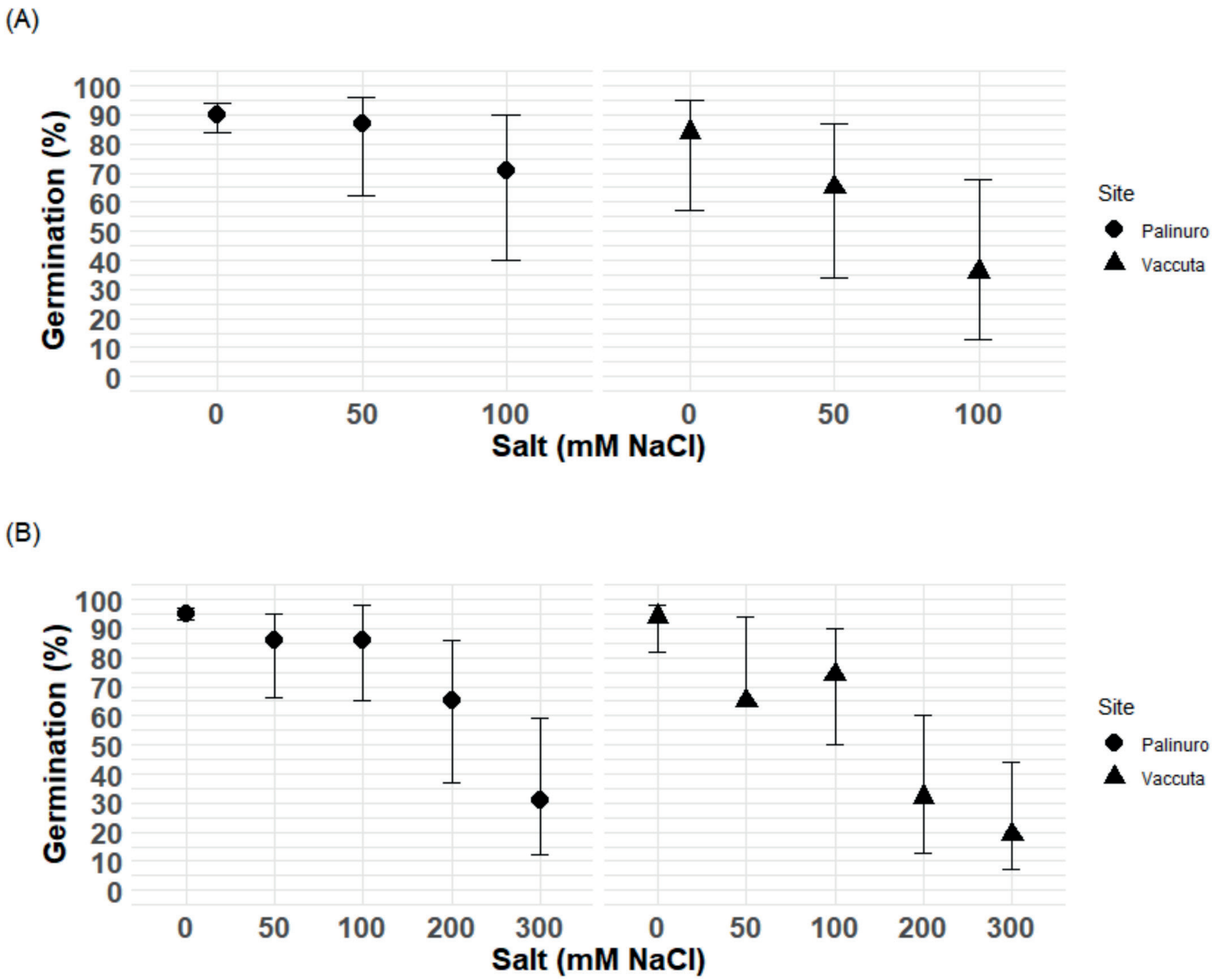

Figure 5. Estimates of maximum germination in percentage of Beta regression model for salt concentration for each sampling site and confidence intervals. the centre represents the model estimates, lines represent the 2.5% and 97.5% confidence intervals. The circle represents the Palinuro sampling site, while the triangle represents the Vaccuta sampling site. a) Experiment conducted at 20-10°C; b) Experiment conducted at 6-15°C.

the species. Temperature and salt were found to significantly impact the germination process and success, determining the capacity of the species to colonise and thrive only in certain sites. Although the environmental conditions in sea-exposed cliffs are warmer and saltier than in more internal areas. In sea proximity, excessive salinity could favour individuals which are more able to germinate under such a limited envelope, especially if combined with high temperatures. Seed size and quantity were also assessed as they are widely recognised as good indicators of seed longevity and environmental influence (Leishman et al., 2000). They revealed a slight advantage of sea-exposed populations. However, analyses did not provide conclusive explanations, requiring follow-up investigations.

Such studies can help elucidate whether the differences in germination responses result from genetic adaptation to local environments or other ecological niche processes. They can concretely contribute to identifying and anticipating the impacts of direct and indirect anthropogenic pressures (e.g. habitat destruction, climate change) against the species and support the definition of conservation and management strategies oriented to specific populations.

Acknowledgments

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We would also like to thank project LIFE SEEDFORCE (LIFE20/NAT/IT/001468) for covering the field sampling costs and project beneficiaries for providing important information during the workshops.

Table 3. Estimates of parameters 'b' and 'e' (fifth column) of time-to-event model for Salt concentration (second column) for each sampling site (third column) and confidence intervals (2.5 fourth column, 97.5 sixth column). The temperature was set a 10-20°C.

Parameter	Salt	Site	CI (2,5%)	Estimates	CI (97.5%)
B	0	Palinuro	-7.39	-6.28	-5.16
B	50	Palinuro	-7.88	-6.66	-5.42
B	100	Palinuro	-7.32	-5.67	-4.03
B	0	Vaccuta	-4.70	-3.95	-3.21
B	50	Vaccuta	-5.02	-3.83	-2.64
B	100	Vaccuta	-21.03	-13.73	-6.42
E	0	Palinuro	12.51	13.24	13.96
E	50	Palinuro	14.36	15.22	16.08
e	100	Palinuro	20.16	21.93	23.70
E	0	Vaccuta	13.37	14.67	15.98
E	50	Vaccuta	22.44	26.12	29.80
E	100	Vaccuta	14.45	18.95	20.46

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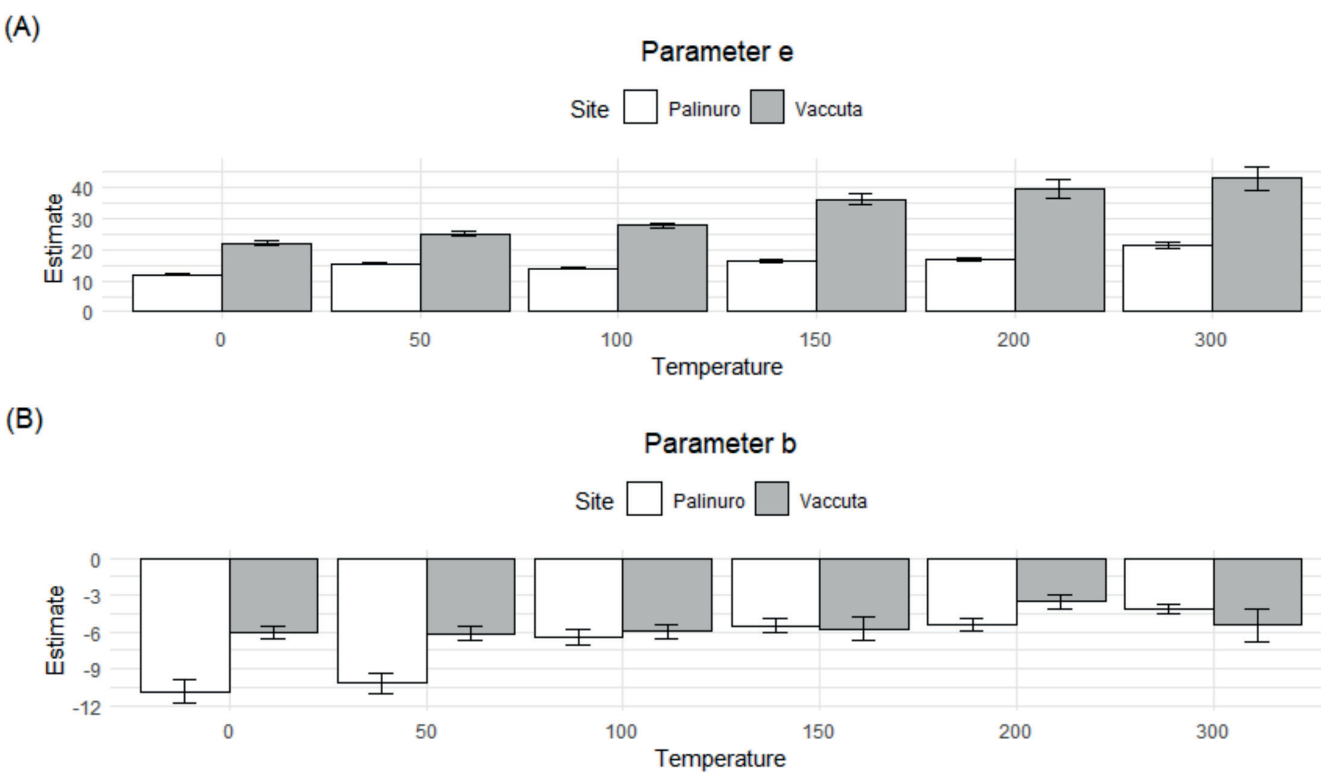

Figure 6. Estimates of parameters 'b' and 'e' of time-to-event model for Salt concentration, at 6-15°C, for each sampling and standard errors.

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EFFECTS OF ECOLOGICAL FACTORS AND REPRODUCTIVE STRATEGIES ON GERMINATION CAPABILITY OF *AILANTHUS ALTISSIMA* (MILL.) SWINGLE

Ecological factors and reproductive strategies in *Ailanthus altissima* germination

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Abstract – The tree of heaven *Ailanthus altissima* is one of the most invasive plant species in Europe and Italy and poses a threat especially in urban environments. A more profound comprehension of how germination traits respond to environmental factors could help elucidate the invasion process. Germination tests on *A. altissima* seeds collected at different altitudes were carried out at different light conditions (12-hr light/12-hr darkness and continuous darkness) and temperature regimes (15-6 °C, 20-10 °C and 25-15 °C). A further test was also performed to assess the germinative response of seeds retained on the plant during winter. Seeds haven't shown clear preferences for temperature and light, nor an effect of the altitude at which they develop on their germination capacity. However, a coast-inland gradient emerged in relation to seeds' sensitivity to cold stratification. Moreover, the continuous release of seeds throughout the year may maximize the chances of a successful germination, offsetting low germinability against a significant number of offsprings. These characteristics endow *A. altissima* with substantial adaptability and highlight the pioneer nature of the species during its reproduction and germination, both in its tolerance to different environmental conditions and in adopting a reproduction strategy oriented towards the extreme of r-strategist species, contributing to the species' invasiveness.

Keywords: altitudinal differences, ecological adaptation, germination, invasive alien species, reproductive strategies.

Introduction

Biological invasions are considered one of the primary drivers of global change (Crutzen, 2002), posing significant threats to the environment, economy and human health (IUCN, 2000; Rai & Singh, 2020). Human activities play a crucial role in introducing numerous species beyond their native ranges, enabling them to overcome biogeographical barriers (Richardson et al., 2000; Nentwig et al., 2018). When a species establishes a self-sustaining population that rapidly spreads from the introduction site, it is classified as an invasive alien species (IAS) (Pyšek et al., 2020). Alien species are the second global cause of extinctions (Bellard et al., 2016), leading to a reduction in biodiversity and to biotic homogenization (Sax & Gaines, 2003; Olden et al., 2016). Often, plant species are introduced for ornamental and horticultural purposes (Heywood, 1989; Pergl et al., 2016), and many of them have become permanent additions to local flora (Seebens et al., 2018). Globalization and intensified trades have contributed to a significant increase in the introduction of exotic species over the last century (Meyerson & Mooney, 2007). In Europe, the number of plant IAS has increased 4-fold (Hulme, 2009), and invasions are particularly prominent in urban areas, where

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28% of the local flora comprises alien species (Aronson et al., 2014; Godefroid & Ricotta, 2018). According to the checklist of the vascular flora alien to Italy by Galasso et al. (2024), 1782 exotic species are present on the Italian territory, and 250 of them are invasive.

A common question in the study of alien species is which attributes contribute to a species' invasiveness (Rejmanek & Richardson, 1996). Factors that are considered crucial for the success of an IAS include dispersal ability, tolerance thresholds, adaptability to different ecological factors and phenotypic plasticity (Cain et al., 2000; Funk, 2008; van Kleunen et al., 2016). The ability to adapt to various environments allows species to display adaptive characteristics, which can influence their ecological distribution and, as a result, their likelihood of invading new territories (Hulme, 2008; Molina-Montenegro et al., 2018). Traits associated with reproduction and growth are also commonly linked to invasion success. Many invasive plant species exhibit high fecundity or efficient mechanisms of vegetative propagation (Pyšek & Richardson, 2008). Seed production, early and rapid germination, and the capacity to germinate under a broad range of environmental conditions are crucial processes that determine the outcome of invasion in new regions (Moravcová et al., 2006; Pyšek & Richardson, 2008). Furthermore, the prolonged viability of seeds and the ability to form persistent seed banks can significantly contribute to determining the invasion potential of alien plants in their new distribution ranges (Gioria et al., 2019).

Many lists of the most harmful alien species have been compiled to raise awareness amongst the general public, politicians and stakeholders (Nentwig et al., 2018). European Parliament has adopted the Regulation No 1143/2014 to prevent, minimize, and mitigate the adverse impact on biodiversity of the introduction and spread of invasive alien species within the Union (EU 2014). In 2019 the plant species *Ailanthus altissima* was included within the species of Union concern. *Ailanthus altissima* (Mill.) Swingle 1916 (Simaroubaceae), also called the "tree of heaven", is a deciduous medium-sized tree native to China and northern Vietnam. Introduced in Europe in 1740 by the French missionary Pierre d'Incarville, it is currently invasive on all continents except Antarctica (Kowarik & Säumel, 2007). Due to its rapid growth and efficient photosynthesis in open, bright areas, it is considered a pioneer ruderal species occupying highly disturbed sites (Knapp & Canham, 2000; Call & Nilsen, 2003; Kowarik & Säumel, 2007). Disturbances such as frost, fires and cutting induce the production of shoots from the roots and the stem (Bory et al., 1991), forming dense monoclonal stands and thus allowing the rapid colonization of the invaded sites. *A. altissima* is tolerant to many ecological factors, such as drought, high temperatures, different soil compositions, salinity and pollution (Kovacs et al., 1982; Kowarik & Säumel, 2007; Sladonja et al., 2015). Nevertheless, it is a shade-intolerant species and it is sensible to severe frost (Knapp & Canham, 2000; Kowarik & Säumel, 2007; Knüsel et al., 2019). *A. altissima* can be found at <1000 m in temperate Europe, but in the Mediterranean climate most of *A. altissima* observations occur at <300 m of altitude (Fotiadis et al., 2011). *A. altissima* is one of the most frequent non-native species in Italy, particularly in urban areas where it thrives on walls, sidewalks, embankments of roads and railways and abandoned lots (Kowarik & Säumel, 2007). Its growth in cities poses a threat as its roots can cause significant damage, particularly to archaeological remains in historically rich Mediterranean cities like Rome (Celesti-Grapow & Blasi, 2004; Casella & Vurro, 2013; Trotta et al., 2020). In the rare cases that the species occupies natural areas, competition with native species and the production of allelopathic substances causes a reduction of local biodiversity (Constán-Nava et al., 2015). It is a dioecious species: female individuals produce a conspicuous number of winged fruits (samaras). A single tree can produce up to 325.000 seeds (Clair-Maczulajtys, 1984), but according to Martin & Canham (2010) a mature individual can produce more than a million seeds per year. Seeds can remain on the tree for an extended period, be dispersed after the subsequent winter or retained until the following autumn.

Due to the high ability of IAS to adapt to different environmental conditions, it is crucial to acquire as much information about their germination strategies as possible. A deeper insight into how germination traits react to environmental conditions can help clarify the mechanisms behind the invasion process. This, in turn, may enhance our ability to predict and anticipate future invasions. As for all plants, seed germination of invasive species is a crucial process in their life cycle and is highly vulnerable to environmental change (Wu et al., 2019). Since biological invasions act in synergy with other global change drivers, climate change can impact each stage of exotic species' invasion pathways depending on the species' ecology (Hellmann et al., 2008; Sage, 2020). Changes in temperature and precipitation regime may indeed strongly impact vulnerable life-history stages such as germination (Vesela et al., 2020). Furthermore, climate change may enable lower altitude species to expand and adapt to higher altitudes (Walther, 2007). Differences in the germination pattern among populations of the same species could provide information about its adaptability to different environments and help predict how the species would behave under the ongoing climate changes. In this study, germination tests were conducted on seeds collected from various environments at different altitudes. The aim was to gain new insights into the adaptability of the germination process of *A. altissima*. Additionally, tests were performed to assess the invasive tree's ability to employ reproductive strategies associated with seed dispersal and seed retention on the parent plant.

Materials and methods

Sampling

Seeds of *A. altissima* were collected from three sites in Rome Province: Castel Fusano (FUS) (41° 43' 12.6"N, 12° 21' 32.8"E; 5 m s.l.m.), Appia Antica Regional Park (Caffarella Valley, CAF) (41° 51' 58.4"N, 12° 31' 29.5"E; 40 m s.l.m.) and Grottaferrata (GRO) (41° 46' 56.7"N, 12° 40' 46.5"E; 365 m s.l.m.) (Fig. 1).

The three sites have a Mediterranean climate. The average total yearly rainfall, mostly distributed in autumn and winter, is 1079,0 mm in Castel Fusano, 700,5 mm in Rome and 723,3 mm in Grottaferrata. The average mean air temperature of the hottest month (August) is 23,13 ± 0,38 °C in Castel Fusano, 26,68 ± 0,35 °C in the city of Rome and 24,90 ± 0,38 °C in Grottaferrata. The average mean air temperature of the coldest month (January) is 6,02 ± 0,49 °C in Castel Fusano, 8,34 ± 0,35 °C in the city of Rome and 7,87 ± 0,40 °C in Grottaferrata (data provided by SIARL, Integrated Agrometeorological Service of Lazio Region; Meteorological Stations of Fiumicino-Maccarese, Roma-Via Lanciani and Grottaferrata-Valle Marciana, for the period 2010 to 2020). Seeds were collected in December 2020. For the site of Caffarella, a second harvest was performed in March 2021 to evaluate the germinative response of seeds retained on the plant during winter. Samaras were sampled from individuals placed at a distance of more than 10 m from each other to prevent the collection of seeds from closely related individuals and thus to ensure the genetic variability of the sample. To avoid the prevalence of certain genotypes, an equal number of seeds was collected for each individual (Bacchetta et al., 2006). Samaras were collected from different points of the tree canopy to avoid biases

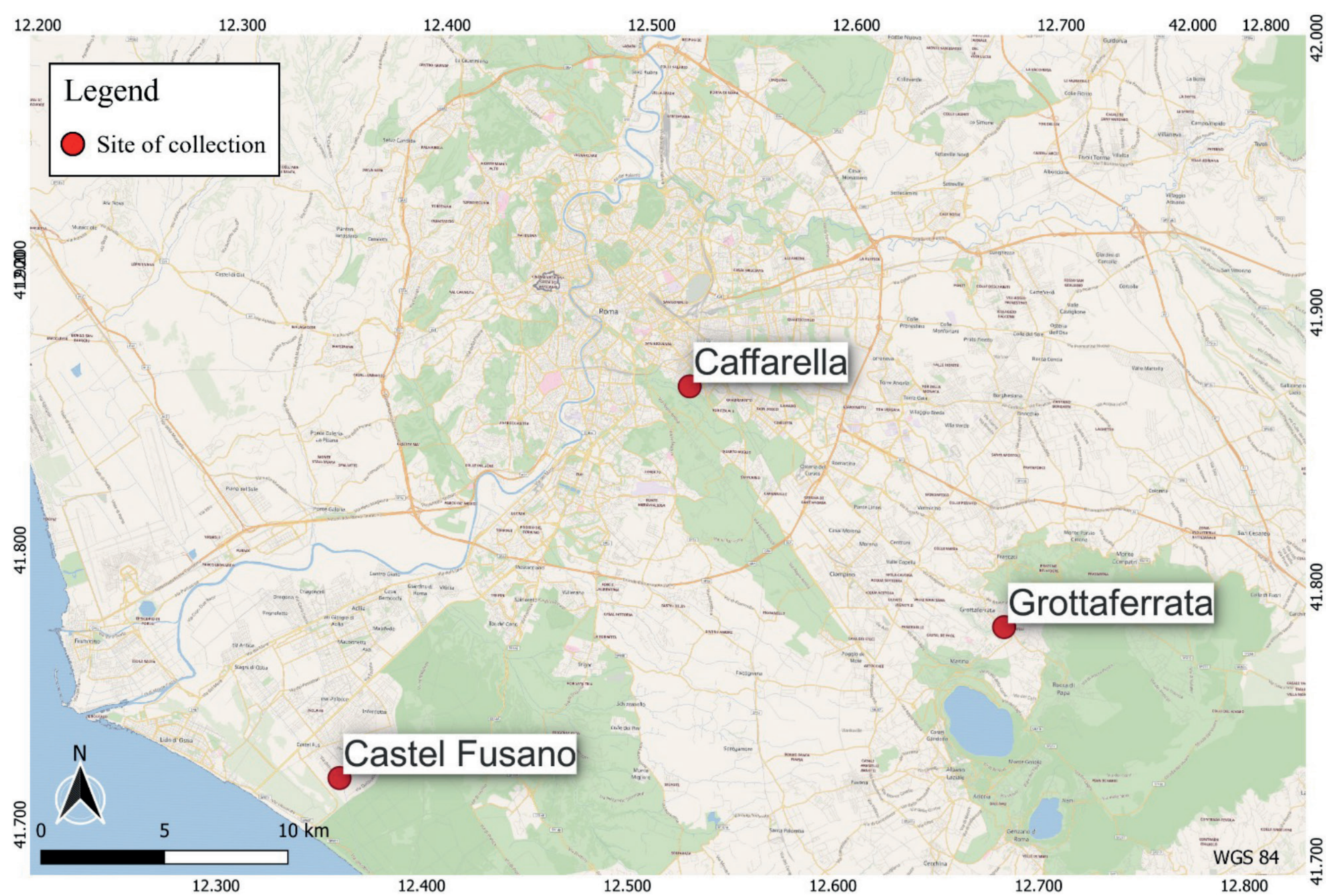

Figure 1. Sites of collection of *A. altissima* seeds. Castel Fusano (FUS) (41° 43' 12.6"N, 12° 21' 32.8"E; 5 m s.l.m.), Appia Antica Regional Park (Caffarella Valley, CAF) (41° 51' 58.4"N, 12° 31' 29.5"E; 40 m s.l.m.) and Grottaferrata (GRO) (41° 46' 56.7"N, 12° 40' 46.5"E; 365 m s.l.

resulting from the operator's ease of collection. The fruits were placed in paper bags during harvesting and immediately transported to the Germplasm Bank of the Botanical Garden, where seeds were manually separated from the fruits and post-ripened in open containers at room temperature of 20 °C and with a relative humidity of 40-60% for 15 days.

Morphometric traits

The following morphometric traits were measured from 100 seeds for each collection site: seed fresh mass (SFM, g), seed dry mass (SDM, g), moisture content (mc, %), major axis (A, mm) and minor axis (a, mm).
SFM and SDM were obtained by weighing 5 samples, each containing 20 seeds, with a Gilbertini precision balance (precision of 0.1 mg). SFM was measured immediately after seed collection, while SDM was determined after drying at 103 °C for 17h the same seeds used to measure SFM. Mc was calculated as a weight loss according to ISTA (2014) ($mc_{\%}$ = 100 x [(SFM-SDM)/SFM]) and expressed as a percentage. A and a were measured individually for 100 seeds per collection site using NIS-Element Br. 2.10 with images from a digital camera Nikon Digital Sight DS-120 U1, mounted on a stereo microscope Carl Zeiss.

Effect of ecological factors on seed germination

For each site of collection, seed germination was tested at three alternating temperature regimes (15-6°C, 20-10°C and 25-15°C), according to Bacchetta et al. (2006). The chosen temperatures simulate the thermal conditions of the different seasons in the region of interest, and they are recommended in literature (Martínez-Díaz et al., 2018; Wu et al., 2019). For each temperature, seeds were exposed to an alternating light/darkness regime (L/D: 12h light – 12h dark) and continuous darkness (D: 24h dark). Since dormancy in *A. altissima* seeds is reported in the literature (Redwood et al., 2019), three trials were carried out to evaluate seed germination. In trial 1, seeds were germinated without undergoing any pre-treatment to overcome their dormant state. Trial 2 was carried out after cold stratifying seeds for three months at 4°C in river sand, and trial 3 after six months of cold stratification under the same conditions. For each trial and at each temperature regime, germination was analyzed for eight experimental units (four in the light and four in the darkness) of 25 seeds. Seeds were placed in Petri dishes on a layer of sterile filter paper. Sterile distilled water was periodically added to each Petri dish to keep the substrate moistened. The Petri dishes destined for light treatments were incubated for 12h light and 12h darkness (L/D), with coolwhite fluorescent tubes providing a photon flux density of 80 µmol (photon) m^{-2} s^{-1}. The 12h light period corresponded to the daily high temperature, thus simulating natural conditions. Petri dishes destined to complete darkness treatments (D) were covered with a double aluminum foil wrapping to shield seeds from light.
For seeds collected in March 2021 (after winter) in Caffarella site (CAFw), a further test (trial 4) was set up in which seeds were subjected to the same combinations of temperature (15-6°C, 20-10°C and 25-15°C) and light (L/D and D) of the previously described germination tests.
All seeds were incubated for 60 days, and germinated seeds were counted every day. A seed was considered germinated when the length of the emerging radicle was >1 mm (ISTA, 1985). Seeds incubated in darkness were examined daily in a darkroom with a green safety light (Joly & Felippe, 1979).

Germination traits

For each treatment, two germination traits were calculated:
Germination percentage (grp, %): the percentage of seeds germinated at the end of germination period. Corresponds to the ratio between the number of germinated seeds and the total number of seeds, multiplied by one hundred. It is calculated as:

$$grp = \left(\frac{\sum_{i=1}^{k} n_i}{N}\right) \times 100 \qquad (1.1)$$

where n_i is the number of seeds germinated on day i, k is the last day of the germination test and N is the total number of seeds present in an experimental unit (Lozano-Isla et al., 2019) ($0 \leq grp \leq 100$).
Mean germination time (mgt, days): the average time a seed takes to germinate. It corresponds to a weighted average of the germination time, where the weight is represented by the number of seeds germinated in the time interval established for the count (in this case one day) (Czabator, 1962). It is calculated as:

$$mgt = \left(\frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}\right) \qquad (1.2)$$

where n_i is the number of seeds germinated on day i, t_i is the number of days passed since the beginning of the experiment and k is the last day of the germination test (Lozano-Isla et al., 2019) ($0 \leq mgt \leq k$).
Germination traits were calculated using R package version 2.1.3 GerminaR: indices and graphs to evaluate the seed germination process (Lozano-Isla et al., 2019).

Data analysis

To compare values of SFM and SDM of the seeds collected in the three sites (CAF, FUS and GRO), a one-way ANOVA was carried out, followed by a post-hoc Tukey test for pairwise multiple comparisons between sites. A Kruskal-Wallis test was used to compare A, a, and mc, followed by a post-hoc Dunn test for multiple pairwise comparisons.

To evaluate the effect of site of collection (three levels: CAF, FUS and GRO), temperature (three levels: 15-6°C, 20-10°C and 25-15°C) and light (two levels: L/D and D) a three-way ANOVA was carried out separately for trials 1, 2 and 3.

A four-way ANOVA was performed to evaluate the effect of cold stratification on grp and mgt. Factors were pre-treatment (three levels: no pre-treatment, three months of cold stratification and six months of cold stratification), site of collection (three levels: CAF, FUS and GRO), temperature (three levels: 15-6°C, 20-10°C and 25-15°C) and light (two levels: L/D and D).

A three-way ANOVA was carried out to compare, for CAF alone, grp and mgt of seeds harvested in December and not pre-treated (CAF0) with those of seeds harvested in March after the winter season (CAFw). The effects of the period of collection (two levels: CAF0 and CAFw), temperature (three levels: 15-6°C, 20-10°C and 25-15°C) and light (two levels: L/D and D) were evaluated.

Data were log-transformed if tests for the ANOVA assumptions of normality and equal variance failed. Following ANOVA, a post-hoc Tukey test was run for pairwise multiple comparisons.

For all the tests the level of significance chosen was $\alpha \leq 0.05$. Data are shown as mean value ± SE.

Results

Morphometric traits

Analyses highlighted differences between seeds collected in the three sites (Tab. 1). GRO showed the highest values of both SFM and SDM, while those traits didn't differ significantly for CAF and FUS. Mc significantly differed between CAF (6,56 ± 0,09 %) and FUS (7,21 ± 0,07 %). CAF seeds were also characterized by the lowest size, since they showed a significantly inferior value for both A and a.

Trial 1: germinability with no cold stratification

Unstratified seeds showed very low germination rates, in all cases less than or equal to 5%, confirming the presence of dormancy in *A. altissima* seeds. The results of the three-way ANOVA (Tab. 2) showed that neither temperature nor site of collection nor light influenced germination percentage of non-stratified seeds.

Table 1. Morphometric traits of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO). Seed fresh mass (SFM), seed dry mass (SDM), moisture content (mc), major axis (A) and minor axis (a) are shown. Different letters indicate significant differences ($p < 0.05$; for SFM and SDM: one-way ANOVA followed by post-hoc Tukey tests; for mc, A and a: Kruskal-Wallis test followed by post-hoc Dunn test).

	SFM (g)	SDM (g)	mc (%)	A (mm)	a (mm)
CAF	0.19 ± 0.00 a	0.18 ± 0.00 a	6.56 ± 0.09 a	4.80 ± 0.05 a	3.78 ± 0.04 a
FUS	0.19 ± 0.00 a	0.18 ± 0.00 a	7.21 ± 0.07 b	5.15 ± 0.03 b	4.09 ± 0.04 b
GRO	0.23 ± 0.01 b	0.21 ± 0.01 b	6.91 ± 2.04 ab	5.05 ± 0.03 b	3.94 ± 0.02 b

Table 2. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO) from trial 1 (not stratified seeds). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). The absence of mgt values is caused by a corresponding grp = 0 %. In cases where "NA" (Not Available) standard error occurs, at least one seed has germinated in only one of the four replicates, so it was not possible to calculate the standard error. Different letters indicate significant differences ($p < 0.05$; three-way ANOVA followed by post-hoc Tukey tests).

Trial 1

	grp (%)		mgt (days)	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF	3 ± 1.00 a	0 a	42.33 ± 2.33 ab	-
FUS	4 ± 1.63 a	0 a	41.17 ± 2.92 ab	-
GRO	4 ± 0.00 a	2 ± 1.16 a	44.75 ± 3.97 ab	48.00 ± 12.00 b
20-10 °C				
CAF	3 ± 1.92 a	0 a	18.00 ± 3.00 ab	-
FUS	0 a	1 ± 1.00 a	-	14.00 ± NA a
GRO	4 ± 1.63 a	1 ± 1.00 a	21.00 ± 3.51 ab	18.00 ± NA ab
25-15 °C				
CAF	4 ± 2.31 a	2 ± 1.16 a	16.00 ± 9.50 a	28.00 ± 6.00 ab
FUS	5 ± 3.79 a	1 ± 1.00 a	15.25 ± 2.25 a	47.00 ± NA ab
GRO	0 a	0 a	-	-

Table 3. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO) from trial 2 (seeds cold stratified for three months). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). The absence of mgt values is caused by a corresponding grp = 0 %. Different letters indicate significant differences ($p < 0.05$; three-way ANOVA followed by post-hoc Tukey tests).

Trial 2

	grp (%)		mgt (days)	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF	0 a	0 a	-	-
FUS	0 a	0 a	-	-
GRO	0 a	0 a	-	-
20-10 °C				
CAF	3 ± 1.00 ab	18 ± 4.16 c	34.33 ± 2.67 a	37.93 ± 5.47 a
FUS	4 ± 1.63 ab	4 ± 1.63 ab	44.00 ± 8.02 a	27.33 ± 6.44 a
GRO	14 ± 3.83 bc	16 ± 3.27 c	42.60 ± 1.85 a	36.65 ± 2.69 a
25-15 °C				
CAF	10 ± 4.76 abc	18 ± 2.58 c	31.83 ± 7.37 a	30.83 ± 3.70 a
FUS	4 ± 2.83 ab	9 ± 2.52 abc	26.83 ± 3.83 a	27.00 ± 3.05 a
GRO	3 ± 1.00 ab	10 ± 1.16 abc	29.00 ± 1.73 a	28.38 ± 2.73 a

Table 4. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO) from trial 3 (seeds cold stratified for six months). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). Different letters indicate significant differences ($p < 0.05$; three-way ANOVA followed by post-hoc Tukey tests).

Trial 3

	grp (%)		mgt (days)	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF	57 ± 5.26 c	55 ± 3.00 c	38.75 ± 1.11 cdef	36.28 ± 0.80 cde
FUS	15 ± 3.42 ab	19 ± 5.26 ab	40.52 ± 1.81 def	35.89 ± 1.75 bcde
GRO	19 ± 4.44 ab	31 ± 5.75 b	44.58 ± 1.60 ef	43.57 ± 3.59 ef
20-10 °C				
CAF	6 ± 1.16 a	18 ± 2.58 ab	39.13 ± 3.62 cdef	31.48 ± 2.57 abcde
FUS	4 ± 2.82 a	8 ± 1.63 a	53.67 ± 0.33 f	43.88 ± 5.04 ef
GRO	9 ± 5.26 a	14 ± 3.46 ab	39.33 ± 4.17 cdef	33.25 ± 3.51 abcde
25-15 °C				
CAF	15 ± 4.12 ab	14 ± 3.83 ab	22.02 ± 2.23 ab	21.53 ± 1.82 a
FUS	4 ± 2.83 a	5 ± 3.79 a	25.50 ± 3.50 abcd	23.13 ± 1.13 abc
GRO	7 ± 4.73 a	15 ± 4.44 ab	29.05 ± 0.55 abcde	36.60 ± 2.14 cdef

Mean germination time values showed a greater variability. However, the analysis didn't recognize a strong significance in the differences between mgt values. The model highlighted the effect of temperature on the average germination time, suggesting the presence of longer germination times for the lowest temperature (15-6°C) and lower for the higher temperatures (20-10°C and 25-15°C): high temperatures, therefore, seem to accelerate the germination times of *A. altissima* seeds; however, the differences were not significant after pairwise comparisons (Tab. 2). At 15-6°C mgt was more than 40 days for all sites, while at 20-10°C and 25-15°C mgt almost never exceeded 30 days. Furthermore, even if not significant, a notable difference can be observed at 25-15°C between L/D and D for CAF (L/D: 16.00 ± 9.50 days; D: 28.00 ± 6.00 days) and FUS (L/D: 15.25 ± 2.25 days; D: 47.00 ± NA days).

Trial 2: germinability after three months of stratification

For grp, the three-way ANOVA highlighted a greater distinction between the temperature of 15-6°C and those of 20-10°C and 25-15°C: at the lowest temperature no germination occurred (Tab. 3). A significant difference in D occurred between the percentage observed for CAF at 15-6°C (0 ± 0.00 %) and those observed at 20-10°C (18 ± 4.16 %) and 25- 15°C (18 ± 2.58%). For GRO there was a significant difference both in L/D and D between seeds incubated at 15-6°C (L/D: 0 ± 0.00%; D: 0 ± 0.00 %) and those incubated at 20-10°C (L/D: 14 ± 3.83 %; D: 16 ± 3.27 %). FUS, on the other hand, didn't show significant variations in grp between different temperatures. The only significant difference between sites was observed at 20-10°C in D: GRO and CAF had a similar behavior (GRO:

16 ± 3.27%; CAF: 18 ± 4.16%), while FUS maintained a lower grp (4 ± 1.63%). The only statistically significant difference between L/D and D occurred for CAF at 20-10°C where in D (18 ± 4.16 %) seeds germinated 6 times more than in L/D (3 ± 1.00 %). Mgt values were all similar and not significantly different to each other: neither temperature nor site of collection nor light conditions affect the mean germination time (Tab. 3).

Trial 3: germinability after six months of stratification

Table 5. Germination percentage (grp, %) for every combination of temperature (15-6 °C, 20-10 °C, 25-15 °C), light (alternating light/dark: L/D, dark: D), site of collection (Caffarella: CAF, Castel Fusano: FUS, Grottaferrata: GRO) and pre-treatment (trial 1: not pre-treated seeds, trial 2: three months of cold stratification, trial 3: six months of cold stratification). Different letters indicate significant differences ($p < 0.05$; four-way ANOVA followed by post-hoc Tukey tests).

grp (%)

	15-6 °C		20-10 °C		25-15 °C	
	L/D	D	L/D	D	L/D	D
Trial 1						
CAF	3 ± 1.00 cde	0 e	3 ± 1.92 cde	0 e	4 ± 2.31 cde	2 ± 1.16 cde
FUS	4 ± 1.63 bcde	0 e	0 e	1 ± 1.00 de	5 ± 3.79 cde	1 ± 1.00 de
GRO	4 ± 0.00 bcde	2 ± 1.16 cde	4 ± 1.63 bcde	1 ± 1.00 de	0 e	0 e
Trial 2						
CAF	0 e	0 e	3 ± 1.00 cde	18 ± 4.16 abc	10± 4.76 abcd	18 ± 2.58 abc
FUS	0 e	0 e	4 ± 1.63 bcde	4 ± 1.63 bcde	4 ± 2.83 cde	9 ± 2.52 abcd
GRO	0 e	0 e	14 ± 3.83 abc	16 ± 3.27 abc	3 ± 1.00 cde	10± 1.16 abcd
Trial 3						
CAF	57 ± 5.26 a	55 ± 3.00 a	6 ± 1.16 bcde	18 ± 2.58 abc	15 ± 4.12 abc	14 ± 3.83 abc
FUS	15 ± 3.42 abc	19 ± 5.26 abc	4 ± 2.82 cde	8 ± 1.63 abcd	4 ± 2.83 cde	5 ± 3.79 cde
GRO	19 ± 4.44 abc	31 ± 5.75 ab	9 ± 5.26 bcde	14 ± 3.46 abc	7 ± 4.73 bcde	15 ± 4.44 abc

The main difference which clearly emerges compared to the previous trials concerns the temperature of 15-6°C, at which seeds achieved the highest germination rates among all the tests performed, for all sites of collection. However, only for CAF (L/D: 57 ± 5.26%; D: 55 ± 3.00%) grp values were significantly higher than those measured at higher temperatures, in both light conditions (Tab. 4). CAF grp values were also significantly greater than GRO and FUS ones. For temperatures of 20-10°C and 25-15°C the three-way ANOVA didn't recognize differences between stations, temperatures, and light conditions. Mgt was significantly lower for seeds incubated at higher temperatures (Tab. 4): in L/D, CAF had a smaller mgt at 25- 15°C (22.02 ± 2.23 days) compared to the other temperature ranges (20-10°C: 39.13 ± 3.62 days; 15-6°C: 38.75 ± 1.11 days). Instead, in D at 25-15°C (21.53 ± 1.82 days) mgt was significantly different only with respect to 15-6°C (36.28 ± 0.80 days). For FUS, at 25-15°C (L/D: 25.50 ± 3.50 days; D: 23.13 ± 1.13 days) mgt values were only significantly lower than those observed at 20-10°C (L/D: 53.67 ± 0.33 days; D: 43.88 ± 5.04 days), for both light conditions. No significant difference between temperatures was observed for GRO. In addition, at 25-15°C and in D, CAF had a lower mgt than GRO (36.60 ± 2.14 days).

Effect of cold stratification on germinability

The four-way ANOVA on all germination tests highlighted a mild effect of stratification on the interruption of seed dormancy. Not stratified seeds showed the lowest grp, while seeds stratified for six months showed the highest grp (Tab. 5). Only in D, three months of stratification caused a significant increase in grp, compared to non-stratified seeds, at incubation temperatures of 20-10 °C and 25-15 °C (Tab. 5). The same stratification did not cause an increase in grp at low temperatures (15-6 °C), making a longer pre-treatment duration necessary. At the lowest temperature, six months of stratification resulted in a significant and particularly large increase of grp, especially in D, while the increase of grp wasn't significant at 20-10 °C and 25-15 °C compared to three months of stratification (Tab. 5).
Thus, the effect of cold stratification on grp was more evident when seeds were incubated at the lowest temperature range, and in complete darkness. Cold stratification didn't influence the timing of germination: mgt values were nearly always not significantly different (Tab. 6).

Trial 4: overwintering germinability of seeds retained on the plant

Statistical analyses highlighted the non-significance of the period of seed collection (Tab. 7); therefore,

Table 6. Mean germination time (mgt, days) for every combination of temperature (15-6 °C, 20-10 °C, 25-15 °C), light (alternating light/dark: L/D, dark: D), site of collection (Caffarella: CAF, Castel Fusano: FUS, Grottaferrata: GRO) and pre-treatment (trial 1: not pre-treated seeds, trial 2: three months of cold stratification, trial 3: six months of cold stratification). The absence of values is caused by a corresponding grp = 0 %. In case "NA" (Not Available) standard error occurs, seeds germinated in only one of the four replicates, so it was not possible to calculate the standard error. Different letters indicate significant differences ($p < 0.05$; four-way ANOVA followed by post-hoc Tukey tests).

mgt (days)

	15-6°C		20-10°C		25-15°C	
	L/D	**D**	**L/D**	**D**	**L/D**	**D**
Trial 1						
CAF	42.33 ± 2.33 abc	-	18.00 ± 3.00 a	-	16.00 ± 9.50 a	28.00 ± 6.00 ab
FUS	41.17 ± 2.92 abc	-	-	14.00 ± NA a	15.25 ± 2.25 a	47.00 ± NA abc
GRO	44.75 ± 3.97 abc	48.00 ± 12.00 bc	21.00 ± 3.51 a	18.00 ± NA a	-	-
Trial 2						
CAF	-	-	34.33 ± 2.67 abc	37.93 ± 5.47 abc	31.83 ± 7.37 abc	30.83 ± 3.70 abc
FUS	-	-	44.00 ± 8.02 abc	27.33 ± 6.44 ab	26.83 ± 3.83 ab	27.00 ± 3.05 ab
GRO	-	-	42.60 ± 1.85 abc	36.65 ± 2.69 abc	29.00 ± 1.73 abc	28.38 ± 2.73 ab
Trial 3						
CAF	38.75 ± 1.11 abc	36.28 ± 0.80 abc	39.13 ± 3.62 abc	31.48 ± 2.57 abc	22.02 ± 2.23 a	21.53 ± 1.82 a
FUS	40.52 ± 1.81 abc	35.89 ± 1.75 abc	53.67 ± 0.33 c	43.88 ± 5.04 abc	25.50 ± 3.50 ab	23.13 ± 1.13 ab
GRO	44.58 ± 1.60 abc	43.57 ± 3.59 abc	39.33 ± 4.17 abc	33.25 ± 3.51 abc	29.05 ± 0.55 abc	36.60 ± 2.14 abc

Table 7. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella in December (CAF0) and March (CAFw) from trial 4 (seeds retained on the plant overwinter). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). The absence of mgt values is caused by a corresponding grp = 0 %. In cases where "NA" (Not Available) standard error occurs, at least one seed has germinated in only one of the four replicates, so it was not possible to calculate the standard error. Different letters indicate significant differences ($p < 0.05$; three-way ANOVA followed by post-hoc Tukey tests).

Trial 4

	grp (%)		mgt (days)	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF0	3 ± 1,00 ab	0 a	42,33 ± 2,33 bc	-
CAFw	1 ± 1,00 a	0 a	53,00 ± NA c	-
20-10 °C				
CAF0	3 ± 1,92 ab	0 a	18,00 ± 3,00 ab	-
CAFw	15 ± 4,73 b	2 ± 2,00 a	40,33 ± 3,89 abc	41,00 ± NA abc
25-15 °C				
CAF0	4 ± 2,31 ab	2 ± 1,16 ab	16,00 ± 9,50 a	28,00 ± 6,00 abc
CAFw	15 ± 4,12 b	13 ± 1,92 b	44,81 ± 4,90 c	29,52 ± 1,04 abc

overwinter permanence of seeds on the tree didn't affect germination rates. However, at higher temperatures an increase of grp was observed between CAF0 and CAFw seeds. The model highlighted the effect of temperature and light on germination rates. At 20-10°C, a significant difference of grp emerged for CAFw between D (2 ± 2.00 %) and L/D (15 ± 4.73 %). For CAFw, in L/D there were significantly higher percentages at 20-10 °C and 25-15 °C compared to those obtained at 15-6 °C. In D instead, 25-15 °C generated significantly higher percentages than those obtained both at 20-10 °C and 15-6 °C. The effect of the collection period on mgt could be observed at 25-15°C in L/D, conditions under which CAF0 seeds (16.00 ± 9.50 days) germinated more rapidly than CAFw ones (44.81 ± 4.90 days) (Tab. 7). In the other conditions no significant differences between CAF0 and CAFw were observed. For CAFw seeds no effect of temperature and light emerged on mgt values.

Discussion

Germination can be considered as part of the invasiveness mechanism (Richardson et al., 2000). Reproductive traits such as abundant seed production, rapid germination, and the ability to germinate under a wide range of environmental conditions characterize many invasive species (Pyšek & Richardson, 2008). Since germination is a critical phase in the life cycle of a plant and plays an important role in seedling establishment, seed characteristics and the adaptation of their germination to the environment are fundamental determinants for the invasion success of an alien plant (Wen, 2015). In order to evaluate the germinative response of many species, it is necessary to break the dormancy of their seeds (Finch-Savage & Leubner-Metzger, 2006). Nevertheless, cold stratification had only a mild effect on seed germinability. Soler & Izquierdo (2024) pointed out that several intrinsic factors could play a crucial role in *A. altissima* seed dormancy, since environmental requirements for its seeds to germinate have been shown to be greatly variable. In the present study the effect of stratification could be observed only under complete darkness. An interaction between stratification and light was also found in the study by Milberg & Andersson (1998) in which, following stratification of seeds of 33 annual species, the response to light changed for 21 of them. They also highlighted the possibility of an interaction between population and stratification for some species. The present study seems to reveal a population-stratification-temperature interaction, as the effect of stratification was different at different temperatures for each site of collection: for FUS stratification had a significant effect on increasing germination percentages only at 15-6 °C, for CAF the effect was significant at 15-6 °C and 20-10 °C, and for GRO it was significant at all temperatures. Seeds collected at higher altitude therefore appear to be more susceptible to the effect of cold stratification, while those collected at the lowest altitude respond to the same pre-treatment only at the lowest germination temperature. This pattern reveals the existence of a gradient from the coast to the inland, possibly indicating an ecotype differentiation. Although *A. altissima* tolerates a wide range of climatic conditions, it is in fact most common and abundant in temperate climatic zones (Knapp & Canham, 2000; Kowarik & Säumel 2007; Clark et al., 2014). In a more typical Mediterranean climate, as that of Italian Tyrrhenian coasts, it thrives less well, thus resulting in a lower germinative response to higher temperatures after cold stratification.

For nearly all conditions, instead, no significant differences were observed in the germination capability among seeds collected from different sites. The average germination time was also not found to be influenced by the site of collection. The germinative characteristics of *A. altissima* seeds seem to be independent of the altitude at which they are produced. In other words, the environmental characteristics in which the mother plant is found may not have an influence on the germinative characteristics of the seeds it produces. This demonstrates that the morphological variability of *A. altissima* seeds observed in the measurements of A, a and SFM may not have an adaptive value to the different environmental conditions in which the seeds are produced, but would rather be the result of inter- and intra-individual variability, as well as of several ecological and genetic factors (Vaughton and Ramsey, 1997; Alonso-Blanco et al. 1999; Halpern, 2005). The absence of an effect of the site of seed production on their germination dynamics may contribute to the high invasive potential of the tree of heaven, as it demonstrates how the species can easily adapt to a variety of environmental conditions without consequences on the early stages of its development. The lower abundance of invasive species at higher elevations (Medvecká et al., 2014; Beniak et al., 2015) may not necessarily reside in their lower adaptability to such environments: it could also be due to a time-lag effect, related to invasion dynamics or to less intense human activities present at higher elevations (McDougall et al., 2011). This could be true for *A. altissima*, which is a typically urban species (Miller, 2003). Urban environments are also characterized by the phenomenon of the heat island, which produces higher temperatures in cities than in surrounding areas (Santamouris, 2007; Godefroid & Ricotta, 2018). However, although *A. altissima* is considered a thermophilic species (Sudnik-Wójcikowska, 1998), no clear preference for higher temperatures has been found during the germination process in the present study.

In fact, the incubation of seeds at different temperatures has highlighted contrasting relationships between temperature and germination capacity, depending on the type of pre-treatment undergone by seeds (Soler & Izquierdo, 2024). In the absence of stratification, temperature had no effect on germination. Seeds stratified for three months showed, for GRO and CAF, an increase in germination percentages at a temperature of 20-10°C, indicating a partial contribution of intermediate temperatures in determining higher germination rates. CAFw seeds showed a significant increase in germination percentages at higher temperatures (at 20-10°C in L/D and at 25-15°C in both L/D and D): for these seeds, germination seems to be favored by higher temperatures (Little, 1974; Kheloufi et al., 2020). Since overwintering seeds are located above the tree canopy and thus unable to germinate during the cold season, displaying higher germination percentages at higher temperatures could be an adaptation to the period when they are dispersed. For seeds stratified for six months, however, lower temperatures generated the highest germination percentages, although this increase was significant only for CAF. This last observation agrees with that of Constán-Nava & Bonet (2012) who found, for seeds incubated in growth chambers, higher germination percentages at 15°C and lower at 30°C. Higher temperatures can in fact determine a greater demand for oxygen by the embryo, and a lack of oxygen could induce secondary or induced dormancy (Hilhorst, 2007). These results demonstrate that temperature is a secondary control factor of the germination of *A. altissima* seeds. The main limiting factor could indeed be water: in the experiments, seeds were not restricted in water availability. The field experiment by Constán-Nava & Bonet (2012) showed that seedling emergence is related to rainfall rather than temperature, while Sevik & Cetin (2015) demonstrated in a laboratory study that *A. altissima* seeds are particularly sensitive to water stress, which affects their germination rates. The absence of a clear trend in germinations in relation to temperature is an indication of the high invasiveness of this species, which is not limited in the initial stages of its development by the temperature factor and thus germinates independently of the environment in which it is found. Nevertheless, as stated above, pre-treatment could highlight a coast-inland gradient: after undergoing cold stratification, seeds from inlands (CAF and GRO) enhance their germination in a wider range of temperatures, while seeds from coastal areas (FUS) are less susceptible to this kind of pre-treatment, increasing their germination rates only at the lowest temperature and resulting in being less sensitive to cold stratification. Germination timing was almost never affected by temperature, in agreement with Kheloufi et al. (2020). A significant effect was observed when the higher temperature of 25-15 °C accelerated the germination of seeds from CAF and FUS stratified for six months, both in L/D and D. Following longer cold stratification, higher temperatures seem to favor faster mgt.

As for temperature, light didn't affect seeds germination, resulting in no differences between L/D and D conditions. Kota et al. (2007) obtained higher germination rates in laboratory conditions with lower light conditions, while in the field germination increased with increasing light. These contrasting trends indicate that the amount of light is not the main regulator of *A. altissima* germination. The heliophilous nature of this species would not depend on its germination, but on subsequent phases: *A. altissima* is in fact a ruderal pioneer species characterized by efficient photosynthesis in open sites (Kowarik and Säumel, 2007). Given that light does not appear to be a limiting factor for germination, the seed's depth in the soil would not influence the species' germination capacity.

Regardless of the different conditions seeds were undergone, the germination tests carried out in the present study produced low germination percentages, almost always less than 20%. The overall low germination rates obtained, in agreement with other studies (Kota et al., 2007; Constán-Nava & Bonet, 2012; Cabra-Rivas & Castro-Dìez, 2016), contrast with the high invasiveness of *A. altissima* (Kowarik & Säumel, 2007). Indeed, numerous studies emphasize the importance of vegetative propagation in the invasion mechanism of *A. altissima* (Bory et al., 1991; Vilà et al., 2006; Kowarik & Säumel, 2007). Seeds are the main mechanism by which this species reaches new habitats, but then the root system contributes to the rapid increase in the tree density and the subsequent expansion of the population nearby, forming populations of scattered clones (Soler & Izquierdo, 2024). However, this species has a high fecundity: a single individual can in fact produce up to a million samaras every year (Wickert et al., 2017). *A. altissima*, in addition to spreading vegetatively, would rely on the large quantity of seeds produced rather than their quality (viability and germinability) in colonizing new areas for its reproductive success. Thus, low germination rates would be offset by a high seed production. According to Rejmànek & Richardson (1996), invasive species are often characterized by a reproductive r-strategy. These organisms invest many resources into reproduction but provide a minimal amount of resources to each offspring, thereby obtaining the highest possible number of progenies (Pianka, 1970) and ensuring the reproductive success of the species. Among plant species, r-strategists are often pioneer or early successional species (Pianka, 1970; Rejmànek & Richardson, 1996), and since most invasions occur in disturbed habitats it is not surprising that invasive species are r-strategists (Whitmore, 1991; Rejmànek & Richardson, 1996). *A. altissima* would therefore adopt such strategy, relying on an extremely high number of seeds rather than their vitality for its reproductive success.

The efficiency of *A. altissima* reproduction through seeds is not only due to the large number of seeds produced, though. In fact, this species seems to implement an additional reproductive strategy aimed at maximizing the probability of success of young seedlings. *A. altissima* exhibits a large variability in samaras' dispersal period. Some trees completely lose their fruits by mid-winter, while others keep samaras on their branches until spring and in some cases even until the following autumn. Germination tests have shown that permanence on tree during winter doesn't determine an increase in seeds germination rates. However, grp obtained are still equal to or greater than those produced by non-stratified seeds, demonstrating that these seeds maintain their viability. The enormous amount of seeds produced allows this species to keep a portion of them on the tree: a "reservoir effect" that results in a delayed release of samaras over time. Seeds that remain on the plant constitute a genetic reservoir of potentially reproductive material, which is gradually released throughout the year. Worldwide, over 1200 species employ the strategy of storing seeds on their mother plants, forming what is known as an aerial seed bank (Silvestro et al., 2020). This phenomenon, referred to as serotiny, has usually been associated with extreme environments as a strategy to reduce the risk of offspring mortality and await better conditions for seedling establishment (Lamont & Enright, 2000; Bastida et al., 2010; Saracino et al., 2017; Silvestro et al., 2020). Furthermore, the prolonged emergence of seedlings resulting from gradual seed release can avoid intense competition among seedlings and act as a mechanism for plant population maintenance in the face of unpredictable environments (Günster, 1994; Gao et al., 2014). For *A. altissima*, the gradual and continuous dispersal of samaras may contribute to its success as an invasive species: the continuous seed release allows the generation of new individuals in different conditions of temperature and water availability, maximizing the chances of success for a greater number of seedlings throughout the year.

In addition to an aerial seed bank, *A. altissima* may also constitute a soil seed bank, a deposit of viable seeds present on or in the soil, which acts as a reserve of propagules and genetic diversity for many species (Gioria et al., 2019). For every site of collection, seeds' internal humidity was less than 15 %, allowing to classify *Ailanthus* seeds as orthodox and possibly favoring their ability to constitute a soil seed bank. The formation of soil seed banks is a common strategy in plants that grow in environments subject to disturbance, such as invasive ones (Miller, 2000; Bacchetta et al., 2006). Since different studies report the ability of *A. altissima* seeds to germinate even in the years following their production (Kota et al., 2007; Cabra-Rivas & Castro-Dìez, 2016; Wickert et al., 2017; Rebbeck & Jolliff, 2018), the formation of a soil seed bank may thus contribute to the invasive potential of this species.

Conclusions and future perspectives

This study provides evidence that seeds of the invasive species *A. altissima* are capable of germinating under a wide range of conditions, showing no preferences for temperature, light or altitude at which they develop. These findings align with previous studies by Kota et al. (2007) and Moore & Lacey (2009), who found that the germination rate of *A. altissima* is not influenced by the habitat type in which its seeds germinate. This characteristic makes *A. altissima* highly adaptable to different environments without being constrained by specific conditions. It suggests that the anticipated temperature increase associated with climate change may not affect the germination capability of this species. The presence of a coast-inland gradient in response to cold stratification may indicate, however, that seeds produced in coastal areas wouldn't perform as effectively at high temperatures as those produced in more temperate-like areas, leading to a possible ecotype differentiation. Adaptability is typical of many invasive species, which are often capable of germinating in a wide range of environmental conditions (Pyšek & Richardson, 2008). It is therefore possible that the nature of pioneer species of *A. altissima* emerges already during its reproduction and germination, a crucial phase in a plant's life cycle, both in its tolerance to different environmental conditions and in the adoption of a reproduction strategy oriented towards the extreme of r-strategist species. The continuous release of numerous seeds throughout the year may maximize the chances of a successful germination. Unlike many pioneer species, however, germination of *A. altissima* is not limited by light, contributing even more significantly to its adaptability and therefore to its invasiveness. Nevertheless, the potential impact of climate change on water availability should be more deeply investigated, as it appears to be a limiting factor for the germination process of this species. Understanding the germination capabilities and environmental requirements of invasive species like *A. altissima* is crucial for developing effective and targeted management strategies, in order to mitigate their spread and impacts.

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PHYTOCHEMICAL ANALYSIS ON THE LEAVES OF *TEUCRIUM CAPITATUM* L. SUBSP. *CAPITATUM* COLLECTED IN THE BOTANICAL GARDEN OF ROME

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Abstract – In this paper, the phytochemical study on the leaves of *Teucrium capitatum* L. subsp. *capitatum* collected in the Botanical Garden of Rome was presented for the first time. Nine secondary metabolites were evidenced *i.e.*, pheophytin *a* (**1**), verbascoside (**2**), alyssonoside (**3**), apigenin (**4**), cirsimaritin (**5**), cirsiliol (**6**), 8-*O*-acetyl-harpagide (**7**), teucardoside (**8**) and quinic acid (**9**). Their presence is perfectly in accordance with previously published general results on the species, thus confirming the phytochemical knowledge on it.

Keywords: *Teucrium Capitatum* L. Subsp. *Capitatum*, Botanical Garden Of Rome, Phytochemical Analysis

Introduction

Teucrium capitatum L. subsp. *capitatum* (Fig. 1) is a suffruticose perennial plant belonging to the Lamiaceae family. It is morphologically characterized by an erected, slightly hairy stem which is woody at the base. The leaves are opposite, entire, and sessile. The inflorescence is branched, gathered in a cob with a white tomentose corolla formed by five small petals. The fruit is a schizocarp constituted by four light brown colored ovoidal mericarps (Pignatti, 1982).

After the recent modifications on its growth area, the species is now distributed from the Mediterranean Basin to Afghanistan (www.powo.science.kew.org). In Italy, it is present everywhere except in Lombardy, Trentino-Alto Adige and Valle d'Aosta, growing wild in garigues, dunes, arid and stony areas till the altitude of 1800 m a.s.l. (Conti et al., 2005).

Different phytochemical studies have been conducted on *T. capitatum* subsp. *capitatum* evidencing the presence of several classes of natural compounds like sesquiterpenoids, triterpenoids, diterpenoids, phenyl-ethanoid glycosides, flavonoids and iridoids (Sadeghi et al., 2022) but none has previously focused on an exemplar collected in the Botanical Garden of Rome. This represents the main reason why this work was begun because performing more phytochemical analyses on different populations of a same species is always necessary since it can provide more precise and complete information on its real phytochemical composition given that this is deeply affected by intrinsic and extrinsic factors (Liebelt et al., 2019). In this paper, a general phytochemical comparison with the previously published results on other populations of the species was also presented.

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Figure 1. Image of *Teucrium capitatum* L. subsp. *capitatum*.

Materials and methods

Plant material

The leaves of *T. capitatum* subsp. *capitatum* (4.5 g) were collected in the Botanical Garden of Rome located in Largo Cristina di Svezia, 23 A - 24, 00165 Roma RM (geographical coordinates: 41°53'32'' N, 12°27'57'' E) on July 13 in 2023. The botanical identification was performed by the botanist of the park and one of the authors (Dr Andrea Bonito) by comparing the morphological features with those available in the literature (Pignatti 1982). A sample of this collection is stored in our laboratory for further reference under the voucher code TCSC13072023.

Solvents and reagents

The following materials and solvents were used during this study: ethanol 96% for the extraction procedure; *n*-butanol, distilled water, methanol, *n*-hexane and ethyl acetate as pure solvents or in mixture among them as eluting systems for the column chromatography separation on silica gel (40–63 µm) used as stationary phase; 2N sulfuric acid for the developments of the TLCs; deuterated solvents ($CDCl_3$, CD_3OD and D_2O) for the identification of the secondary metabolites by means of NMR spectroscopy; HPLC-grade methanol for the identification of the secondary metabolites by means of mass spectrometry. All the solvents having RPE purity grade, if not differently specified, together with the deuterated solvents, the TLCs and HPLC-grade methanol were purchased from Merck (St. Louis, Missouri, USA) whereas silica gel was purchased from Fluka Analytical (Bergamo, Italy).

Instrumentation

NMR spectra were recorded at 298 K on a Jeol JNM-ECZ 600R spectrometer with a magnet operating at 14.09 T corresponding to a proton resonance frequency of 600.19 MHz and equipped with a Jeol multinuclear *z*-gradient inverse probehead. ^1H NMR spectra were acquired with 32 transients, a spectral width of 9013.7 Hz (corresponding to 15 ppm) and 64K data points for an acquisition time of 7.3 s. The recycle delay was set to 7.7 s to achieve complete resonance relaxation between successive scans. The chemical shifts were referenced to TMS (s, 0 ppm) for spectra in $CDCl_3$, the internal solvent signal of CD_2HOD (m5, 3.31 ppm) was the reference for spectra in CD_3OD while the HDO signal (s, 4.79 ppm) was set as reference for spectra in D_2O. MS spectra were acquired with a triple quadrupole mass spectrometer PE-Sciex API-3000® (Perkin Elmer Sciex, Toronto, ON, Canada), equipped with an ESI source operating in the negative and/or positive ion mode. The capillary ion voltage was set at 5000 V for the positive ionization and -4500 V for the negative one. High-purity nitrogen was used as a curtain gas (5 L/min) while air was employed as the nebulizer (2 L/min) and drying gas (30 psi). The temperature to heat the drying gas was set at 100 °C. The flow rate of sample infusion was 20 µL/min. MS spectra were acquired with 20 acquisitions per sample. The full width at half maximum (FWHM) was set at *m/z* 0.7 ± 0.1 in each mass-resolving quadrupole to operate with a unit resolution. The mass spectrometer operated in Full Scan mode in a mass spectral range of 100–1000 *m/z*. Data were acquired and elaborated by Analyst® 1.6 software (AB Sciex, Washington, USA).

Extraction, separation, and identification procedures

The leaves of *T. capitatum* subsp. *capitatum* (4.00 g) were extracted with ethanol 96% (about 300 mL) three times after a maceration of 96 h, each. The ethanol was evaporated at reduced pressure at 50 °C until a water suspension was obtained. Throughout the concentration procedure, pH of the extracting solution was checked on litmus paper to verify that it was not too acid or basic (meaning between the range 5.5–8.5) because an extreme acidity or alkalinity might cause unwanted secondary reactions in the extract such as the hydrolysis of ester and glycosidic bonds. In this case, pH was about 8. The obtained dried dark green extract weighed 1 g. The whole of this was subjected to a first chromatographic separation on silica gel (40 g, ratio about 1:40 *w/w*). The eluting system consisted of a mixture of *n*-butanol and distilled water at the concentration ratio of 82:18 *v/v* (400 mL). During the chromatographic run, the polarity of the eluting system was raised to let the elution of more polar compounds by passing to a mixture of *n*-butanol, methanol, and distilled water at the concentration ratio of 70:10:30 *v/v/v* (300 mL). From this first chromatographic separation, six compounds were identified by comparison with spectroscopic and spectrometric data reported in the literature: pheophytin *a* (**1**) (Frezza et al., 2019a) in mixture with lipids (ratio 1:15 *w/w*) from the combined fractions 7-22 (70.0 mg); verbascoside (**2**) (Frezza et al., 2019b) in mixture with alyssonoside (**3**) (Çalis et al., 1992) (ratio 6:1 *w/w*) from the combined fractions 39-42 for the total weight of 5.7 mg; 8-*O*-acetyl-harpagide (**7**) (Venditti et al., 2016a) in mixture with alyssonoside (**3**) (Çalis et al., 1992) in ratio 1:10 *w/w* from the combined fractions 65-78 (7.7 mg); teucardoside (**8**) (Frezza et al., 2023) in mixture with 8-*O*-acetyl-harpagide (**7**) (Venditti et al., 2016a) and saccharides (ratio not calculable) from the combined fractions 79-91 (25.2 mg); quinic acid (**9**) (Frezza et al., 2019a) as almost pure compound from the methanol column wash for the weight of 71.1 mg. Since not all the secondary metabolites could be perfectly identified from this first chromatographic procedure, a second chromatographic step was performed on the assembly of fractions 7-38 for the total weight of 251.4 mg using 10 g of silica gel as stationary phase (ratio about 1:40 *w/w*). The initial eluting system consisted of a mixture of *n*-hexane and ethyl acetate in ratio 95:5 *v/v* (50 mL) but during the chromatographic run, the polarity of this eluting mixture was raised passing to concentration ratios of 9:1 *v/v* (100 mL), 8:2 *v/v* (100 mL), 7:3 *v/v* (100 mL), 6:4 *v/v* (100 mL), 1:1 *v/v* (100 mL), 4:6 *v/v* (100 mL), 2:8 *v/v* (100 mL) in order to let the elution of more polar compounds. From this chromatographic step, three further compounds were identified: apigenin (**4**) and cirsimaritin (**5**) (Frezza et al., 2023) in mixture in ratio 1:6 *w/w* from the combined fractions 122-124 (10 mg); apigenin (**4**), cirsimaritin (**5**) and cirsiliol (**6**) (Frezza et al., 2023) in mixture (ratio not calculable) from the combined fractions 142-166 (6.3 mg).

NMR and MS data of the identified metabolites

Pheophytin *a* (**1**): ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.50 (1H, s, H-10), 9.37 (1H, s, H-5), 8.53 (1H, s, H-20), 7.97 (1H, dd, *J* = 18.2/11.7 Hz, H-3’), 6.17 (1H, d, *J* = 12.2 Hz, H_b-3’’), 5.15-5.10 (1H, overlapped, H-22), 3.87 (3H, s, H-13^{IV}), 3.67 (3H, s, H-12’), 3.38 (3H, s, H-2’), 3.22 (3H, s, H-7’), 1.87 (3H, s, H-18’), 1.71-1.64 (6H, overlapped signals, Me-8’, Me-23), 0.80-0.75 (12H, overlapped signals, H-24, H-25, H-26, H-27).
ESI-MS: *m/z* 893.31 $[M+Na]^+$.
Verbascoside (**2**): ^{1}H NMR (600 MHz, CD_3OD) δ: 7.60 (1H, d, *J* = 15.9 Hz, H-β), 7.06 (1H, d, *J* = 2.0 Hz, H-2’), 6.96 (1H, dd, *J* = 8.1/2.0 Hz, H-6’), 6.78 (1H, d, *J* = 8.1 Hz, H-5’), 6.69 (1H, d, *J* = 2.3 Hz, H-2’’), 6.68 (1H, d, *J* = 8.1 Hz, H-5’’), 6.57 (1H, dd, *J* = 8.1/2.3 Hz, H-6’’), 6.28 (1H, d, *J* = 15.9 Hz, H-α), 5.18 (1H, d, *J* = 1.7 Hz, H-1’’’), 4.38 (1H, d, *J* = 7.9 Hz, H-1), 4.00-3.35 (overlapped signals of saccharides), 2.83-2.78 (1H, overlapped, H-β’), 1.08 (3H, d, *J* = 6.2 Hz, Me- Rha).
ESI-MS: *m/z* 623.34 $[M-H]^-$.
Alyssonoside (**3**): ^{1}H NMR (600 MHz, CD_3OD) δ: 7.59 (1H, d, *J* = 15.8 Hz, H-β), 7.19 (1H, d, *J* = 2.0 Hz, H-2’), 7.11 (1H, dd, *J* = 8.2/2.0 Hz, H-6’), 6.75 (1H, d, *J* = 8.2 Hz, H-5’), 6.67 (1H, d, *J* = 2.1 Hz, H-2’’), 6.65 (1H, d, *J* = 8.1 Hz, H-5’’), 6.59 (1H, dd, *J* = 8.1/2.1 Hz, H-6’’), 6.29 (1H, d, *J* = 15.8 Hz, H-α), 5.16 (1H, d, *J* = 1.7 Hz, H-1’’’), 4.92 (1H, d, *J* = 2.0 Hz, H-1^{IV}), 4.35 (1H, d, *J* = 7.8 Hz, H-1), 4.00-3.35 (overlapped signals of saccharides), 3.88 (3H, s, 3’-OMe), 2.83-2.78 (1H, overlapped, H-β’), 1.06 (3H, d, *J* = 6.2 Hz, Me- Rha).
ESI-MS: *m/z* 793.78 $[M+Na]^+$; *m/z* 769.71 $[M-H]^-$.
Apigenin (**4**): ^{1}H NMR (600 MHz, CD_3OD) δ: 7.86 (1H, d, *J* = 8.9 Hz, H-2’ and H-6’), 6.95 (1H, d, *J* = 8.9 Hz, H-3’ and H-5’), 6.61 (1H, s, H-3), 6.47 (1H, d, *J* = 2.1 Hz, H-8), 6.22 (1H, d, *J* = 2.2 Hz, H-6).
ESI-MS: *m/z* 293.21 $[M+Na]^+$; *m/z* 269.16 $[M-H]^-$.
Cirsimaritin (**5**): ^{1}H NMR (600 MHz, CD_3OD) δ: 7.91 (1H, d, *J* = 8.9 Hz, H-2’ and H-6’), 6.95 (1H, d, *J* = 8.9 Hz, H-3’ and H-5’), 6.84 (1H, s, H-8), 6.68 (1H, s, H-3), 3.99 (3H, s, 7-OMe), 3.84 (3H, s, 6-OMe).
ESI-MS: *m/z* 337.30 $[M+Na]^+$.
Cirsiliol (**6**): ^{1}H NMR (600 MHz, CD_3OD) δ: 7.44 (1H, d, *J* = 8.3/2.3 Hz, H-6’), 7.41 (1H, d, *J* = 2.3 Hz, H-2’), 6.92 (1H, d, *J* = 8.3 Hz, H-5’), 6.81 (1H, s, H-8), 6.61 (1H, s, H-3), 3.99 (3H, s, 7-OMe), 3.84 (3H, s, 6-OMe).
ESI-MS: *m/z* 353.31 $[M+Na]^+$.
8-*O*-acetyl-harpagide (**7**): ^{1}H NMR (600 MHz, CD_3OD) δ: 6.39 (1H, d, *J* = 6.4 Hz, H-3), 6.07 (1H, d, *J* = 1.0 Hz, H-1), 5.00 (1H, overlapped with solvent signal, H-4), 4.60 (1H, d, *J* = 7.9 Hz, H-1’), 3.95-3.38 (overlapped signals of saccharide), 2.86 (1H, br. s, H-9), 2.21-2.12 (1H, m, H-7a), 2.09-2.03 (1H, m, H-7b), 2.02 (3H, s, OAc), 1.45 (3H, s, H-10).
ESI-MS: *m/z* 429.58 $[M+Na]^+$; *m/z* 405.47 $[M-H]^-$.
Teucardoside (**8**): ^{1}H NMR (600 MHz, D_2O) δ: 6.50 (1H, d, *J* = 6.3 Hz, H-3), 6.14 (1H, br. s, H-7), 6.01 (1H, d, *J* = 1.3 Hz, H-1), 5.35 (1H, d, *J* = 1.7 Hz, H-1’’), 5.06 (1H, dd, *J* = 6.3/0.7 Hz, H-4), 4.69 (1H, overlapped with solvent signal, H-1’), 4.02-3.44 (overlapped signals of saccharides), 2.34 (3H, s, H-10), 1.24 (3H, d, *J* = 6.3 Hz, Me-Rha).
ESI-MS: 513.61 *m/z* $[M+Na]^+$.
Quinic acid (**9**): ^{1}H NMR (600 MHz, D_2O) δ: 4.15-4.07 (1H, m, H-4), 3.80-3.72 (1H, m, H-5), 3.64-3.54 (1H, m, H-3), 2.26-2.15 (4H, m, H_a-2, H_b-2, H_a-6, Hb-6).
ESI-MS: 214.58 *m/z* $[M+Na]^+$; *m/z* 191.31 $[M-H]^-$.

RESULTS

The phytochemical analysis on the leaves of *T. capitatum* subsp. *capitatum* collected in the Botanical Garden of Rome led to the identification of nine compounds: pheophytin *a* (**1**), verbascoside (**2**), alyssonoside (**3**), apigenin (**4**), cirsimaritin (**5**), cirsiliol (**6**), 8-*O*-acetyl-harpagide (**7**), teucardoside (**8**) and quinic acid (**9**) (Fig. 2).
These compounds belong to five different classes of natural compounds *i.e.*, degradation products of chlorophylls (**1**), phenyl-ethanoid glycosides (**2-3**), flavonoids (**4-6**), iridoids (**7-8**) and organic acids (**9**).

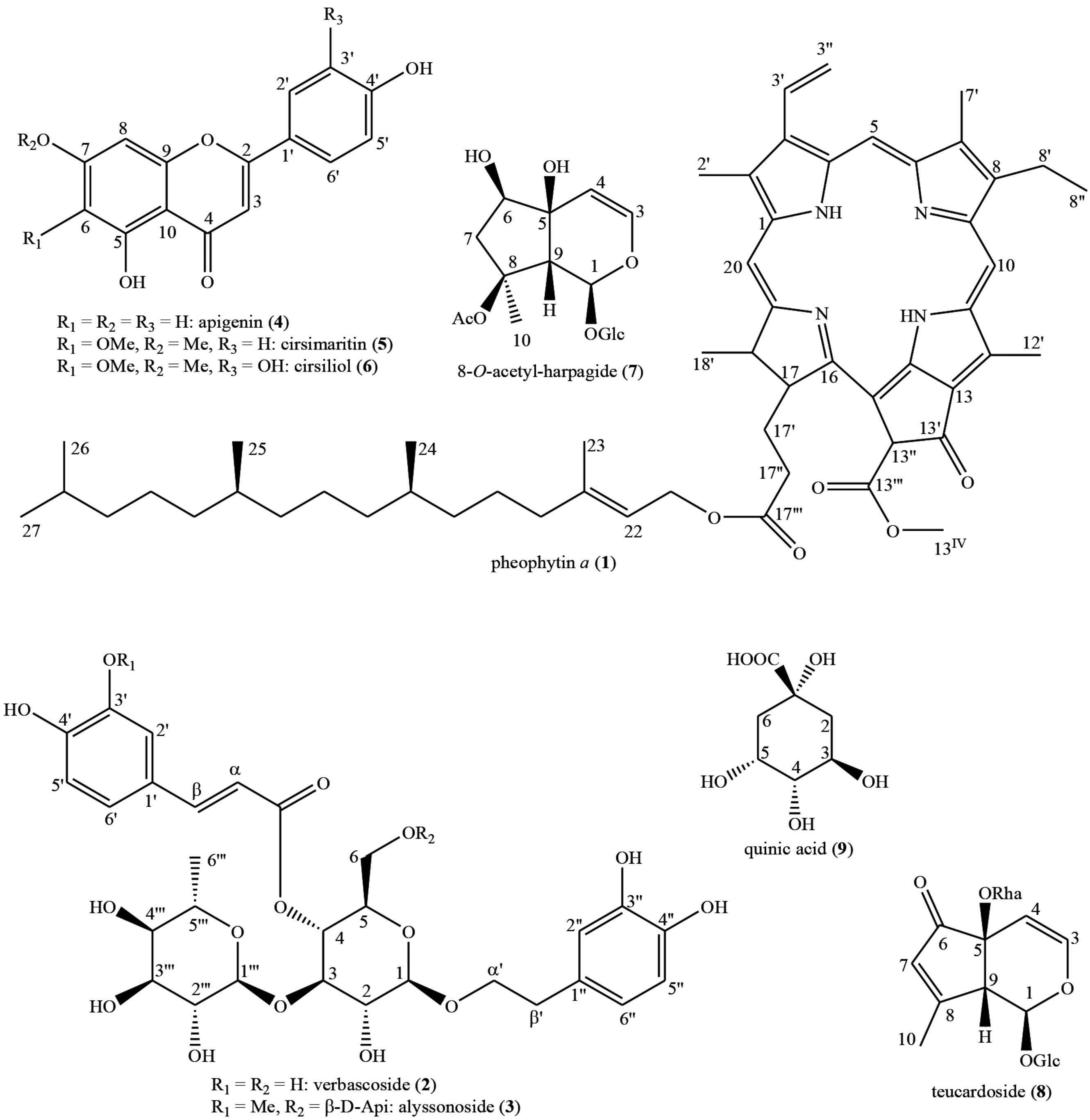

Figure 2. Structures of the identified compounds in *T. capitatum* subsp. *capitatum*.

Discussion

Except for pheophytin *a* (**1**) which derives from chlorophyll *a* and is probably an artifact of extraction (Venditti et al., 2020), verbascoside (**2**), alyssonoside (**3**), apigenin (**4**), cirsimaritin (**5**), cirsiliol (**6**), 8-*O*-acetyl-harpagide (**7**), teucardoside (**8**) and quinic acid (**9**) have been already evidenced in several populations of the species collected in many areas of the world (De Marino et al., 2012; Goulas et al., 2012; Mitreski et al., 2014; Venditti et al., 2017a, 2017b; Mihailović et al., 2020; Abdullah et al., 2022; Toplan et al., 2022), thus confirming their wide occurrence in this *taxon*. In particular, the presence of 8-*O*-acetyl-harpagide (**7**) and teucardoside (**8**), alone and along with verbascoside (**2**) and alyssonoside (**3**), is extremely important since they all confirm the botanical classification of the studied sample as a member of the *Teucrium* genus, of the Lamiaceae family and of the Asterids given that they represent their typical phytochemical compounds (Frezza et al., 2019c; Jensen et al., 1992). The absence of *neo*-clerodane diterpenes having a furan ring, typical phytochemical compounds of *Ajugoideae* sub-family (Frezza et al., 2019a) where this species is included, is also noteworthy and it is different from previous results (Abdullah et al., 2022; Bedir et al., 1999; Bruno et al., 2003; Sadaka et al., 2017). Yet, this peculiarity has been already observed in different *Ajugoideae* species collected in the continental Italy (Venditti et al., 2016a, 2016b; Frezza et al., 2017, 2018, 2019d, 2023) unlike the Islands (Fernández et al., 1985; Bruno et al., 2002). The reason behind this is currently under investigation even if preliminary considerations lead to hypothesize that the environment may indeed be responsible since the same methodology has always been adopted and in the species collected in Iran, these compounds have been found (Venditti et al., 2017a, 2017b). This aspect surely needs further studies and in-depth analyses.

Conclusions

The phytochemical analysis on the leaves of *T. capitatum* subsp. *capitatum* collected in the Botanical Garden of Rome led to the identification of nine compounds, four of which, also in combination, are typical phytochemical compounds of the species, family, clade. Their occurrence in this *taxon* has already been evidenced, thus confirming the previous phytochemical results. On the other hand, the absence of *neo*-clerodane diterpenes having a furan ring is an important difference from the previous studies even if this has been already recorded in Italian *Ajugoideae* populations, maybe due to the environment.

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INVENTORY, ASSOCIATION, AND HABITAT CHARACTERISTICS OF HUPERZINE A NATURAL RESOURCES IN THE CIBODAS BOTANICAL GARDENS, WEST JAVA, INDONESIA

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Abstract – Huperzine A (HupA) is a lycopodium alkaloid that is important in the treatment of alzheimer's symptoms. Until now, the raw material for this compound comes from the Lycopodiceae family, which is harvested directly from nature. However, not too much ecological information exists for this plant family, while responsible utilization would require a sufficient baseline. This research aims to carry out an inventory of HupA natural resources and study their association and habitat characteristics in the Cibodas botanical gardens. A survey was conducted on 29 host trees using a purposive sampling method. Three species of HupA natural resources were identified, including *Phlegmariurus pinifolius* (Trevis.) Kiew, *Phlegmariurus squarrosus* (G. Forst.) Á. Löve & D. Löve, and *Phlegmariurus phlegmaria* (L.) Holub. The most abundant and evenly distributed species was *P. pinifolius*, while the least abundant and restricted distribution was *P. phlegmaria*. *Phlegmariurus pinifolius* showed no association with other vascular epiphytes, while *P. squarrosus* and *P. phlegmaria* showed associations. The species diversity of vascular epiphytes is significantly correlated with elevation and relative air humidity. Based on ecological aspects, we suggest *P. pinifolius* and *P. squarrosus* be developed in further bioprospecting studies.

Keywords: huperzine a, lycopodiaceae, phlegmariurus, association, vascular epiphytes, alzheimer

Introduction

The genus *Phlegmariurus* (Herter) Holub, commonly known as tassel-ferns, is a member of the Lycopodiaceae family, a group of delicate pendant ferns with branching strobili (Field, 2011). In the history of its taxonomy, tassel-ferns have been placed in several genera, even once placed in a separate family, Huperziaceae (Rothmaler, 1944; Ching, 1978). Initially, the tassel ferns were classified by Linnaeus into the genus *Lycopodium* using *Lycopodium phlegmaria* L. from the Paleotropics (Linnaeus, 1753). This broader definition of *Lycopodium* was generally adopted in the taxonomic literature until the 1950s, including the taxonomic literature of Lycopodiaceae in the Indonesian region (van Alderwerelt van Rosenbergh, 1915, 1917; Backer & Posthumus, 1939). Furthermore, the tassel-ferns have been placed in some genera, such as *Urostachys* Herter (Herter, 1908, 1949a, 1949b, 1950; Nessel, 1939) and *Huperzia* Bernhardi

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(Bernhardi, 1801; Trevisan De Saint-Leon, 1874; Holub, 1985; Ollgaard, 1987). In one of the famous revisions of the global Lycopodiaceae, Ollgaard (1987) assigned the epiphytic tassel-ferns to the genus *Huperzia* Bernh based on their morphological similarity. As a result, *Huperzia* s.l. (broad definition) is still often adopted today in various flora literature and some applied research, such as biochemical and biopharmaceutical. However, based on the results of various phylogenetic studies using both molecular and non-molecular character analysis, the epiphytic tassel-ferns clearly separate from the genus *Phlegmariurus* (Herter) Holub, which is part of the subfamily Huperzioideae, together with *Phylloglosum* Kunze and *Huperzia* s.s. (narrow definition) plants (Ollgaard, 2015; Field et al., 2016; PPG, 2016). At this moment, *Phlegmariurus* (Herter) Holub consists of epiphytic tassel-fern species from the tropics and the Southern Hemisphere as well as unique secondary terrestrial species from the Andes (Field et al., 2016; Testo et al., 2018).

Phlegmariurus contains roughly 250 species and is the most numerous in the Lycopodiaceae (PPG, 2016; Testo et al., 2018). *Phlegmariurus* species are widely distributed in tropical and subtropical regions, occupy various altitudes ranging from sea level to 5000 m ASL, and have different habits, such as epiphytes, terrestrial, and rupicolous (Testo et al., 2018). *Phlegmariurus* grows in moist habitat and is commonly found in mountain forests and alpine grasslands. *Phlegmariurus* also grows very slow, taking 15 to 20 years from spore germination to maturity (Ma et al., 2006; Luo et al., 2010). These plants, like other members of Lycopodiaceae, have a mycoheterotrophic gametophyte that gets a carbon source from a symbiotic relationship with fungi by forming mycorrhizal associations (Merckx et al., 2013).

Several species of *Phlegmariurus* have been used for medicinal purposes and food supplements, including for the treatment of fever, rheumatism, pain of the joints and waist, injuries from falls, swelling due to poisoning, bone fractures, and cancer (Ma et al., 2006; Silalahi et al., 2015; Xu et al., 2019). There are studies to investigate medicinal chemical compounds from *Phlegmariurus*. One of the important findings was the Huperzine A (HupA), an alkaloid compound that can act as a strong acetylcholinesterase inhibitor and is promising as a treatment for Alzheimer's symptoms (Ma et al., 2006; Yang et al., 2016). The compound was originally found in *Huperzia serrata* but in lower content compared to some of *Phlegmariurus* species, i.e. *P. squarrosus*, *P. pinifolius*, and *P. phlegmaria* (Ma et al., 2005; Ishiuchi et al., 2013). However, it is important to note that *Phlegmariurus* populations in some areas are disrupted due to slow growth, habitat loss, and their high potency, resulting in overharvesting for medicinal purposes (Ma et al., 2006).

The association and ecology of *Phlegmariurus* highlight the importance of the relationships between plants, fungi, and other organisms in maintaining healthy ecosystems (Gilliam, 2006; Higgins et al., 2007; Debbab et al., 2012; Bunce et al., 2013; Jia et al., 2016). Furthermore, Cibodas Botanic Gardens (CBG), located in the lower montane zone (1300–1425 meters above sea level) on the slopes of Mount Gede Pangrango, is a conservation area that has wild and introduced huperzioid plant collections, including *Phlegmariurus* and *Huperzia* species. CBG has a total land area of 84.9 hectares, comprising garden and collection areas as well as 8.43 hectares of remnant forest (Nurdiana & Buot JR, 2021). Nasution (2014) reported that there are five species of *Phlegmariurus* (recorded with the *Huperzia* name) in CBG, i.e., *Huperzia carinata, H. gnidioides, H. phlegmaria, H. serrata,* and *H. squarrosa.* However, there is no updated information related to the ecological aspects of *Phlegmariurus* species in the CBG area. This research focuses on the inventory and habitat characteristics of potential natural resources of HupA in the Cibodas Botanical Gardens area. Location selection was based on previous research in the Cibodas Botanical Garden (Nasution 2014). He recorded that several *Phlegmariurus* species inhabit this garden. However, previous studies did not explain the abundance and habitat characteristics. These data are important as a consideration in the bioprospecting of these species. We assume that botanical gardens can be used as a model to study the trends in the abundance and habitat preferences of *Phlegmariurus* to support bioprospecting. This initial information is important to support bioprospecting research on this group of plants. This data is also needed to support sustainable use and avoid extinction in nature due to overharvesting.

Material and methods

Study area

This research was carried out from January to April 2023 at the Cibodas Botanical Gardens (CBG), West Java, Indonesia (Fig. 1). Study sites included a collection garden and remnant forests inside the CBG area. The CBG region is located on the slopes of Mount Gede Pangrango at 1300–1425 meters above sea level (S 06^{0}44.515', E 107^{0}00.290'). According to Mutaqien & Zuhri (2011), the average annual rainfall for CBG is 2,950 mm, the average air temperature is 20 ^{0}C, and the average relative air humidity is 80%. This garden covers an area of 85 hectares, and around 10% is a remnant forest with a tropical mountain rainforest ecosystem.

Sampling method

We conducted a comprehensive survey of the Cibodas Botanical Garden area, including the collection garden area and remnant forest. The purposive sampling method was used to determine the plot location. The first tree recorded as a *Phlegmariurus* habitat was made into a sampling plot. The sampling plot is rectangular with dimensions of 0.5 x 2 m (1 m^2). The plot was placed on the trunk of the host tree where *Phlegmariurus* was found. We limited the sampling location to tree trunks because some of the host trees were tree ferns that did not have branches and twigs. Then, we inventoried all *Phlegmariurus* species and other vascular epiphyte species in the plot to determine their associations. We continued our inventory of the four nearest trees and also recorded plant species in the 1 m^2 plot. Subsequently, we shifted our focus to another *Phlegmariurus* host tree and repeated the same process by sampling its four closest neighbours.

Herbarium specimens were collected for identification. We referred to Varenflora voor Java (Backer & Posthumus, 1939) and van Alderwerelt van Rosenbergh (1915; 1916) as references for the identification of *Phlegmariurus* and ferns. Meanwhile, for identification of other plant species, we referred to the Flora Malesiana Series and van Steenis (1972). We also measured habitat characteristics. The parameters that were measured included host tree diameter, tree bark thickness, air temperature, relative air humidity, elevation, and light intensity.

Data analysis

We used species-area curves to analyze whether the number of plots sampled represented all species diversity at the study site (Krebs 1999). In order to analyze vegetation data, we calculated the important value index (IVI) proposed by Ellenberg & Mueller-Dombois (1974). The Shannon-Wienner diversity index (Odum et al., 1971) was also utilized to analyze the vegetation data. To assess association, we used a contingency table analysis (Ellenberg & Mueller-Dombois, 1974). We analyzed the Spearman rank correlation between measured parameters and species abundance and species diversity of vascular epiphytes using R software. The strength of the correlation referred to Kuckartz et al. (2013). There were five categories based on the value of r: no correlation (0.0 < 0.1), low correlation (0.1<0.3), medium correlation (0.3<0.5), high correlation (0.5<0.7), and very high correlation (0.7<1).

Results

Vegetation Data

A comprehensive survey was carried out in the Cibodas Botanical Gardens area. This initial survey was conducted to

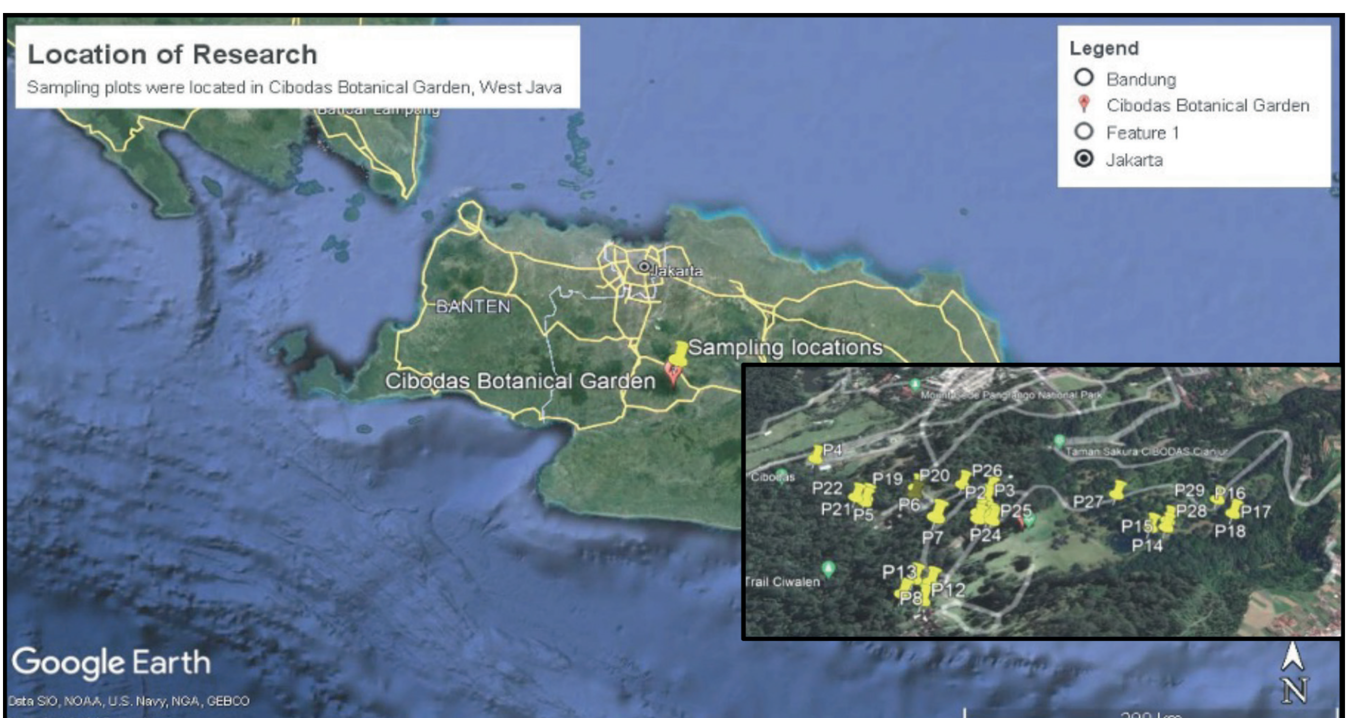

Figure 1. Location of sampling plots in Cibodas Botanical Gardens, West Java, Indonesia.

determine the first host trees to be sampled. We established the first sampling plot on the host tree of *Phlegmariurus* that we first recorded. Then, we continued until 29 host trees were obtained. The location of these plots is in the southern part of the Cibodas Botanical Garden area (Fig. 1).

We did not find any *Phlegmariurus* species in the northern part of the Cibodas Botanical Gardens. A total of 64 species of vascular epiphytes were recorded, and three species of *Phlegmariurus* were identified (Tab. 1).

Table 1. List of species, families and Important Value Index of vascular epiphytes on sampling sites in Cibodas Botanical Garden

No.	Species	Families	RD (%)	RF (%)	IVI (%)
1	*Aeschynanthus radicans* Jack	Gesneriaceae	7.41	8.17	15.58
2	*Phlegmariurus pinifolius* (Trevis.) Kiew	Lycopodiaceae	6.83	5.77	12.60
3	*Haplopteris elongata* (Sw.) E.H.Crane	Pteridaceae	6.26	5.29	11.54
4	*Phlegmariurus squarrosus* (G.Forst.) Á.Löve & D.Löve	Lycopodiaceae	5.97	4.33	10.29
5	*Davallia hymenophylloides* (Blume) Kuhn	Davalliaceae	5.00	4.81	9.81
6	*Liparis cespitosa* (Lam.) Lindl.	Orchidaceae	7.31	2.40	9.72
7	*Peperomia tetraphylla* (G.Forst.) Hook. & Arn.	Piperaceae	5.58	3.85	9.43
8	*Davallia denticulata* (Burm.f.) Mett.	Davalliaceae	3.46	5.29	8.75
9	*Ageratina riparia* (Regel) R.M.King & H.Rob.	Asteraceae	5.10	1.44	6.54
10	*Dendrobium crumenatum* Sw.	Orchidaceae	3.37	2.88	6.25
11	*Goniophlebium percussum* (Cav.) W.H.Wagner & Grether	Polypodiaceae	2.60	2.88	5.48
12	*Selaginella involvens* (Sw.) Spring	Selaginellaceae	4.33	0.96	5.29
13	*Ceratostylis* sp.	Orchidaceae	3.27	1.92	5.20
14	*Heptapleurum scandens* (Blume) Seem.	Araliaceae	1.06	3.37	4.42
15	*Loxogramme scolopendrioides* (Gaudich.) C.V.Morton	Polypodiaceae	2.79	1.44	4.23
16	*Eria* sp.	Orchidaceae	1.92	1.92	3.85
17	*Nephrolepis davallioides* (Sw.) Kunze	Nephrolepidaceae	2.12	1.44	3.56
18	*Asplenium nidus* L.	Aspleniaceae	1.15	2.40	3.56
19	*Antrophyum reticulatum* (G.Forst.) Kaulf.	Pteridaceae	2.79	0.48	3.27
20	*Phlegmariurus phlegmaria* (L.) Holub	Lycopodiaceae	1.83	1.44	3.27
21	*Asplenium salignum* Blume	Aspleniaceae	0.87	2.40	3.27
22	*Octarrhena parvula* Thwaites	Orchidaceae	2.50	0.48	2.98
23	*Medinilla laurifolia* Blume	Melastomataceae	1.44	1.44	2.89
24	*Medinilla alpestris* (Jack) Blume	Melastomataceae	0.96	1.92	2.89
25	*Ficus villosa* Blume	Moraceae	0.67	1.92	2.60
26	*Goniophlebium subauriculatum* (Blume) C.Presl	Polypodiaceae	0.67	1.92	2.60
27	*Microsorum membranifolium* (R.Br.) Ching	Polypodiaceae	0.67	1.92	2.60
28	*Ficus ampelos* Burm.f.	Moraceae	0.58	1.92	2.50
29	*Pyrrosia albicans* (Blume) Ching	Polypodiaceae	1.06	0.96	2.02
30	*Belvisia mucronata* (Fée) Copel.	Polypodiaceae	0.58	1.44	2.02
31	*Ficus cuspidata* Reinw. ex Blume	Moraceae	0.58	1.44	2.02
32	*Davallia pentaphylla* Blume	Davalliaceae	0.96	0.96	1.92
33	*Davallia repens* (L.f.) Kuhn	Davalliaceae	0.87	0.96	1.83
34	*Ficus punctata* Thunb.	Moraceae	0.87	0.96	1.83
35	*Vaginularia trichoidea* Fée	Pteridaceae	0.29	1.44	1.73
36	*Asplenium laserpitiifolium* Lam.	Aspleniaceae	0.58	0.96	1.54
37	*Goniophlebium persicifolium* (Desv.) Bedd.	Polypodiaceae	0.77	0.48	1.25
38	*Grammitis* sp.	Grammitidaceae	0.29	0.96	1.25

39	*Coelogyne ventricosa* (Blume) Rchb.f.	Orchidaceae	0.67	0.48	1.15
40	*Agalmyla parasitica* (Lam.) Kuntze	Gesneriaceae	0.19	0.96	1.15
41	*Belvisia spicata* (L.f.) Mirb. ex Saff.	Polypodiaceae	0.48	0.48	0.96
42	*Haplopteris ensiformis* (Sw.) E.H.Crane	Pteridaceae	0.38	0.48	0.87
43	*Bulbophyllum* sp.	Orchidaceae	0.29	0.48	0.77
44	*Poikilospermum suaveolens* (Blume) Merr.	Urticaceae	0.29	0.48	0.77
45	*Davallia sessilifolia* Blume	Davalliaceae	0.19	0.48	0.67
46	*Hoya* sp.	Apocynaceae	0.19	0.48	0.67
47	*Medinilla eximia* (Jack) Blume	Melastomataceae	0.19	0.48	0.67
48	*Viola javanica* W.Becker	Violaceae	0.19	0.48	0.67
49	*Begonia cucullata* Willd.	Begoniaceae	0.10	0.48	0.58
50	*Blumea balsamifera* (L.) DC.	Asteraceae	0.10	0.48	0.58
51	*Coelogyne miniata* (Blume) Lindl.	Orchidaceae	0.10	0.48	0.58
52	*Elaphoglossum* sp.	Lomariopsidaceae	0.10	0.48	0.58
53	*Ficus benjamina* L.	Moraceae	0.10	0.48	0.58
54	*Ficus variegata* Blume	Moraceae	0.10	0.48	0.58
55	*Neonauclea excelsa* (Blume) Merr.	Rubiaceae	0.10	0.48	0.58
56	*Oxalis corniculata* L.	Oxalidaceae	0.10	0.48	0.58
57	*Pothos scandens* L.	Araceae	0.10	0.48	0.58
58	*Paspalum* sp.	Poaceae	0.10	0.48	0.58
59	*Psilotum nudum* (L.) P.Beauv.	Psilotaceae	0.10	0.48	0.58
60	*Selliguea enervis* (Cav.) Ching	Polypodiaceae	0.10	0.48	0.58
61	*Smilax zeylanica* L.	Smilacaceae	0.10	0.48	0.58
62	*Tetrastigma* sp.	Vitaceae	0.10	0.48	0.58
63	*Vanda tricolor* Lindl.	Orchidaceae	0.10	0.48	0.58
64	*Youngia japonica* (L.) DC.	Asteraceae	0.10	0.48	0.58

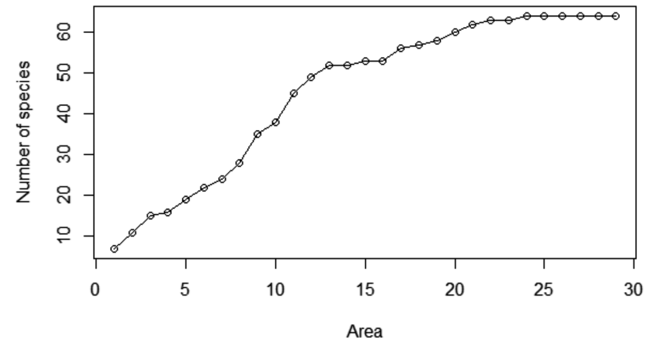

Figure 2. The species-area curve showed flat curve when sampling area reached 29 plot (29 m^2).

The three species belong to the Lycopodiaceae family, including *Phlegmariurus pinifolius* (Trevis.) Kiew, *Phlegmariurus squarrosus* (G. Forst.) Á. Löve & D. Löve, and *Phlegmariurus phlegmaria* (L.) Holub. We analyzed the number of sampling plots made with species-area curves. The species-area curve shows a horizontal line when the number of samplings has reached 29 plots (Fig. 2).

We analyzed the role of the inventoried vascular epiphytes by calculating the importance value index (IVI). In Table 1, *Aeschynanthus radicans* had the highest IVI and dominated compared to other species, followed by *P. pinifolius* and *H. elongata*. *Phlegmariurus pinifolius* was the most abundant and had the most even distribution among the three *Phlegmariurus* species, followed by *P. squarrosa* in the second place. Meanwhile, *P. phlegmaria* had the lowest IVI value compared to the two previous *Phlegmariurus* species. This species also had a lower value of relative density and relative frequency among the three *Phlegmariurus* species.

Association of ***Phlegmariurus***

We used a contingency table to calculate the X^2 value in the association analysis between *Phlegmariurus* species and other vascular epiphytic plants in the plot. There is a significant association if the X^2 value is greater than the X^2 table. Even though *P. piniflius* was the most abundant and had the highest IVI compared with two other *Phlegmariurus*, we didn't find any association of this species with other vascular epiphytes in our plot (Tab. 2).

There was also no association between *P. pinifolius*, *P. squarrosus* and *P. phlegmaria*.

We have discovered an association between *P. squarrosus* and other vascular epiphytes within the observation plots (Tab. 3). Eleven species of vascular epiphytes are significantly associated with *P. squarrosus*. The most frequent family associated with *P. squarrosus* is Moraceae, which encompasses three distinct species: *Ficus cuspidata*, *F. villosa*, and *F. ampelas*.

Table 2. Association analysis of *P. pinifolius* with other vascular epiphytes in the sampling plot (X^2 table = 3.84)

No.	Species	Family	X^2	Association*
1	*Coelogyne miniata*	Orchidaceae	2.779502315	Not Associated
2	*Davallia denticulata*	Davalliaceae	2.541771886	Not Associated
3	*Goniophlebium subauriculatum*	Polypodiaceae	1.937217262	Not Associated
4	*Ficus variegata*	Moraceae	1.937217262	Not Associated
5	*Ceratostylus* sp.	Orchidaceae	1.937217262	Not Associated
6	*Peperomia tetraphylla*	Piperaceae	1.712165179	Not Associated
7	*Haplopteris ensiformis*	Pteridaceae	1.701736111	Not Associated
8	*Pyrrosia albicans*	Polypodiaceae	1.701736111	Not Associated
9	*Blumea balsamifera*	Asteraceae	1.701736111	Not Associated
10	*Liparis caespitosa*	Orchidaceae	1.701736111	Not Associated

*Associated if $X^2 > X^2$ table

Table 3. Association analysis of *P. squarrosus* with other vascular epiphytes in the sampling plot (X^2 table = 3.84)

No	Species	Family	X^2	Association*
1	*Schefflera scandens*	Araliaceae	15.8887987	Associated
2	*Ficus cuspidata*	Moraceae	14.25385802	Associated
3	*Ageratina riparia*	Asteraceae	14.25385802	Associated
4	*Medinilla laurifolia*	Medinillaceae	14.25385802	Associated
5	*Aeschynanthus radicans*	Gesneriaceae	9.298744658	Associated
6	*Ficus villosa*	Moraceae	6.132579365	Associated
7	*Ficus ampelos*	Moraceae	6.132579365	Associated
8	*Davallia hymenophylloides*	Davalliaceae	5.686523908	Associated
9	*Pyrrosia albicans*	Polypodiacae	5.542989418	Associated
10	*Selaginella caulescens*	Selaginellaceae	5.542989418	Associated
11	*Agalmyla parasitica*	Gesneriaceae	5.542989418	Associated
12	*Grammitis* sp.	Grammitidaceae	5.542989418	Associated

*Associated if $X^2 > X^2$ table

The association of *P. phlegmaria* with other vascular epiphytes was also observed in all plots. Table 4 presents the associations based on X^2 values, wherein six species belonging to six distinct families were found to associate with *P. phlegmaria*. Notably, among these associated species were two fern species: *Goniophlebium percissifolium* and *Haplopteris elongata*.

Habitat Characteristics

We measured and recorded several parameters of habitat characteristics in each sampling plot. A summary of the measurement results is presented in Tab. 5.

We found that *P. phlegmaria* had a limited distribution and was only found in three plots. Meanwhile, the other two species of *Phlegmariurus* had a more even distribution. *Phlegmariurus pinifolius* was present in 12 plots, and *P. squarrosus* was found in nine plots. These plots were spread across collection gardens and remnant forest areas. *Phlegmariurus pinifolius* was mostly found in collection garden plots. A total of nine plots of this species, or around 75% of the sampling area, were found in the garden collection. On the other hand, *P. phlegmaria* is more commonly found in remnant forest plots. *Phlegmariurus squarrosa* was almost equal between plots in the garden (five plots) and in the remnant forest (four plots). About half of the hosts for *P.*

Table 4. Association analysis of *P. phlegmaria* with vascular epiphytes in sampling plot (X^2 table = 3.84)

No.	Species	Family	X^2	Association*
1	*Goniophlebium percissifolium*	Polypodiaceae	22.82886905	Associated
2	*Smilax zeylanica*	Smilacaceae	22.82886905	Associated
3	*Tetrastigma* sp.	Vittaceae	22.82886905	Associated
4	*Bulbophyllum* sp.	Orchidaceae	22.82886905	Associated
5	*Haplopteris elongata*	Pteridaceae	13.12813283	Associated
6	*Schefflera scandens*	Araliacae	5.296266234	Associated
7	*Aeschynanthus radicans*	Gesneriaceae	2.145864152	Not associated
8	*Davallia hymenophylloides*	Davalliaceae	1.111204147	Not associated
9	*Nephrolepis davallioides*	Nephrolepidaceae	0.937232906	Not associated
10	*Davallia denticulata*	Davalliaceae	0.538257576	Not associated

*Associated if $X^2 > X^2$ table

pinifolius and *P. squarrosus* were tree ferns (Cyatheaceae). However, we did not find any *P. phlegmaria* on the tree fern. We conducted a Spearman correlation analysis to investigate the relationship between habitat characteristics with the species abundance and diversity of vascular epiphytes at our research site. Six parameters were analyzed, including tree diameter, tree bark thickness, air temperature, air relative humidity, elevation, and light intensity. Correlation analysis between species abundance of vascular epiphytes and measured parameters is displayed in Fig. 3.

In our sampling plot, we only found a significant correlation between vascular epiphytes abundance with elevation. The correlations of the other five parameters were weak and insignificant. We found that vascular epiphytes diversity had a significant correlation with elevation and humidity (Fig. 4). The correlation strengths were medium. There was no significant correlation between species diversity and the other four measured parameters.

Table 5. The summary of *Phlegmariurus* spp. habitat characteristics in Cibodas Botanical Garden

No	Parameters	All species of Vascular epiphytes	*P. pinifolius*	*P. squarrosus*	*P. phlegmaria*
1	Tree host diameter (cm)	62.51±35.41	49.03±30.20	47.49±25.90	100.17±17.18
2	Bark tree thickness (mm)	5.33±1.91	5.73±1.58	5.62±1.30	4.78±1.71
3	Air Temperature (^{0}C)	24.04±1.32	23.44±0.91	24.18±1.44	23.50±0.52
4	Air Relative Humidity (%)	73.90±6.35	76.83±6.93	77.00±5.94	77.33±6.35
5	Elevation (m ASL)	1387.28±20.72	1397.83±18.38	1393.33±22.62	1377±21.93
6	Light intensity (Lux)	3654.24±2072.64	4023.25±1993.05	4137.44±2206.04	2727±2234.09
7	Plot location	Garden (14), remnant forest (15)	Garden (9), remnant forest (3)	Garden (5), remnant forest (4)	Garden (1), remnant forest (2)
8	Tree host species		*Helicia formosana* , *Echinocarpus australis, Spathodea campanulata, Ficus variegata, Cyathea contaminans* (2), *Cyathea junghuhniana* (3), *Cyathea raciborskii* , *Magnolia grandiflora, Magnolia sumatrana*	*Ficus variegata, Ostodes paniculata, Cyathea contaminans, Cyathea junghuhniana* (3) *Magnolia grandiflora, Macropanax dispermus, Magnolia sumatrana*	*Melaleuca alternifolia, Magnolia sumatrana, Sloanea sigun*

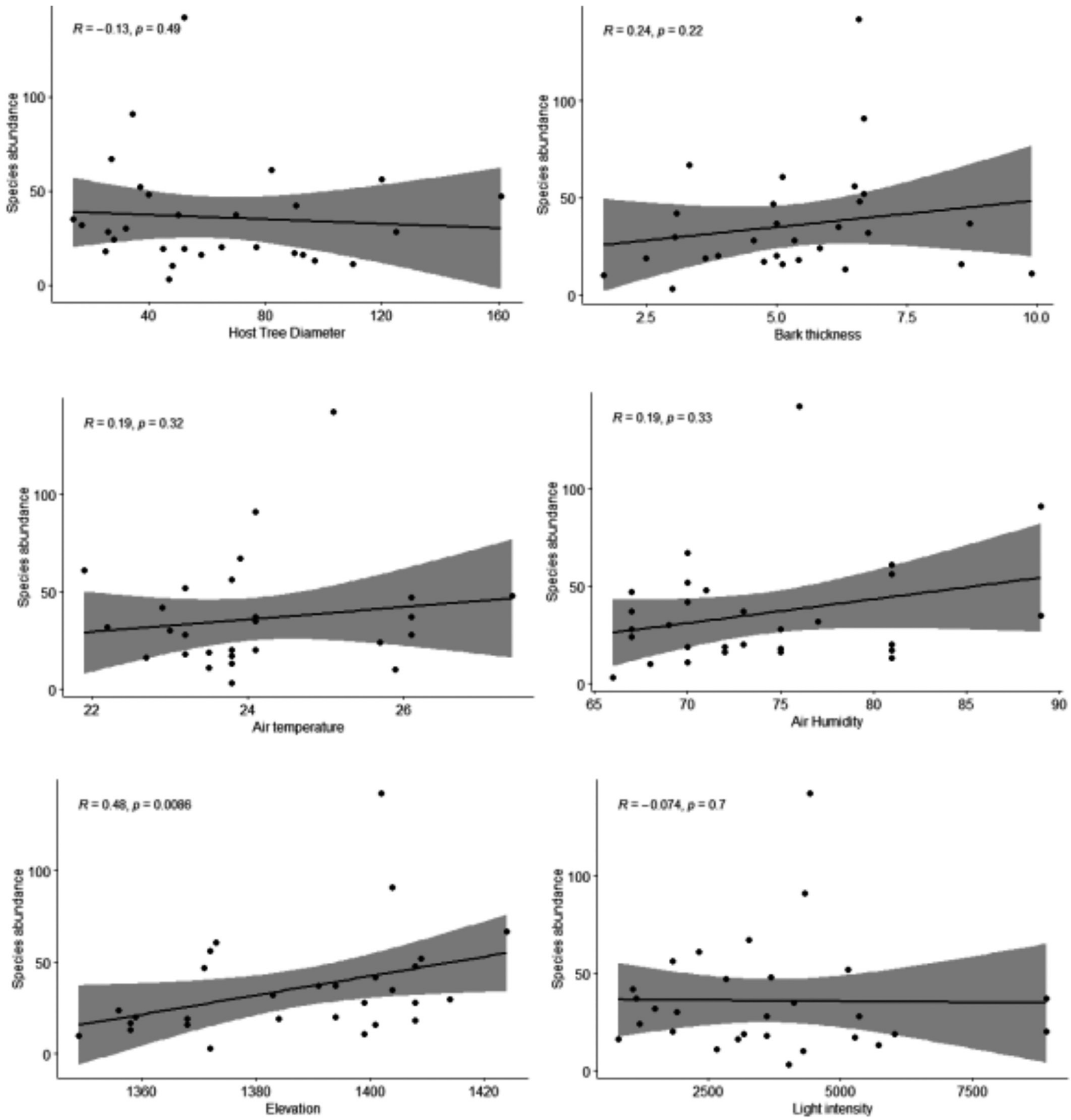

Figure 3. Correlation of vascular epiphytes abundance with habitat characteristics in the Cibodas Botanical Gardens.

Discussion

We found 64 species of vascular epiphytes and identified three species of *Phlegmariurus* that potentially become Huperzine A natural sources. The three species were *Phlegmariurus pinifolius* (Trevis.) Kiew, *Phlegmariurus squarrosus* (G. Forst.) Á. Löve & D. Löve, and *Phlegmariurus phlegmaria* (L.) Holub. All three species are epiphytic plants, classified into the *Phlegmariurus* genus and the Lycopodiceae family. The identification of these three species was in line with previous studies that stated that these three species were distributed in the mountains of Java (van Alderwerelt

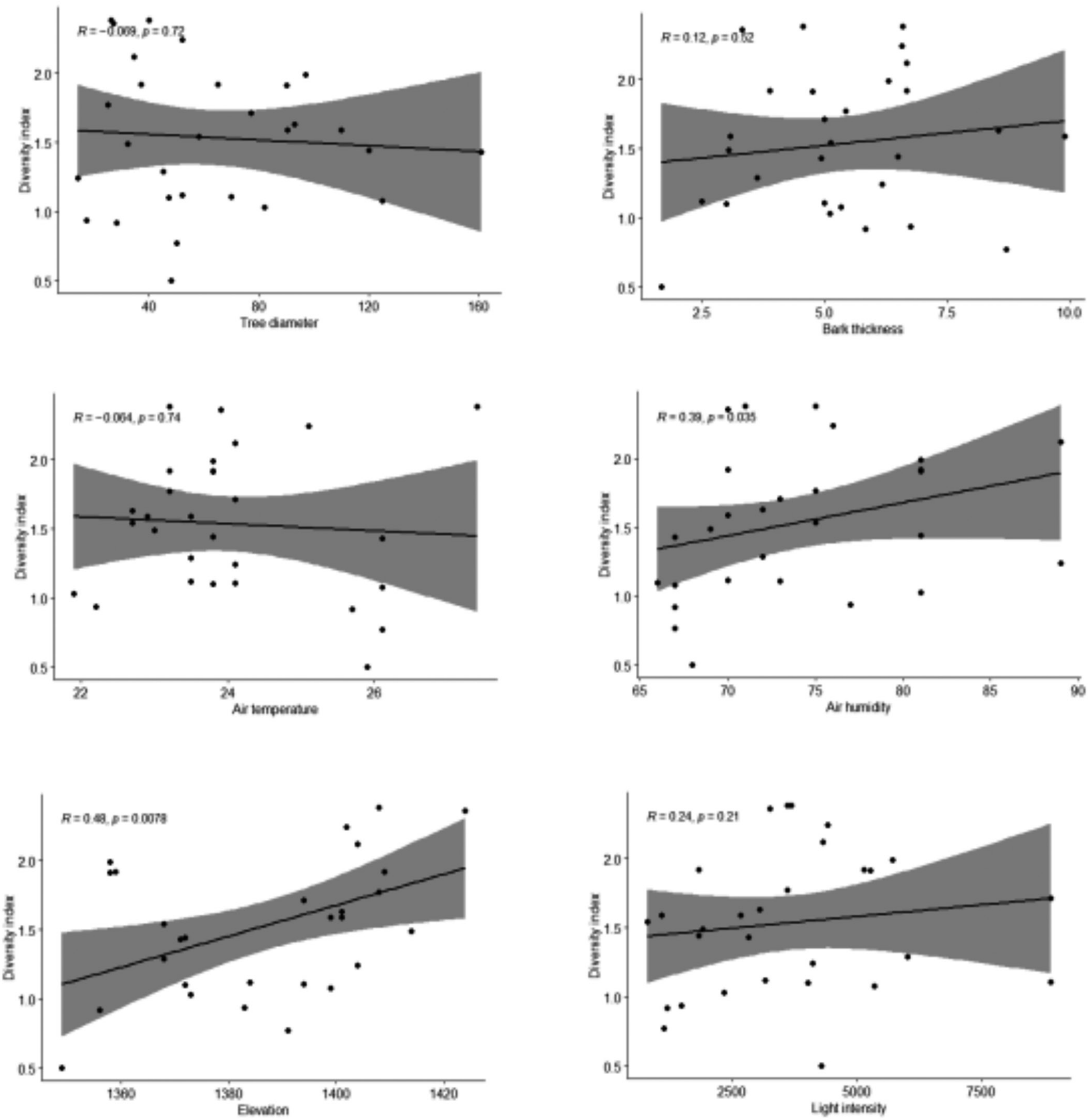

Figure 4. Correlation of vascular epiphytes diversity with habitat characteristics in the Cibodas Botanical Gardens.

van Rosenbergh, 1915, 1917; Backer & Posthumus, 1939; Nasution, 2015; Hartini, 2015). Meanwhile, HupA producers from other genera, such as *Huperzia*, were not found in our sampling plot.

Differences in habitat factors at least influence the distribution of the three species of *Phlegmariurus* in the garden and remnant forest. All *P. phlegmaria* were recorded on the bigger tree, which provided a lower air temperature, higher relative air humidity and lower light intensity (Tab. 5). The garden area is more open than the remnant forest area, thus influencing its microclimatic factors. It can be concluded that *P. phlegmaria* prefers higher humidity habitat. According to

Hoshizaki and Moran (2001), *P. phlegmaria* prefers moist and wet habitats, while *P. squarrosus* and *P. pinifolius* prefer habitats with better drainage.

We sampled 29 host trees and took an inventory of vascular epiphytes in a 1 m^2 plot on the tree trunk. The species-area curve shows the relationship between the size of the sampling area and the number of vascular epiphytes. In this study, the curve has reached an asymptote (Fig. 2), so it is predicted that the number of sampling plots is sufficient to inventory species diversity in the research area. In another study by Nurahma et al. (2005), at the same location, the curve reached an asymptote before reaching 30 sampling plots.

We calculated the IVI of 64 vascular epiphytes, which is the sum of relative frequency and relative density. The highest IVI value is occupied by *A. radicans*, followed by *P. pinifolius*, *H. elongata*, *P. squarrosus*, and *D. hymenophylloides*. These five species had higher IVI because they had a high relative density and relative frequency compared to other vascular epiphytes. A higher relative density indicates that the species has a high abundance, while a high relative frequency indicates that the species is more evenly distributed than other species in the study area.

Phlegmariurus pinifolius exhibited a higher relative density and relative frequency, followed by *P. squarrosus* and *P. phlegmaria*, respectively. *Phlegmariurus phlegmaria* showed the lowest IVI value compared to other *Phlegmariurus*. The lowest IVI value of *P. phlegmaria* is influenced by its lower abundance and limited distribution in the research location. This species only occurred in three plots, with a total of 19 individuals. It also has the lowest relative frequency, which indicates that the distribution of *P. phlegmaria* is more limited than two other species, most likely due to its preference of the shadier and more humid habitat (Hoshizaki and Moran 2001). This is supported by the microhabitat data (Tab. 5), which shows a lower average air temperature, higher air humidity, and lower light intensity for this species. *Phlegmariurus phlegmaria* also has a lower abundance than the other two species of *Phlegmariurus*. This low abundance is probably due to differences in microhabitat characteristics.

A total of 64 species were identified and belonged to 27 families. The families with the highest number of species are Polypodiaceae (9 species), Orchidaceae (9 species), Moraceae (6 species), Davalliaceae (5 species), and Pteridaceae (4 species). Ferns dominated the family with the largest number of species, including Polypodiaceae, Davalliaceae, and Pteridaceae. Research on vascular epiphytes in mountain forests in Guatemala was also dominated by orchid and fern species (Catling & Lefkovitch, 1989). The high abundance of ferns is supported by suitable microclimatic conditions in the study area. Ferns need moist conditions to grow well, especially in the gametophyte phase. The measurement results at the research location presented in Table 5 have shown relatively low temperatures and high humidity at the research location. Large trees are suitable hosts for vascular epiphytes, especially ferns. According to Zhao et al. (2015), the size of the host tree had a significant effect on the presence of vascular epiphytes. The study site is located at an elevation 1349 – 1424 m above sea level, which is classified as a submontane or lower montane forest ecosystem van Steenis, 1972. The submontane forest zone has relatively low average air temperature, high air humidity, and low light intensity that are suitable for *Phlegmariurus*. *Phlegmariurus phlegmaria* prefers shadier and more moist habitat compared to *P. squarrosus* (Hoshizaki and Moran, 2001; Hartini, 2015).

An association study was carried out to determine whether the *Phlegmariurus* species are associated with each other. Association analysis showed that there was no association between the three species of *Phlegmariurus*. This indicates that the three species of *Phlegmariurus* are independent of each other and are thought not to use the same resources in their habitat. However, an association was found between *Phlegmariurus* and several vascular epiphyte species in the observation plot. *Phlegmariurus pinifolius* does not show associations, while both *P. squarrosus* and *P. phlegmaria* associate with other epiphytic plants on host trees. The highest X^2 value for *P. pinifolius* is less than the X^2 table value (3.84). Table 2 displays the ten species with the highest X^2 value, with the results being less than the X^2 table value. As a result, it is possible to conclude that *P. pinifolius* has no association with other vascular epiphytes. Meanwhile, based on the X^2 values shown in Table 3 and Table 4, we discovered an association for both *P. squarrosus* and *P. phlegmaria* with other vascular epiphytes in the plots. *Phlegmariurus squarrosus* was found to be associated with 11 vascular epiphytes. It is more numerous than *P. phlegmaria* (six species). This is conceivable since *P. phlegmaria* has a restricted distribution, appearing in only three plots. Two fern species and four spermatophytes are associated with *P. phlegmaria*. Meanwhile, seven of the 11 vascular epiphytes associated with *P. squarrosa* were spermatophytes, two species of ferns, and two species of Lycopods. In comparison to the other two *Phlegmariurus* species, *P. squarrosus* had more extensive associations with vascular epiphytes at the research site. The association between two species shows whether the two species grow in the same environment, have the same distribution, and depend on each other (Kusmana, 1995). *Phlegmariurus pinifolius* does not show associations with other species, so it can be concluded that this species is more adaptive and does not depend on other species. This is also supported by the higher IVI and more abundant compared to other *Phlegmariurus* species.

We calculated the Shannon-Wiener diversity index on each plot and analyzed the correlations of the measured factors. Only the elevation parameter shows a significant correlation with the diversity and abundance of vascular epiphytic species. Meanwhile, the correlation between elevation and abundance was medium-strong and positive. A positive relationship indicated that abundance would increase with increasing elevation. Meanwhile, humidity only significantly correlates with species diversity. The correlation strength was medium. We suspect that the lack of significant correlation between the other parameters measured was due to the location of the sampling plots close to each other. A significant correlation between diversity and elevation was also proposed by Zhao et al. (2015). They also found that the diameter of the tree host also showed a significant correlation with the vascular epiphyte diversity. This study did not show the same result because the sampling was limited to a 1 m^2 sampling plot and located on tree trunks. We did not inventory branches and twigs because some of the host trees at the research location were tree ferns that did not have branches and twigs. The correlation between elevation and humidity on diversity and abundance is in accordance with the statements of Hartini (2015) also Hoshizaki and Moran (2001), which state that the habitat of *Phlegmariurus* is in the submontane zone with shady and humid conditions. In line with increasing elevation, the temperature will decrease, and the humidity will increase. This is a suitable habitat condition for the growth of *Phlegmariurus*. The implication for bioprospecting is that it is necessary to consider that the harvesting location chosen is a suitable habitat so that it continues to grow optimally even when harvesting activities are carried out.

The *Phlegmariurus* species exhibiting optimal abundance and even distribution were proposed for further bioprospecting studies. Drawing from the ecological data and HupA content from previous studies, we strongly recommend *P. pinifolius* and *P. squarrosus* in further bioprospecting studies. Meanwhile, we do not suggest *P. phlegmaria* due to its low abundance and restricted distribution in Cibodas Botanical Garden.

Conclusions

We identified three species of *Phlegmariurus* that are potential natural resources of Huperzine A, including *P. pinifolius*, *P. squarrosus*, and *P. phlegmaria,* in the Cibodas Botanical Gardens. *Phlegmariurus pinifolius* had the highest abundance and was evenly distributed, while *P. phlegmaria* showed low abundance and limited distribution. *Phlegmariurus squarrosus* and *P. phlegmaria* showed associations with other vascular epiphytes, while *P. pinifolius* did not show significant associations. In our sampling plot, elevation showed a significant correlation with species diversity and species abundance. Meanwhile, relative air humidity only showed a significant correlation with species diversity. We suggested further bioprospecting research on *P. pinifolius* and *P. squarrosus* and do not recommend *P. phlegmaria* due to its low abundance and limited distribution in the study area.

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EFFECTS OF LANDSCAPE PLANTS ON PUBLIC HEALTH: THE CASE OF BURSA-MUDANYA

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Abstract – Plants are a part of life, and they are elements that are an inseparable part of landscape designs, especially in areas that are widely used by large masses of people. It is of great importance to know and choose the properties of plants that affect public health due to their aesthetic, functional, and psychological effects, as well as some components they contain. Toxic components in different organs of plants cause adverse effects for humans and animals by touching and ingesting. Furthermore, many plants can cause allergic reactions because of their pollens.
This study examined open green areas designed for public use in Bursa province of Turkey, Mudanya district, and the taxa of woody landscape plants in these areas were evaluated in terms of their toxic properties for public health.
In the study, it was determined that 79.4% of the 107 taxa of woody landscape plants showed toxic and allergenic properties, while 20.6% did not show toxic properties. On the other hand, it was observed that the rate of non-toxic but allergenic taxa was 18.6 %. As a result of statistical studies with the obtained data, a significant relationship among the parameters examined was determined in the study.
Landscape designs made by considering the toxicological properties of plants may reduce the cases of poisoning caused by plants, and the measures to be taken for existing designs may raise awareness of the public on this issue.

Keywords: Toxicological classification of plants, toxic plants, allergenic plants, importance of plant selection, Mudanya.

Introduction

Plants are living organisms that form a remarkable part of the ecosystem, have great functions within the environment and human factors, have dynamic features, and are constantly evolving (Eroglu et.al., 2005; Acar & Sari, 2010). Plants, which cover a large part of the ecosystem, are important in creating livable green spaces, especially in urban and rural areas (Akdeniz et.al., 2017; Domina et.al., 2024).

Landscape plants, which are used in open and green areas designed to meet the needs of people for green space and located among the concrete building blocks in cities as a result of rapid population growth and unplanned urbanization, help to keep the ongoing mutual interaction between the human and environmental system in balance with effects such as reducing air pollution, hide unattractive vistas, directing pedestrian traffic, preventing soil erosion, creating space that are contributing to urban aesthetics and

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are having psychological, emotional, and mental functions on people (Booth, 1990; Scarfone, 2007; Karasah & Var, 2012; Sari & Karasah, 2018; Beckmann-Wübbelt et.al., 2021; Lausi et.al., 2022).

Many features, especially dendrological, ecological, aesthetic, and functional features, are considered in the selection of landscape plants for landscape designs (Paganová & Jureková, 2012). Due to the increase in health problems (allergic reactions, etc.) in humans and animals in recent years, it is of great importance to consider the toxic components of plants. Some of the plants known for their unique properties in the 'Plant Universe' show toxic effects due to the components they contain, and their effects arise as a result of interactions with humans and animals in different ways (Çelik, 2020; Domina et.al., 2024).

A poisonous plant is defined as a plant that creates negative effects through the poisonous substances it contains (Wagstaff, 2008). There are cases of poisoning as a result of ingestion of different parts of the plant or contact with the skin and these poisonings can cause cases whose symptoms disappear spontaneously, as well as serious cases that end up with death (Benzeid et al., 2018; Serrano, 2018; Nithaniyal et al., 2021). Toxic components are found in many genera and species in different families. The components which are toxic to humans and animals are the primary components that plants synthesize to maintain their basic metabolism, as well as the secondary components they produce, such as protein, fat, and carbohydrates. Some of them are alkaloids, glycosides, oxalates, saponins, terpenes, tannins, polyenes, resinous components, fatty acids, volatile oils, toxic amino acids, proteins, and peptides. These components, which can have toxic effects on humans and animals, also take on the task of protecting plants against pathogens. The severity of poisoning in humans and animals varies according to gender, age, body weight, type of toxic substance, the amount contained in the plant, and the way and amount of these components are taken into the body (Ginsburg & Deharo, 2011; Serrano, 2018). It is also possible that the concentration of a toxic substance is greater in some communities of a plant within the same species. As a result, the severity of symptoms seen in living things affected by plants in different communities can vary. Responses to plant toxins may also be influenced by human genetic variation that can detoxify many of these substances (Peterson, 2011). The ingestion of the leaves, stems, and roots of some plants or contact with their sap can result in toxic effects. Depending on the degree of toxicity, this can cause serious illnesses (Knight, 2007; Poppenga, 2010; Filmer, 2012; Domina et.al., 2024). Especially in this context, plants that cause high toxicity can cause serious damage and even death. On the other hand, ingestion or chewing of plants with low toxicity may cause minor discomforts such as vomiting and diarrhea, while plants containing oxalate crystals in their sap cause symptoms such as mouth, tongue, and throat irritation, swelling, burning, and stomach disorders. Moreover, the sap or thorns of plants that cause dermatitis cause itching, redness, or irritation on the skin through their villus. Plants that cause animal toxicity can have toxic effects, especially for pets. It is also possible that the concentration of a toxic substance is greater in some communities of a plant within the same species (Nelson et.al., 2007; Knight, 2007; Poppenga, 2010; Filmer, 2012; Zencirkiran et.al., 2018).

On the other hand, in addition to the toxic components contained in the plant, the pollen produced by the trees at different times of the year, mostly in the spring, can cause allergies such as rhinitis, asthma, and conjunctivitis in some people. It is estimated that approximately 10-30% of the world's population has pollen-related rhinitis. It's revealed the necessity of focusing on whether the landscape plants to be used in plant designs are also allergenic due to the effects of tree pollens on asthma and their cross-reactivity (Stach et.al., 2008; Steinman, 2008; Lauriola & Talluri, 2022).

In this context, within the scope of this study, which was carried out based on the hypothesis that "human and animal health needs to know the toxic properties of landscape plants that will be included in the designs of open green areas where there is an intense public use, and to consider this issue in designs", woody landscape plants of the light green areas of Mudanya district of Bursa province were evaluated according to their toxicity status.

Materials and methods

This research was carried out in Mudanya district of Bursa province. Mudanya district is located between 28°- 29° east

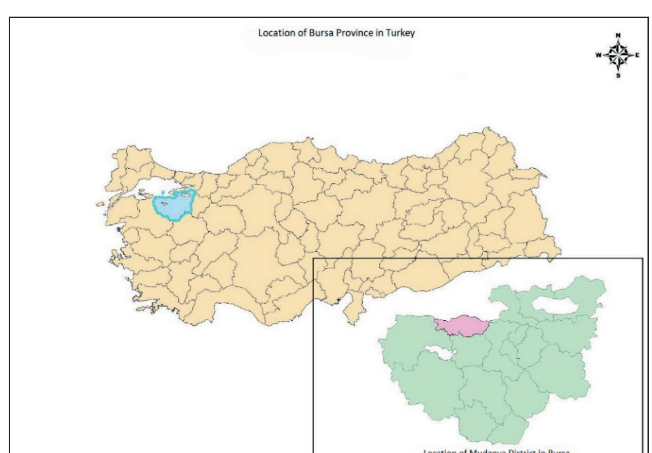


Figure 1. Bursa province and Mudanya district location map.

longitude and 40°- 41° north latitude and has a surface area of 366 km^2. Mudanya district has a coast to the southeast of the Sea of Marmara (Fig. 1) and is adjacent to the district of Karacabey in the west, Nilüfer and Osmangazi in the south, and Gemlik in the east (Anonymous, 2022).

The main material of the study consists of woody landscape plants in 123 open green areas located within the borders of Mudanya district of Bursa province. Observation technique and document analysis methods were used. In the field study, it was carried out within the scope of the observation technique (Ozdemir, 2010; Baltaci, 2019; Kiral, 2020), which is a technique that provides access to the data first and records the observations of the researcher who approaches the subject impartially without making any changes (Ozdemir, 2010; Baltaci, 2019; Kiral, 2020). The visits were made by the study team 40 times in total, once every two weeks during the vegetation period (spring - summer), and the samples were taken from the shoots, leaves and flowers of woody landscape plants. During sampling, each plant was photographed with a Nikon D5200 camera to obtain visual material. The samples were dried by natural drying method. This method was applied by placing the plants between papers and changing the papers daily in an environment where the air was active and direct sunlight was not received (Uma & Duzenli, 2012). For this purpose, the plant samples were placed in papers of newspaper in an orderly manner, and pressed in wooden presses, and dried in the laboratory of the department. After drying, the plants were placed on white cardboard sheets and identified. Plant identifications were carried out by the study team using the document analysis method (Ozdemir, 2010; Kiral, 2020), which enables the examination and systematic analysis of all documents, including printed and electronic materials. In this context, different sources (Davis, 1965-1988; Kayacik, 1980, 1981, 1982; Krusmann, 1984-1986; Dirr, 1992; Pamay, 1992, 1993; Yaltirik, 1993; Anonymous, 1998; Mataraci, 2002; Zencirkiran, 2004; Zencirkiran, 2009; Zencirkiran, 2013; Zencirkiran & Akdeniz, 2017; Çelik 2020) and samples from Bursa Uludag University Faculty of Science and Literature Herbarium were used.

Plants were included in more than one toxicity group (Tab. 1) depending on different parameters such as the toxic components it contains, the amount of toxic substance entering the body of the affected organism, the way the toxic substance taken into the body. The woody taxa identified in the study were classified and evaluated under 7 groups in terms of toxicity and allergenicity (Baytop, 1989; Knight, 2007; Nelson et al., 2007; Steinman, 2008; Wagstaff, 2008; Filmer, 2012; Atasoy, 2012; Zencirkiran et al., 2018; DiTomaso, 2019; Friday, 2019; Çelik, 2020; Çelik & Zencirkiran, 2021; Kušen et al., 2022).

Table 1. Toxicity groups and their effect.

Group 1. Major Toxicity	These plants may cause serious illness or death.
Group 2. Minor Toxicity	Ingestion of these plants may cause minor illnesses such as vomiting or diarrhea.
Group 3. Oxalates	The juice or sap of these plants contains oxalate crystals. These needle-shaped crystals can irritate the skin, mouth, tongue, and throat, resulting in throat swelling, breathing difficulties, burning pain, and stomach upset.
Group 4. Dermatitis	The juice, sap or thorns of these plants may cause a skin rash or irritation.
Group 5. Animal Toxicity	Plants in this group are toxic to animals such as cats and dogs.
Group 6. Allergen	Plants in this group can cause allergic disorders such as rhinitis, asthma, conjunctivitis in humans due to the pollen they produce.
Group 7. Non-toxic	Plants in this group are not harmed.

SPSS 28 (IBM, 2022) program was used for statistical analysis of the obtained data. Differences between taxa and toxic groups were determined by one-way and two-way analysis of variance, Duncan's test (Duncan, 1955) was used for differences between groups, and the significance levels were lettered as $p \leq 0.05$. On the other hand, Pearson Correlation analysis was used to determine the relationships between toxic groups and taxa.

Results

107 taxa of woody landscape plants were identified in 123 open green areas examined in the district of Mudanya. Observations revealed that 84 of the identified taxa were in the Angiospermae subdivision and 23 in the Gymnospermae subdivision.

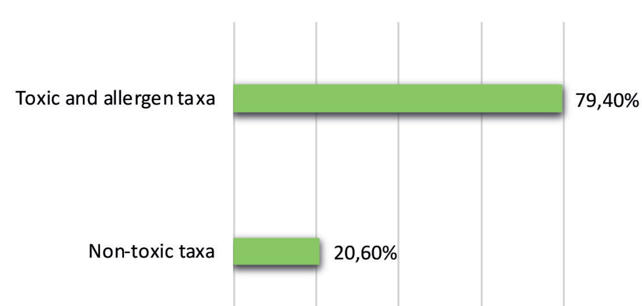
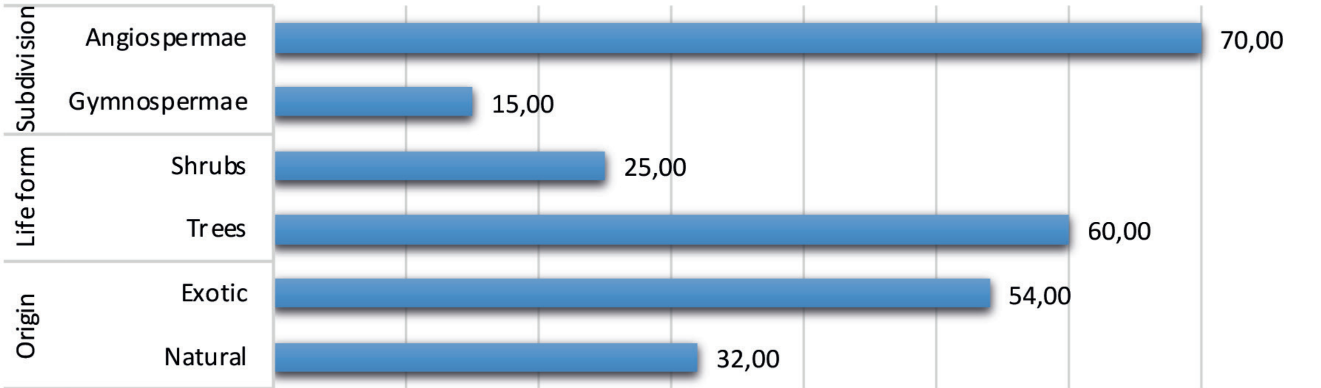


Figure 2. Distribution of toxic - allergen and non-toxic taxa.



	Origin		Life form		Subdivision	
	Natural	Exotic	Trees	Shrubs	Gymnospermae	Angiospermae
Number of Toxic Taxon	32,00 b	54,00 a	60,00 a	25,00 b	15,00 b	70,00 a*

Figure 3. Number of toxic taxa by subdivision, origin and life forms (*The letters indicate different groups at the p ≤ 0.05 level).

Table 2. Number of taxa by toxic groups.

Toxic Groups	Number of taxa
Group 1	13 d*
Group 2	30c
Group 3	1 e
Group 4	32 b
Group 5	42 a
Group 6	41 a
Group 7	42 a

(*The letters indicate different groups at the p ≤ 0.05 level).

79.40% (85 units) of the 107 woody taxa detected showed toxic and allergenic properties, while 20.60% (22 units) were non-toxic (Fig. 2). On the other hand, 20% (17 units) of the detected toxic taxa were allergen-toxic and 23.52% (20 units) were allergen-non-toxic.

The study determined that the distributions of toxic taxa numbers differed significantly in terms of subdivision, life form, and origin at p ≤ 0.05 level.

70 taxa in the subdivision of Angiospermae, 60 taxa in tree form, and 54 taxa of exotic origin showed toxic properties (Fig. 3).

As a result of statistical analyses, there were significant differences between the toxic groups at the p ≤ 0.05 level. The

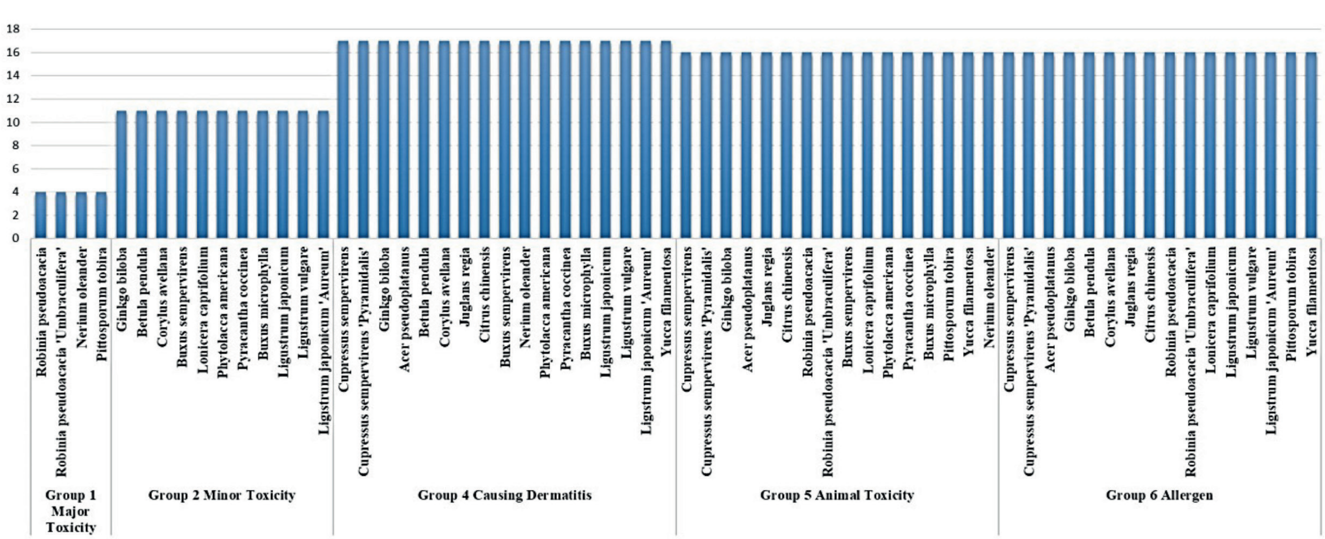


Figure 4.Distribution of taxa in three or more toxic groups according to toxic groups.

study determined that the highest number of taxa were in Group 7 and Group 5, and the least number of taxa were in Group 3 (Tab. 2). Taxa in three or more toxic groups are shown in Fig. 4. The correlation analysis between taxa and toxicity groups revealed a positive relationship between the total number of taxa and the number of toxic taxa, and the number of toxic taxa increased depending on the increase in the number of taxa (Tab. 3).

Table 3. The correlation analysis between total taxa in toxic groups and toxic taxa.

Total Taxa	Toxic Taxa	
	Pearson Correlation (r)	1,000**
	Significant (Sig.)	0.000

** Correlation is significant at the 0.01 level (2-tailed).

On the other hand, an evaluation of the relationship between the total number of taxa and toxic groups revealed a linear relationship since the correlation coefficient was (+) positive and the number of taxa in toxic groups increased as the total number of taxa increased (Tab. 4).

The results of the correlation analysis between the toxic groups exhibited that the toxic groups were positively (+) correlated with each other. Accordingly, an increase in the number of taxa in each group also increases the number of taxa in other groups (Tab. 5).

The results of the two-way analysis of variance revealed that the interactions of subdivision x toxic groups, life form x toxic groups, and origin x toxic groups were significant at the $p \leq 0.05$ level. An examination of the interaction of subsection x toxic groups revealed that the highest number of taxa was in Angiospermae subdivision x group 5, followed by Angiospermae subdivision x group 6. The study determined no taxon in Gymnospermae subdivision x group 3 (Tab. 6).

The analysis of the interaction of life form x toxic groups exhibited that the highest number of taxa was in Trees x group 7, followed by Trees x group 6. (Fig. 5).

The examination of the origin x toxic groups interaction revealed that the highest number of taxa was in exotic x group 5, followed by exotic x group 7. No taxa were determined in natural x group 3 (Tab. 7).

Conclusion

Plants can cause toxic effects for humans and animals due to some of the components they contain, and they can also cause allergic reactions with the pollen they spread. The results of research have demonstrated that species can bear different toxic properties, and that toxic compounds can also vary across species (Al-Qura'n, 2005; Çelik & Zencirkiran, 2021; Domina et.al., 2024). Also, a species can cause different toxic effects due to the components it contains. Poisoning caused by plants varies depending on various factors such as the amount and type of poison contained in the plant, and the age of the affected living being, and this may result in mild or even fatal situations. Emergent cases have been reported in the worldwide for species such as *Datura stramonium*, *Euphorbia* sp., *Nerium oleander* and *Ricinus communis* (Nithaniyal et al., 2021; Domina et.al., 2024).

For this reason, determining and knowing the toxicity status of plants is vital for well-being and a healthy environment. Accordingly, within the example of Mudanya district, 123 woody landscape design plants used in open green areas were identified and each taxon was examined in terms of toxicological properties and divided into different groups according to their properties.

The interactions of these groups with each other were revealed by making various analyses. As a result, the percentages of the 107 woody taxa were determined as 78.5% (with 84 taxa) in Angiospermae, 21.5% (with 23 taxa) in the subgroup Gymnospermae, 37.4% (with 40 taxa) in the subgroup natural and 62.7% (with 67 taxa) in exotic origin. In addition, it was also determined that 79.4% of the taxa showed toxic and allergenic properties, and 20.6% did not show toxic properties. On the other hand, the observations revealed that 18.6% of the taxa showed allergenic properties although they did not show toxic properties.

Turkey is a very rich country in terms of biodiversity with approximately 12.000 plant species, and registered poisonous plant species with properties that may pose a danger to human and animal health increasing day by day. It's also known that there are approximately 200 toxic plants registered in the country (Baytop, 1989; Ozturk et al., 2008; Gokkur & Dogan, 2018).

Domina et al. (2024) and Sebald et al. (2023) reported 137

Table 4. The correlation analysis between toxic groups and taxon numbers.

		Major Toxicity	Minor Toxicity	Oxalates	Dermatitis	Animal Toxicity	Allergen	Non-Toxic
Total taxon	Pearson Correlation	1,000**	1,000**	1,000**	1,000**	1,000**	1,000**	1,000**
	Sig. (2-tailed)	0,000	0,000	0,000	0,000	0,000	0,000	0,000

** Correlation is significant at the 0.01 level (2-tailed).

Table 5. The relationships between toxic groups

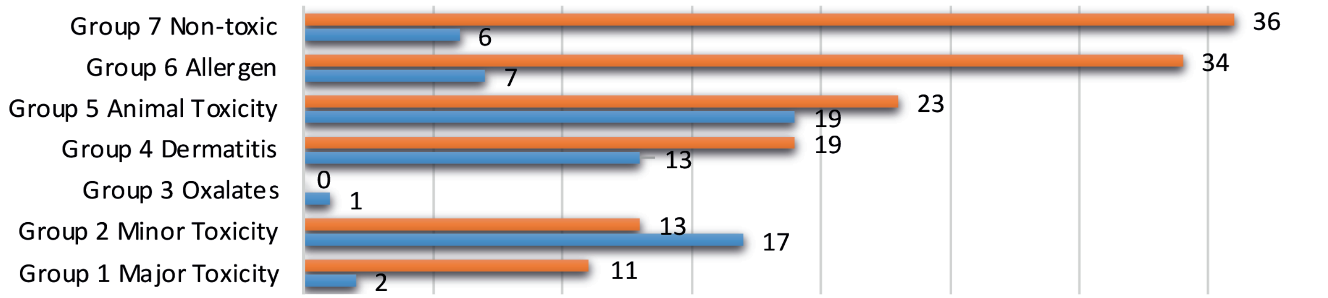
		Total taxon	Major Toxicity	Minor Toxicity	Oxalates	Dermatitis	Animal Toxicity	Allergen	Non-Toxic
Total Taxon	Pearson Correlation	1	1,000**	1,000**	1,000**	1,000**	1,000**	1,000**	1,000**
	Sig. (2-tailed)		,000	,000	,000	,000	,000	,000	,000
Major Toxicity	Pearson Correlation		1	1,000**	1,000**	1,000**	1,000**	1,000**	1,000**
	Sig. (2-tailed)			,000	,000	,000	,000	,000	,000
Minor Toxicity	Pearson Correlation			1	1,000**	1,000**	1,000**	1,000**	1,000**
	Sig. (2-tailed)				,000	,000	,000	,000	,000
Oxalates	Pearson Correlation				1	1,000**	1,000**	1,000**	1,000**
	Sig. (2-tailed)					,000	,000	,000	,000
Dermatitis	Pearson Correlation					1	1,000**	1,000**	1,000**
	Sig. (2-tailed)						,000	,000	,000
Animal Toxicity	Pearson Correlation						1	1,000**	1,000**
	Sig. (2-tailed)							,000	,000
Allergen	Pearson Correlation							1	1,000**
	Sig. (2-tailed)								,000
Non-Toxic	Pearson Correlation								1
	Sig. (2-tailed)								

**. Correlation is significant at the 0.01 level (2-tailed).

Table 6. Distribution of taxa among subdivision x toxic groups.

Subdivision	**Toxic groups**	**Number of taxa**
Gymnospermae	Group 1 Major Toxicity	1 j
	Group 2 Minor Toxicity	4 ı
	Group 3 Oxalates	0 j
	Group 4 Dermatitis	9 g
	Group 5 Animal Toxicity	6 h
	Group 6 Allergen	7 h
	Group 7 Non-toxic	11 f
Angiospermae	Group 1 Major Toxicity	12 f
	Group 2 Minor Toxicity	26 d
	Group 3 Oxalates	1 j
	Group 4 Dermatitis	23 e
	Group 5 Animal Toxicity	36 a
	Group 6 Allergen	34 b
	Group 7 Non-toxic	31 c

(*The letters indicate different groups at the $p \leq 0.05$ level)



	Group 1 Major Toxicity	Group 2 Minor Toxicity	Group 3 Oxalates	Group 4 Dermatitis	Group 5 Animal Toxicity	Group 6 Allergen	Group 7 Non-toxic
Trees	11 g	13 f	0 j	19 d	23 c	34 b	36 a*
Shrubs	2 ı	17 e	1 ıj	13 f	19 d	7 h	6 h

Figure 5. Distribution of taxa among life form x toxic groups (*The letters indicate different groups at $p \leq 0.05$).

and 106 toxic plants in their studies in parks and urban community gardens, while Akdeniz & Zencirkiran (2024) reported that 36 of 79 species identified in hospital gardens with a dense user base were toxic. However, plant poisoning is difficult to diagnose, and the epidemiology of plant poisonings is geographically specific (Ng et al., 2019).

According to the American Association of Poison Control Center report, in 2015, between 1.80 - 2.29% of the poisoning complaints were caused by plants, plants ranked 10th in the poisonings seen in children aged 0-5, and plants were reported to be among the top 25 in the general ranking (Mowry et al., 2016; Zencirkiran et.al., 2018). Since the spring and summer months, when urban open green spaces are frequently used, coincide with the period when the fruits and flowers of many plants with higher toxic components, the poisoning caused by plants increases (Gul & Topcu, 2017). The most common cause of poisoning from plants is the unconscious use of plants without being aware of their toxic properties (Kocabas, 2020).

In this study, it was proven that toxic groups were related to each other, and it was also seen that 41 of the toxic taxa were allergens and 42 were taxa with animal toxicity. It can be said that this situation may have negative effects on the users. Similarly, Frenguelli & Passaleva (2003) and Stach et al. (2008) emphasized in their studies that there is a periodic release of pollen in urban open green areas and that reactions such as pollen allergy, rhinitis and conjunctivitis recur every year. Ozgen (1987) stated that it is necessary to use surface air flow maps to control the effects of allergenic pollen.

Plants, which are an inseparable part of the universe, have been used by people for centuries to make medicine, host animals, and provide emotional and psychological contributions to people even are much more than their appearance and functions. For this reason, knowing more about them will be beneficial for humanity in every sense.

Designs should be made by taking into account the aesthetic and functional features of existing plants in urban open green areas such as children's playgrounds, hospital gardens, city parks, etc., especially where the user mass is intense, as well as their toxic and allergenic properties. However, in order to activate people's awareness and draw attention, it is

Table 7. Distribution of taxa among origin x toxic groups.

Origin	Toxic groups	Number of taxa
Natural	Group 1 Major Toxicity	2 ı
	Group 2 Minor Toxicity	9 h
	Group 3 Oxalates	0 j
	Group 4 Dermatitis	15 f
	Group 5 Animal Toxicity	12 g
	Group 6 Allergen	19 d
	Group 7 Non-toxic	18 de
Exotic	Group 1 Major Toxicity	10 h
	Group 2 Minor Toxicity	21 c
	Group 3 Oxalates	1 ıj
	Group 4 Dermatitis	17 e
	Group 5 Animal Toxicity	30 a
	Group 6 Allergen	21 c
	Group 7 Non-toxic	24 b

(*The letters indicate different groups at the $p \leq 0.05$ level).

important to put the warning signs on the taxa in these areas. These signs should contain information such as toxic and allergenic properties of the plant, and also toxic parts of the plants. Considering that there are few studies on the toxicity and allergenicity properties of plants, it is obvious that this study will be instructive for future applications.

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HISTORICAL USE OF MEDICINAL PLANTS AND FUTURE POTENTIAL: FROM PHYTOTHERAPY TO PHYTOCHEMICALS

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Abstract – Since prehistoric times, humans have understood that plants possessed healing properties. The knowledge of herbal medicine has been accumulated for millennia by traditional healers and has been passed down from generation to generation. Phytotherapy was used as the main therapy until the 18th century. Even today, between 40% and 80% of pharmaceuticals are phytochemicals or of plant origin and many of these have significantly changed or are still changing medical therapy. Most people in the world are or have been treated with phytochemicals or drugs derived from phytochemicals. Examples include antineoplastic agents (vinblastine, vincristine, etoposide, teniposide, paclitaxel, irinotecan, topotecan), antibiotics and antivirals (cephalosporins and oseltamivir), antiparasitic drugs (quinine and artemisinin), Intensive Care Unit (ICU) drugs (morphine, neuromuscular blockers, ephedrine), cardiovascular drugs (digoxin, quinidine, atropine, reserpine), antidiabetics (metformin), and many others. Many of these agents are included in the WHO list of essential medicines. Plant-derived medicines have changed human history, contributing fundamentally to the survival and improvement of our species' life expectancy. Phytochemicals, derived from interactions between plants and the environment, are substances often created over millennia. If humans had not drawn on these substances for medical therapy, they probably would never have been able to produce substances so complex and effective in treating disease. Since knowledge of the healing properties of plants is present in traditional medicines, ancient manuscripts should be studied as potential sources of contemporary pharmacotherapy. Unfortunately, in recent years several trends have started to threaten biodiversity and consequently also phytochemical resources. From this perspective, the "One Health" approach is further justified and could also encourage the discovery of new revolutionary phytochemicals.

Keywords: phytochemicals, phytotherapy, antineoplastic agents, hypoglycemic agents, one health, medicine chinese traditional.

Introduction

Since the prehistoric times, humans have understood that plants, in addition to their nutritional properties, possessed healing abilities (Halberstein, 2005; Kaur et al., 2019). Knowledge of the healing properties of plants, sought in the bark, seeds, and fruiting bodies, has been accumulated over millennia by traditional healers through trial and error, and has been passed down from generation to generation (Khan, 2014; Petrovska, 2012). Indeed evidence, in the form of written documents, monuments and even original medicines, indicates that medicinal plants represent the oldest and most widespread form of healing in human history (Khan, 2014; Petrovska, 2012). Plant medicine has been the basis of medicine (Hassan, 2015) in fact it was used as the main therapy until the 18th century (Halberstein, 2005). Herbal medicine declined in the 19th century, and only in the late 20th century did a renewed interest in herbal medicine begin (Ijinu et al., 2022).

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Phytomedicine and Phytochemicals

French physician Henri Leclerc coined the term **phytomedicine** in 1913. The term phytomedicine or **phytotherapy** denotes a medical practice, recognized by official science, involving the use of botanical raw materials such as fruiting bodies or flowers, roots, bark, seeds, and leaves for medicinal and therapeutic purposes (Falzon & Balabanova, 2017). The phytotherapeutic effects of plants can be traced to combinations of so-called secondary metabolites present in the plant (Falzon & Balabanova, 2017; Guerriero et al., 2018). Unlike primary metabolites, which are directly required for plant growth, secondary metabolites, also called **phytochemicals**, mediate plant-environment interactions (Al-Khayri et al., 2023; Erb & Kliebenstein, 2020). Phytochemicals act by protecting plants from pathogens such as germs, fungi, insects, animals, and other threats (Dar et al., 2017), thus enabling plants to adapt and survive in their environment (Erb & Kliebenstein, 2020; Kaur et al., 2019). For this reason, phytochemicals have evolved in as biologically active compounds capable of bringing about pharmacological or toxicological effects in humans and animals (Iyer et al., 2023). There are three main groups of secondary metabolites in plants, namely terpenes, phenols, and nitrogen compounds (Al-Khayri et al., 2023; Erb & Kliebenstein, 2020; Twaij & Hasan, 2022).
Today, between 40% and 80% of pharmaceutical products are derived from plants (Fitzgerald et al., 2019; WHO, 2023b) and many of the landmark drugs are phytochemicals (or derivatives of phytochemicals) discovered in plants or fungi and have decisively changed the history of medicine, including modern medicine (just to name a few: antineoplastic agents, antibiotics and antiviral drugs, antiparasitic drugs, Intensive Care Unit (ICU) drugs, cardiovascular drugs, antidiabetics etc.) (Awosika, Below, et al., 2024). Furthermore according to data from the World Health Organization (WHO), in developing countries 80% of the population uses phytotherapy as the first source of treatment (Alves & Rosa, 2005; Aziz et al., 2018; International Agency for Research on Cancer, 2002). Even in developed countries, the use of traditional herbal medicines is a rapidly growing phenomenon. To give some examples, in China 30-50% of total drug consumption is represented by traditional herbal preparations (Aziz et al., 2018). In Nigeria, Ghana, Zambia and Mali, herbal medicines are used in 60% of cases as the first intervention in children suffering from malaria (Aziz et al., 2018). In Saudi Arabia 80% of people use herbal medicine for medications (Ullah et al., 2020). China is currently the most advanced country in terms of the number of publications on the use of medicinal plants in medicine, although all countries in the world have their own traditions in the use of medicinal plants in medicine (Fitzgerald et al., 2019; Liu et al., 2016; Lu et al., 2022). Today, herbal medicines are marketed in very different ways around the world. In Germany, for example, they are subject to the same efficacy, safety and quality criteria as other pharmaceutical products and are sold as "phytomedicines". In the United States, on the contrary, most herbal products are marketed as food supplements and are therefore not subject to efficacy, safety and quality criteria (International Agency for Research on Caner, 2002).

History of Phytotherapy

It is impossible to trace a complete history of the use of plants in the treatment of human diseases since it is as old as humankind and has been practiced in virtually all cultures. In fact numerous sources attest, with ample evidence, that primitive people used phytotherapy (Halberstein, 2005; Kaur et al., 2019; Kong et al., 2003). In all countries and ethnic groups analyzed around the world, organized and systematized traditional herbal remedies have been described by anthropologists and ethnobotanists (Halberstein, 2005). In ancient times, in Chinese, Indian, Egyptian, Native American and European cultures, the use of plants for healing purposes was widespread (Kaur et al., 2019). Written evidence of the use of phytotherapy can be found in Sumerian clay tablets dating back to 5,000 BC, in the Old Testament, in Egyptian papyri, in the books of the Greek physicians Hippocrates and in the works of the Roman writers Pliny, Dioscorides, Galen, Theophrastus and others (Šantić et al., 2017). In numerous ancient cultures these preparations were used both as a cure for physical illnesses and as psychoactive substances (Merlin, 2003). This ancient knowledge through the Middle Ages, Renaissance and modern history has been passed down to the present day. In the 19th century there was a breakthrough, with the beginning of the extraction of phytochemicals from plants and the understanding of the action of individual molecules on the organism (Kaur et al., 2019).

Zoopharmacognosy

It would appear that humans learned to use plants to treat diseases both from direct experiences by gaining knowledge through trial and error (for example, humans who used plants in their diet began to discover that they could have side effects, be toxic, or sometimes improve certain symptoms (Šantić et al., 2017)) but also by observing how animals used plants for healing purposes (De la Fuente et al., 2022; Halberstein, 2005; Huffman, 1997). This second hypothesis was described as early as 2000 BC in the Rigveda, one of the oldest Indian texts, which states that humans acquired knowledge about differentiating edible from poisonous plants by observing animals (Prasathkumar et al., 2021). Today, indeed, it is known that primates use some plants as treatments that act as anti-inflammatories, immunostimulants, analgesics, antimicrobials, antidiarrheals, digestive aids, and fertility regulators (Huffman, 1997). Furthermore, humans, gorillas, chimpanzees, and monkeys use the same plants to manage similar diseases and health problems (Halberstein, 2005). For example, the plant Vernonia amygdalina is used by chimpanzees to treat parasitic infections. The same plant is used by the human inhabitants of Tanzania to cure fever (Huffman, 1997). Not only primates, but also other animals, use fruits, leaves, bark, roots and flowers of plants for both curative and preventive purposes (Šantić et al., 2017). Precisely, to study the activity of sick animals that seek remedies in nature, a field of scientific study was created, **Zoopharmacognosy** (Shurkin, 2014). Even today, the observation of self-medication by animals can be a source of discovery of new drugs (Domínguez-Martín et al., 2020; Hardy, 2021). For example very recently, a Sumatran orangutan was described treating an open wound on its cheek with a poultice made from a medicinal plant (Vaidyanathan, 2024). This behavior was the first documented case of a wild animal treating a wound with a plant species known to contain biologically active substances and provides new information on the origins of human wound care, as well as providing research insights into the healing properties of the plant (Laumer et al., 2024). Below we illustrate how the use of plants for healing purposes has been practiced throughout history in some human cultures.

India

Traditional Indian medicine or Ayurveda (a Sanskrit word literally meaning "knowledge of life"), which dates to 6000 BC. approximately, contains, in the Atharvaveda text collection, among the oldest references to the medicinal use of plants in Asia (Ijinu et al., 2022; Khan, 2014; Prasathkumar et al., 2021; Šantić et al., 2017). Ayurveda is a theory of the functioning of the human body, developed by the ancient Rishis of India, which defines man as composed of 7 fundamental tissues that work in harmony, while disease occurs due to the imbalance of these components (Ijinu et al., 2022; Khan, 2014). Ayurveda aims to achieve the prevention and cure of diseases by coordinating the connection between body and mind with the help of a vegetarian diet, use of medicinal herbs, physical exercise and meditation (International Agency for Research on Cancer, 2002). The properties of medicinal plants described in the Vedic literatures, particularly in the Rig Veda and the Atharvaveda, constitute the first written documents available in the history of Indian medicine (Ijinu et al., 2022; Petrovska, 2012). Regarding Ayurveda, the Susrutha Samhita and the Charaka Samhita are the most accepted texts on surgical and internal medicine aspects respectively (Ijinu et al., 2022). Ayurvedic medicine continued to develop until the Mughal period in India and began its decline during the regime of the Europeans (Ijinu et al., 2022). The Sushruta Samhita, dating back to the 4th-6th century BC, describes the medicinal values of around 700 plant species (Kaur et al., 2019; Khan, 2014; Loukas et al., 2010; Prasathkumar et al., 2021).

China

One of the oldest treatment systems is Traditional Chinese Medicine (TCM) which is unique in terms of theories, treatments and therapies (Khan, 2014). Fu Xi (2953 BC) is considered the pioneer of TCM (Khan, 2014). Later, emperors Shen Nung and Hong Ti, developed the system more significantly (Khan, 2014). The writings of Shen Nung, in 2838 BC, are considered between the oldest written documents providing guidelines on the treatment and use of medicinal plants (Šantić et al., 2017). The book "Pen Ts'ao",on root and herb, written by Emperor Shen Nung (3000 BC), lists 365 medicinal plants and their uses, including ginseng, jimsonweed, camphor, cinnamon and ephedrine, many of which are still in use as medicinal plants (Kaur et al., 2019; Petrovska, 2012). An author who made important contributions to TCM is Wang Tao (702-772). In his work "Waitai Miyao" described approximately 6,000 herbal prescriptions (Kopp et al., 2003). Another great Chinese physician and naturalist, Li Shizen, wrote an even more inclusive pharmacopoeia, Ben Ca Gang Mu, which was published in 1596. This work contains 1894 herbal preparations and is still used as a reference and guide for research and education in China (Khan, 2014). TCM was a knowledge passed down from generation to generation until the 1950s when it was introduced into university teaching (Khan, 2014). Although TCM also uses substances of mineral and animal origin to produce its treatments, the main source of remedies in this ancient form of medicine relies on substances of plant origin (International Agency for Research on Cancer, 2002). TCM consists of the clinical practice of a diagnosis followed by the prescription of a complex and often individualized remedy. To date, this type of medical approach is still widespread in China with more than half of the population regularly using traditional remedies, especially in rural areas. Traditional remedies account for about a fifth of the entire Chinese pharmaceutical market (International Agency for Research on Cancer, 2002; Xu et al., 2021).

Mesopotamian

In Nippur Mesopotamia, in present-day Iraq, clay tablets dating back to 5000 BC were found, which described the use of plants for medicinal preparations (Khan, 2014; Šantić et al., 2017). These tablets, which constitute between the oldest written testimony of the use of plants as medicines contain the description of 12 medicines prepared using over 250 plants including myrrh, bay, opium, cumin, henbane and mandrake (Hassan, 2015; Kaur et al., 2019; Khan, 2014; Petrovska, 2012; Šantić et al., 2017). These plants and their phytochemical derivatives are still used today worldwide for medicinal purposes (Hassan, 2015; Kaur et al., 2019; Khan, 2014; Petrovska, 2012; Šantić et al., 2017). Further evidence of the use of plants for healing purposes was also found in Mesopotamia on clay tablets with cuneiform characters dating back to subsequent periods ranging from 2600 BC. to 1800 BC. These documents describe the uses of oils derived from myrrh, cedar, licorice, poppy, and cypress (Dar et al., 2017; Hassan, 2015; Prasathkumar et al., 2021).

Egyptians

In the period between 3000 and 6000 years ago, the Egyptians developed an elaborate and effective pharmacological collection obtained from natural resources (Halberstein, 2005). The medical knowledge of this people is demonstrated by writings found in tombs and papyri dating back to the Old Kingdom of Egypt (Hassan, 2015). The Ebers papyrus, written around 1550 BC, is the most important of these testimonies and describes the use of over 850 plants for medicinal purposes by the Egyptians (Attorre & Bruno, 2022; Hassan, 2015; Khan, 2014; Prasathkumar et al., 2021; Šantić et al., 2017). The Egyptians used plants such as mandrake, garlic, juniper, cannabis, aloe, pomegranate, castor, senna, garlic, onion, fig, willow, coriander, juniper, centaury and preparations such as wine and beer for medical purposes (Halberstein, 2005; Kaur et al., 2019; Petrovska, 2012). For example, mandrake was used to relieve pain and garlic was used to try to treat heart and circulatory disorders (Halberstein, 2005; Kaur et al., 2019; Petrovska, 2012). Herbal preparations were administered enterally, applied topically and administered by fumigation and vapor inhalation (Halberstein, 2005). It would also appear that the ancient Egyptians were the first to develop the concept of posology that is, to describe the use of specific dosages to be used for each individual drug (Attorre & Bruno, 2022).

Bible

In the Bible, specifically in the Old Testament, written in 1200 BC, and in the Jewish sacred book Talmud numerous medicinal plants are described (Hassan, 2015; Petrovska, 2012; Šantić et al., 2017). An example of the use of plants for healing purposes cited in the Bible is “poultice” (poultice, also called cataplasm, is a soft and moist mass, often heated and medicated, which is spread on a cloth on the skin to treat a sore, inflamed or painful part of the body (Yadav et al., 2021); these types of preparations, improved, are still used today to accelerate the healing of wounds especially of secondary intention (Nagappa & Cheriyan, 2001; Samiee-Rad et al., 2022) prepared with figs that Isaiah applied to Hezekiah to heal a wound (Attorre & Bruno, 2022).

Ancient Greek

Among the populations of the Mediterranean basin, knowledge of phytomedicine deepened further, from 3000 to 1500 years ago, in ancient Greece (Halberstein, 2005). Homer around 800 BC in the poems Iliad and Odyssey, described 63 plant species of Minoan, Mycenaean, Assyrian and Egyptian pharmacotherapy. Some of these plants were given the names of mythological characters from the Homeric poems. For example, the Elecampane Inula helenium (Inula helenium of the family Asteraceae) is named in honor of Helen, who was at the center of the Trojan War (Petrovska, 2012). Hippocrates, lived from 460 to 337 BC. (is considered the father of modern medicine because he was the first to have hypothesized that diseases did not derive from supernatural influences, but from disturbances of the normal physiology of the human body and the first to have based clinical reasoning on observation and clinical signs) used many herbal remedies in his practice (Kaur et al., 2019; Yapijakis, 2009). He highlights almost 400 samples of medicinal substances of plant origin based on their action on the organism (Hassan, 2015; Khan, 2014): for example he used wormwood and centaury against fever; garlic against intestinal parasites; opium, henbane, nightshade and mandrake as narcotics; fragrant hellebore and alfalfa as emetics; spring onion, celery, parsley, asparagus and garlic as diuretics and finally oak and pomegranate as astringents (Petrovska, 2012). Theophrastus lived between 371 B.C. and 287 BC. and is considered the father of botanical science for his great merits in the classification and description of plants and medicinal plants (Petrovska, 2012). He wrote the first systematic treatise on pharmaceutical botany, De historia plantarum (Solinas, 2009) and the book De Causis Plantarum (Petrovska, 2012). In these works, he drew up a classification of over 500 medicinal plants. He seems to have been the first to describe that humans can become accustomed to the toxic action of plants through gradually increasing doses (i.e. drug tolerance) (Petrovska, 2012).

America

The healers of the Aztec and Mayan cultures of Mexico and Central America developed a vast and effective pharmacopoeia with medicines made from animals, minerals and especially plants. They knew the use of at least 132 medicinal herbs to treat specific ailments ranging from nosebleeds to gout and epilepsy. Remedies obtained from a combination of different herbal products were used against respiratory and gastrointestinal infections and some preparations were prescribed to prevent some diseases (Halberstein, 2005) .

Romans

Celsus (25 BC – 50 AD), described around 250 medicinal plants in his book "De re medica" (Hassan, 2015). Greek military physician, Dioscorides, wrote De Materia Medica and is considered the most important writer of phytomedicine of antiquity. Is indeed regarded as the "father of **pharmacognosy**" (the science that deals with the natural drugs obtained from organisms such as most plants, microbes, and animals) (Khan, 2014; Orhan, 2014; Petrovska, 2012; Šantić et al., 2017; Upton, 2022). He lived between 60 and 78 AD, was a military physician in the army of the Roman Empire under Nero and studied medicinal plants wherever he traveled with the Roman army (Petrovska, 2012). He is known to have written about over 600 healing plants (Halberstein, 2005). Dioscorides' treatise De Materia Medica has been used as a reference for phytotherapy in Europe for more than a millennium and translated into several languages (Hassan, 2015). Dioscorides described 944 medicines of which 657 were of plant origin. In addition to the external appearance, he described the collection method, where to find them, also the name in other languages, how to prepare the medicine and its therapeutic effect. Among the plants described in his work we find mainly plants with a mild effect, but there is no shortage of references to plants with a greater pharmacological effect such as those containing alkaloids (scented hellebore, false hellebore, poppy, buttercup, jimsonweed, henbane, nightshade). He described, for example, different uses of chamomile, which was used as an antiphlogistic to treat wounds, stings, burns and ulcers, and to clean and rinse eyes, ears, nose and mouth. He was also responsible for the false belief, also inherited from the Arabs, that chamomile had abortifacient properties. In Dioscorides we also find the description of the use of willow (from which acetylsalicylic acid derives) as an antipyretic (Petrovska, 2012). A contemporary of Dioscorides was Pliny the Elder (23 AD-79), who traveled through Germany and Spain and in his book "Historia naturalis" described about 1000 medicinal plants. The writings of Dioscorides and Pliny the Elder contained all the knowledge of the time on phytomedicine (LacusCurtius, retrieved 2024). Galen (131 AD-200 AD) wrote and compiled "De succedanus", the first list of interchangeable drugs with similar or identical action (even if this list is not valid from today's pharmacological point of view) (Elufioye & Badal, 2017). Furthermore, Galen introduced into therapy some plants that his predecessors did not know such as Uvae ursi folium as uroantiseptic, still used today with this same indication and as a mild diuretic (Petrovska, 2012).

The Arabs

After the fall of the Roman Empire, Arab scholars translated the books of Greek and Roman authors and made great advances in science and medicine. They were the first to divide the work of the physician (diagnosis and treatment) from that of the pharmacist (drug extraction and formulation), further accelerating the development of these two fields (Khan, 2014). For example Jaber Bin Hayan extracted and isolated various chemical substances such as alcohols, nitric acids, sulfuric acids (Azaizeh et al., 2006). A famous Muslim scientist, Ali Ibn Rabban Al Tabri (782-855 AD) wrote a work, the Firdous Al Hikmat, which deals with various topics, one of which focuses on drugs and poisons (Khan, 2014). Abu Ali Al Hussan Ibn Sina – also known as Avicenna, 980-932 AD is considered the creator of the Greco-Arabic school of medicine. In his work Canon of Medicine, he condensed and summarized the experience of many centuries of Greek, Indian and Central Asian medicine and the medicine of the Middle Ages, as well as pharmacology, pharmacy and pharmacotherapy (Buranova, 2015). He described many medicinal plants that are firmly rooted in the practice of traditional medicine in many countries and, some of them, even in modern medicine (Buranova, 2015). Abu Musa Jabir ben Hayyan wrote a comprehensive book on different plant poisons and antidotes: The Book on Poisons and Antidotes. This highlights how the Arabs, in addition to the therapeutic and curative characteristics, also described the toxic aspects of various plants (Khan, 2014). The Arabs, having numerous relations with India, introduced many new plants from that country into pharmacotherapy (Khan, 2014). The Arabs used aloe, nightshade, henbane, coffee, ginger, strichno, saffron, turmeric, pepper, cinnamon, rheuum, senna and so on. They were among the first to replace some strong-acting drugs, such as the purgatives Heleborus odorus and Euphorbium, mainly used until then, with milder-acting drugs, such as the mild laxative Sennae folium (Petrovska, 2012).

Middle Ages and Renaissance

In the Middle Ages, monasteries became the central place in Europe where therapeutic skills, the cultivation of medicinal plants and the preparation of medicines were concentrated (Attorre & Bruno, 2022; Petrovska, 2012; Šantić et al., 2017). Thus the so-called monastic medicine developed (Attorre & Bruno, 2022; Petrovska, 2012; Šantić et al., 2017). The phytotherapeutic treatments were mainly based on 16 medicinal plants (sage, anise, mint, Greek seeds, savory, tansy, etc.) which the medical monks commonly cultivated in the monasteries (Šantić et al., 2017). Charlemagne (742 AD-814) considered the founder of the renowned medical school of Salerno, in his "Capitularia" ordered which medicinal plants should be grown on state land. Charlemagne especially appreciated sage whose name in Latin means "to save, to cure". Even today, sage is an obligatory plant in Catholic monasteries (Petrovska, 2012). Among the Slavic populations in the 7th century AD, Rosmarinus officinalis, Ocimum basilicum, Iris germanica and Mentha viridis were used in cosmetics, Alium sativum as a remedy, Aconitum napellus as a poison in hunting and Veratrum album, Cucumis sativus, Urtica dioica, Achilea millefolium, Artemisia maritime, Lavandula officinalis, Sambuci against lice, fleas, moths, mosquitoes and spiders (Petrovska, 2012). As previously mentioned, throughout the Middle Ages European physicians consulted Arab works in which over 1000 medicinal plants were described, namely "De Re Medica" by John Mesue (850 AD), "Canon Medicinae" by Avicenna (980-1037) , and the "Liber Magnae Collectionis Simplicicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197-1248) (Petrovska, 2012). The travels of Marco Polo (1254-1324), the discovery of America (1492), and the travels of Vasco De Gama in India (1498), led to the import of many medicinal plants into Europe (Šantić et al., 2017). Botanical gardens sprang up throughout Europe and attempts were made to cultivate domestic and imported medicinal plants from the old and new worlds. With the discovery of America, a large number of new medicinal plants enriched the materia medica: China, Ipecacuanha, Cacao, Ratanhia, Lobelia, Jalapa, Podophylum, Senega, Vanilla, Mate, tobacco, chili pepper, etc (Petrovska, 2012). Paracelsus (1493-1541) was a European physician and alchemist and great supporter of medicines chemically prepared from raw plants and mineral substances (Michaleas et al., 2021). However, he was firmly convinced that the collection of those substances had to be determined astrologically, highlighting how the sciences of the time had yet to emancipate themselves from magical beliefs. He supported the "Signatura doctrinae": that is, following this belief he asserted that god had directly designated through his "signature" the use of healing substances for certain diseases. According to this theory, the appearance, or precisely the "sign", with which each natural element of animal, vegetable or mineral origin presents itself, reveals by analogy its therapeutic function of the parts of the human body most similar to it (Petrovska, 2012).

Recent Modern History

In the 17th century, the use of the bark of the tree Cinchona succirubra under the name of countess powder, following the legend that the Countess of Chinchon was the first to use it, was introduced into European medicine to treat malaria. It immediately became one of the best herbal remedies ever (Achan et al., 2011). Quinine as an active pharmaceutical ingredient of the cinchona tree, however, was only isolated in 1820 (Hassan, 2015). In the 18th century, an important breakthrough was achieved with the taxonomic work of the revolutionary Swedish physician and naturalist Carolus Linnaeus, who classified botanical species in a standardized way. With his works Systema Naturae Genera Botanica, Critica Botanica and Philosophica Botanica he founded modern biological taxonomy, introducing the binomial denomination, and the identification of plants and their characteristics, also proceeding with the cataloging of all the species known at the time, i.e. over 5900 species of plants (Britannica, 2024; Reid, 2009).

His writings are so important that they are still consulted by botanists, herbalists, horticulturists and taxonomists today (Halberstein, 2005). An important discovery of the 18th century in the field of phytotherapy was the application of digitalis by the English doctor William Withering who used the plant (Digitalis purpurea) to treat edema associated with heart failure. Digitalis-derived digoxin is still in use today to treat chronic heart failure, particularly in association with atrial fibrillation.

The 19th century was a turning point because several phytochemicals were isolated as "pure" drugs extracted from plants (e.g. quinine, morphine and ephedrine) (Hassan, 2015) that marked the beginning of scientific pharmacological research (Petrovska, 2012). Subsequently, other pharmacological substances such as glycosides, tannins, saponosides, essential oils, vitamins and hormones were discovered and isolated. Towards the end of the nineteenth century and the beginning of the twentieth century, alkaloids and glycosides isolated in pure form increasingly replaced the medicinal plants from which they had been isolated. This occurred mainly because the isolated phytochemicals produced a greater effect than their phytotherapeutic counterparts (Petrovska, 2012).

Phytochemicals (and active ingredients derived from phytochemicals) successful in modern medicine

In humanity's long struggle against diseases, the human being has always sought remedies using what he had available, therefore also using plants. For this reason, throughout history, phytotherapy have been fundamental in helping humans in the fight against diseases. With the advent of modern pharmacology however, phytotherapy has lost part of the central role it had held in the past. Despite this, even in the 20th century plants contributed decisively to the development of new revolutionary drugs and are still important, if not fundamental, for the development of new drugs.

Between 1950s and 1990s, just to name a few examples, the following active ingredients derived from plants entered the market (Dar et al., 2017):

The vinca alkaloids, **Vincristine and Vinblastine**, both derived from Catharanthus roseus of the Apocynaceae family, also known as Madagascar Periwinkle. Vincristine is the drug of choice for the treatment of acute lymphoblastic leukemia and in a combination regimen it is used to Treat Hodgkin's Disease, Wilm's Tumor, Neuroblastoma, and Rhabomyosarcoma (Agrawal, 2007), while vinblastine has indications for the treatment of testicular carcinoma, Squamous cell carcinoma of head and neck, Hodgkin's lymphoma, Kaposi's sarcoma, histiocytic lymphoma, Mycosis fungoides and histiocytosis X (Agrawal, 2007).

Reserpina from Rauvolfia serpentina of the Apocynaceae family, is one of the first agents developed to treat hypertension in clinical practice (Cheung & Parmar M., 2023) and is still used as an antihypertensive in clinical practice. Introduced in the 1950s, not currently available in many countries, but still considered able to reduce blood pressure to the same level as frontline antihypertensives (James et al., 2014; Lemieux et al., 1956; Weir, 2020).

Artemisinin from the plant Artemisia Annua (Chinese name – Qinghao) of the Asteraceae family, is an antimalarial effective against all Plasmodium species and is particularly useful in cases of infections with chloroquine-resistant or multi-resistant parasites (WHO, 2015).

Teniposide and Etoposide both from the plants Podophyllum hexandrum and Podophyllum peltatum of Berberidaceae family. Teniposide is an antineoplastic agent used primarily for the treatment of childhood acute lymphoblastic leukemia, while Etoposide an antineoplastic agent used for the treatment of several types of cancer including testicular cancer, lung cancer, lymphoma, leukemia, neuroblastoma, and ovarian cancer, and hemophagocytic lymphohistiocytosis (Reyhanoglu & Tadi, 2024; Yoneda & Cross, 2010).

Irinotecan and **Toptecan** derivates of camptothecin, an alkaloid extracted from the Chinese plant Camptotheca acuminata belonging to the Nyssaceae family. Irinotecan is antineoplastic agent used for the treatment of metastatic carcinoma of the colon or rectum and pancreatic adenocarcinoma (Robert & Rivory, 1998) while Toptecan is antineoplastic agent used for the treatment of ovarian cancer, small cell lung cancer, or cervical cancer.

Paclitaxel, from Taxus brevifolia of the family Taxaceae, an antineoplastic agents that as indication for several cancers, including breast, ovarian, bladder, lung, prostate, melanoma, esophageal, Kaposi sarcoma, and various other solid tumors (Awosika, Farrar et al., 2024).

Today in the Western world, drugs are designed by chemists and biologists using computer models. The pharmaceutical industry mainly relies on screening libraries of inorganic compounds for the discovery of new drugs, as complexities exist in libraries based on natural products. However, this approach has led to a decrease in the entry of new drugs into the market. Therefore, interdisciplinary approaches based also on natural products have become a necessity (Atanasov et al., 2015).

The wealth of possible therapeutic substances for humans present in plants is remarkable. It is estimated that approximately 70,000 plant species have the potential to treat various human diseases (Kuruppu et al., 2019). As demonstrated by history, plants and fungi are an abundant source of potential new drugs and often act as chemical models for the design of new drugs for the treatment of humanity's most serious ailments. We provide some instructive examples below:

Spindle Poisons Agents – Cancer is the second leading cause of death globally (WHO, 2022). Despite this, improvements in treatment have meant that cancer is now considered a chronic disease. In fact, cancer survivors represent one of the fastest growing subgroups of people entering health care systems. In this context, it should be emphasized that 60% of currently used anticancer agents come from natural sources, including plants (Efferth et al., 2007; Martino et al., 2018).Vinca alkaloids, for example, are the first discovered components, in the 1950s, of a new class of drugs called spindle poisons, which work by attacking the microtubule spindle that separates chromosomes during cell mitosis (Duffin, 2000; Hüsemann et al., 2020). They directly kill tumor cells and make them more susceptible to other chemotherapy treatments, which is why they are used in combination therapy. The class of spindle poisons also includes podophyllotoxins, derived from Podophyllum hexandrum and Podophyllum peltatum, and Paclitaxel, obtained from the Taxus brevifolia (Duffin, 2000; Hüsemann et al., 2020).

Vinblastine and vincristine – Vinca alkaloids (Vincristine and Vinblastine) were originally isolated in the 1950s by Canadian scientists Robert Noble and Charles Thomas Beer from the Madagascar periwinkle plant, Catharanthus roseus of the Apocynaceae family (Arora et al., 2024; Martino et al., 2018). Periwinkle is a small perennial with attractive white or pink flowers, is a popular ornamental plant in gardens and homes around the world and it is native to the island of Madagascar. In India, the Philippines and South Africa, the infusions obtained from the leaves of the plant have been used by the traditional healers to treat various diseases, especially diabetes. Vincristine currently has indications as chemotherapy for various types of cancer: leukemia, lymphoma, neuroblastoma and Wilms tumor. It is also used off-label to treat adult central nervous system (CNS) tumors, Ewing sarcoma, gestational trophoblastic tumors, multiple myeloma, ovarian cancer, primary central nervous system lymphoma, small cells and advanced thymoma (Awosika, Below, et al., 2024). Vinblastine is indicated to treat leukemia, Hodgkin's and non-Hodgkin's lymphoma, breast cancer, and testicular carcinoma (Arora et al., 2024). Vincristine and vinblastine belong to a class of drugs called spindle poisons that work by preventing cancer cells from dividing properly. These drugs act by interfering with the polymerization of microtubules, interrupting mitosis, and inhibiting cell division (Duffin, 2000; Hüsemann et al., 2020). They particularly act on rapidly dividing cells, such as cancer cells, which are highly dependent on the proper functioning of microtubules during cell division (Awosika, Below, et al., 2024). Normal cells, that have a lower division frequency, are relatively less affected by the action of these drugs, contributing to the reduction of some adverse effects. Despite being discovered in the 1950s, Vinca alkaloids still have a prominent place in combination chemotherapy. They are in fact among the first and most effective long-acting anti-tumor agents (Arora et al., 2024; Duffin, 2000; Hüsemann et al., 2020). The history of the discovery of Vinca alkaloids is closely linked to the history of the search for drugs to treat diabetes. In fact, the older brother of Robert Noble, one of the two scientists who described the anticancer activity of Vinca alkaloids, worked in the research group of J.J.R. Macleod, one of the discoverers of insulin, at the University of Toronto (Duffin, 2000). It was from Macleod that Robert Noble was introduced to endocrinological research, although he would have preferred to do cancer research. However, it was by studying the 'activity of a plant that in traditional medicines (Ayurveda and TCM) had been described as acting as an antidiabetic and antimalarial agent, the Madagascar periwinkle, that Noble discovered that this plant had potent anticancer activities (Martino et al., 2018). Researchers around the world at that time were in fact looking for oral hypoglycemic agents that would eliminate the needle injections required by insulin (Duffin, 2000). Noble's research group in 1949, in the Collip laboratory dedicated to research in endocrinology, was studying the hormonal properties of various plant extracts used in traditional medicines as antidiabetics. It was at that time that CD. Johnston sent him tea material from Jamaica, made from a West Indian shrub (Catharanthus roseus) that was supposed to help control diabetes (Noble et al., 1958). So he and his team began to study its activity as an antidiabetic in laboratory mice (Duffin, 2000). Although periwinkle extract was not very effective at reducing glycaemia in mice, the researchers noticed that the mice died of septicemia. They later realized that the extract was able to rapidly reduce the number of white blood cells, induce granulocytopenia and profoundly depressed bone marrow (Noble et al., 1958). Researchers who evaluated Madagascar periwinkle extracts hypothesized that they contained phytochemicals useful against certain types of cancer, such as leukemia, which actually involves the proliferation of cancerous white blood cells called blasts (Duffin, 2000). Robert Noble's team then began working with Ely Lilly researchers to delve deeper into the plant's anti-tumor capabilities and a phytochemical study led to the separation and identification of vinblastine – the prototypical Vinca alkaloid – which had been shown to cause myelosuppression in xenografts mouse models of leukemia (Martino et al., 2018). In the 1960s, this remarkable discovery paved the way for a new therapeutic approach against cancer. In those years, vinca alkaloids were approved with indications for various types of cancer including leukemia (Lucas et al., 2010). Nearly 70 years later, vinca derivatives maintain an essential place in combination chemotherapy, making them among the first and longest-lasting effective anticancer agents. Five Vinca derivatives are in clinical use today: the natural vinblastine and vincristine, the semisynthetic derivatives vindesine, vinorelbine and vinfluine, a bis-fluorinated derivative for the second-line treatment of metastatic and advanced urothelial cancer (Martino et al., 2018). The chemical structures of vinca alkaloids are quite complex: they are part of the monoterpene indole alkaloid (MIA) family, a diverse family of complex plant secondary metabolites with many medicinal properties (Zhang et al., 2022). Until 2022 the global vinblastine supply chain was based on the low-throughput extraction and purification of vindoline and cataranthin precursors from the plant Catharanthus roseus (Ishikawa et al., 2009; Jeong & Lim, 2018). Therefore until 2022 access to these chemicals was only possible through extraction from the Madagascar periwinkle and approximately 500 kg of dried leaves were required to produce 1 g of vinblastine. Only in 2022 a group of researchers led by Jay Keasling managed to engineer a yeast for the production of MIA and vinca derivatives (the same group engineered Escherichia coli for the production of artemisinin which, like vinblastine, until 2003 was mainly derived directly from the plant), resulting in a notable step forward for the supply of these drugs (Martin et al., 2003; Zhang et al., 2022; Zhao et al., 2022).

Etoposide – Belongs to the class of topoisomerase II inhibitor drugs. This class of antineoplastic agents acts by blocking the ligation phase of the cell cycle, which generates single- and double-strand DNA breaks, leading to cell death by apoptosis (Swedan et al., 2023). Is a medication used in the management and treatment of various cancers such as Hodgkin's and non-Hodgkin's lymphoma, brain cancer, testicular cancer, prostate cancer, bladder cancer, stomach and lung cancer (Reyhanoglu & Tadi, 2024). Its pharmacodynamics outlines the action and contraindications of etoposide as a valuable agent in the management of the various cancers listed above and other cancer forms (Reyhanoglu & Tadi, 2024). Etoposide is derived from the podophyllotoxins of the plants Podophyllum peltatum (also known as American mandrake), Podophyllum Emodi of the Berberidaceae family, and other plants (Imbert, 1998). It is known that podophyllotoxins have been used as a treatment for more than a thousand years (Slevin, 1991). The mandrake, despite being a poisonous plant and having a foul odor, has a centuries-old history of use by Native Americans. The natives used an aqueous extract of the roots of the mandrake as an emetic, anthelmintic or laxative (Moerman, 1991). Furthermore, the plant was used against snake bites and as a means of suicide, but some Native tribes also used it to treat cancer (Imbert, 1998). The inhabitants of the Himalayas used the extracts of the Podophyllum Emodi, for uses similar to those of the Native American (Kelly & Hartwell, 1954). In England, during the early Middle Ages, the roots of wild chervil, which contain deoxypodophyllotoxin, were used to treat cancer (Cockayne et al., 1864). The use of the plant as an emetic, laxative and cholagogue (not as an anticancer) was later borrowed from the American pioneers (Imbert, 1998). In this way the use of the plant as an emetic laxative and cholagogue entered the American pharmacopoeia in 1820 and the English one in 1864. By 1850 the plant extract, podophyllin, was commercially available, supplanting the use of the plant itself. The structure of podophyllotoxin was first established in the 1930s (Imbert, 1998). In 1944 it was described as capable of curing venereal warts (Culp & Kaplan, 1944), a few years later its antimitotic abilities were described (Kern & Fanger, 1950), and it was subsequently demonstrated that it was capable of killing experimental cancers in animals (Imbert, 1998). In the 1970s, starting from podophyllin, Sandoz Pharmaceuticals synthesized a derivative, etoposide (Slevin, 1991). The drug is now included in the World Health Organization's list of essential medicines (a list that contains the drugs considered most effective and safe to meet the most important needs for the treatment of diseases (WHO, 2023a)) with worldwide sales expected to exceed $1 billion by 2029.

Artemisinin – It is currently the drug of first choice in combined therapy in the treatment of malaria, the most widespread parasitic disease in the world today, which causes 500 million cases and between 1.5 and 2.7 million deaths every year (Buck & Finnigan, 2024). It is effective against all Plasmodium species and is particularly useful in cases of infections with chloroquine-resistant or multi-resistant parasites (WHO, 2015). The drug is also effective in the treatment of toxoplasmosis, leishmaniasis and infections due to some species of Babesia. Artemisinin endoperoxide bridge is thought to be essential for antimalarial activity, as it causes free radical damage to the parasite's membrane systems, including: inhibition of the parasite's sarcoplasmicendoplasmic reticulum calcium ATPase, interference with mitochondrial electron transport, with parasite transport proteins, and disruption of parasite mitochondrial function (Meshnick, 1998). It also appears to have potential as an anticancer agent. Artemisinin was discovered in China by studying Artemisia Annua (Chinese name – Qinghao) of the Asteraceae family (Tu, 2011). In the book "52 prescriptions" found in a tomb of the Han Mawangdui dynasty dating back to 2000 years ago, the first evidence of medicines used to treat intermittent fevers (some of which were probably malarial) was found (Nosten et al., 2022). The text mentions the use of the medicinal herb Qinghao, a remedy later found in the manual of prescriptions for emergencies by Ge Hong in 341 AD (Nosten et al., 2022). Between the 1950s and 1980s, Chinese scientist Tu Youyou worked on Chinese herbal medicine at the Chinese Academy of Chinese Medical Sciences (formerly known as the Academy of TCM) (Tu, 2011). Malaria, caused by Plasmodium falciparum, has been a deadly disease for thousands of years. After the failure of international attempts to eradicate the disease in the 1950s, it began to spread again across much of the world due to the appearance of parasites resistant to existing antimalarial drugs, such as chloroquine (Tu, 2011). This has led to an urgent need for new antimalarial drugs. In 1967 Tu became head of Project 523, a Chinese national research project launched to find a cure for malaria. The scientist's institute consisted of both phytochemical and pharmacological researchers. The group began working on Chinese herbal materials by extracting and isolating components with possible antimalarial activities. In their work they studied more than 2,000 Chinese herbal preparations, among which they identified 640 that had possible antimalarial activities. The extracts were evaluated in a mouse model of malaria (Tu, 2011). The scientist's research group ultimately tested more than 240,000 compounds for use as antimalarials (Nature portfolio, 2024). The turning point occurred when the team found an extract of Artemisia Annua that showed a promising degree of inhibition against parasite growth. Their discovery, however, was not immediately reproducible in subsequent experiments and was in contradiction with what was reported in the literature. So Tu and colleagues carried out an in-depth review of the literature on qinghao, also researching ancient texts of TCM. And they found the solution to the problem in the text of Ge Hong's Emergency Prescription Manual (Fig. 1), in which the use of the plant's medicinal preparation was described as carried out cold. In fact they found that the heating involved in the conventional extraction stage of the preparation used to obtain the drug resulted in the destruction of its active components. (Nature portfolio, 2024; Tu, 2011). Then they used a low-temperature extraction method. On October 4, 1971, they obtained an extract 100% effective against parasitemia in mice infected with Plasmodium berghei and in monkeys infected with Plasmodium cynomolgi. According to the World Health Organization, since 2000 more than three million lives have been saved by using antimalarials containing artemisinin. Tu received the Nobel Prize for his work in 2015 (Nature portfolio, 2024; Tu, 2011).

Finally, some interesting facts about the discovery of artemisinin:

The effectiveness of artemisinin against malaria was discovered in the 1970s in China. The results of its effectiveness were presented to the WHO in the 1980s (Miller & Su, 2011). However, given Western countries' distrust of China and lack of cooperation from the Chinese government, artemisinin did not prove effective against malaria outside China until 2006, when it became the first-line drug in combination with other antimalarials (Maude et al., 2010). The discovery of artemisinin by Tu Youyou remained unknown both in China and the rest of the world until 2011, when Miller LH and Su X traced Tu Youyou as the originator of the discovery (Miller & Su, 2011). The discovery remained secret because it occurred during the years of the Cultural Revolution in China, as an initiative of the Chinese government to help the North Vietnamese in their war against the United States (Miller & Su, 2011). At the time, malaria caused by chloroquine-resistant Plasmodium falciparum was a great problem, and both the United States and China were searching for drugs that could defeat the disease. Thus the United States discovered mefloquine, a single-dose curative compound against chloroquine-resistant parasites (Trenholme et al., 1975). The North Vietnamese turned to the Chinese government for help. Therefore, in 1967 the secret national program called Project 523 was initiated in China (Miller & Su, 2011). This led, in the short term, to the discovery of three drugs against malaria in 1969 and, in the long term, to the discovery of artemisinin.

It should be mentioned that Linnaeus was aware of the use of quinine but also, less known and rather surprising, of artemisia annua in the treatment of malaria (Miller et al., 2023).

Cephalosporins – Are a class of β-lactam antibiotics derived from the fungus Cephalosporium acremonium. Discovered by Giuseppe Brotzu, an Italian physician and hygienist working in Cagliari around 1945. His studies and clinical interests were in the treatment of malaria and typhoid infections. By studying the presence of Salmonella Typhi in the sewers of the city of Cagliari and mapping the most contaminated areas, he observed that Cagliari

Figure 1. Ming dynasty version (1574 CE) of the handbook Emergency Prescription Manual by Ge Hong (Tu, 2011).

residents who bathed in the city sea, where sewage was discharged, didn't get typhoid fever (Bo, 2000). In order to understand this peculiarity, not assuming that sewage would be diluted once in the sea, he tried to understand where seawater's self-purifying capacity came from. In fact, Sardinians who bathed in the sea did not develop typhoid fever or skin disease except very rarely. By culturing a water sample taken from that sea area in July 1945, he first isolated Cephalosporium Acremonium (Brotzu, 1948). During his studies, he realized that the mycete produced a substance that could inhibit the growth of various pathogens in vitro. Brotzu named the substance mycetin (a beta-lactam inhibitor of bacterial wall synthesis like penicillins) and immediately began testing its validity on patients suffering from various types of staphylococcal infections, brucellosis, etc., achieving remarkable improvements and sometimes cures. He had discovered cephalosporins. The importance of this discovery is best understood in light of history: Fleming had discovered penicillin in 1928, but large-scale production of the drug had not begun until 1945. Despite this, in 1945 the drug was still not available worldwide as World War II had just ended. Until 1945, moreover, infectious diseases, particularly pneumonia, tuberculosis, and gastroenteritis, were the leading causes of death in all age groups worldwide (Adedeji, 2016; Office for National Statistics, 2017; Sakai & Morimoto, 2022). Before 1945, in fact, there were no cures for infectious diseases. Brotzu had therefore made a revolutionary discovery and was aware of it. To better purify and identify the substance with antibiotic properties he discovered, he applied for funding from Italian institutions, but unfortunately the funding was denied. (Bo, 2000). At that time, a British medical officer, serving in the occupation forces in Sardinia, had asked to visit the Institute of Hygiene in Cagliari. Through him, Professor Brotzu sent a sample of the fungus to Sir Howard Florey of Oxford University, head of the Anglo-American Antibiotic Research Group. Thanks to Brotzu's work, after 18 years of research in 1962 Edward Abraham's research group isolated the Chealosporin C. This was the progenitor molecule of a new generation of antibiotics, namely cephalosporins. Cephalosporins are antibacterial agents that are still widely used, mainly because of their broad spectrum of activity and low toxicity, and are indicated against both Gram-positive and Gram-negative bacterial infections. They are classified into 5 generations, and some of the newer derivatives of this class are being evaluated in clinical trials. In addition, they are currently among the few antibiotics still effective in the treatment of MRSA (methicillin-resistant Staphylococcus aureus) (Bavaro et al., 2024; Bui et al., 2024). This class of antibiotics is still critically important in the treatment of infections, as antimicrobial resistance (AMR) poses a serious threat to global public health, even in developed countries. In fact, it is estimated that AMR is responsible for 1.27 million deaths worldwide and contributes to 4.95 million deaths.

Morphine – The opium poppy "Papaver somniferum" has played an important role in human history. It appears to have been cultivated as early as the Neolithic period, in 6000 BC. (Brook et al., 2017; Hassan, 2015; Šantić et al., 2017). In cuneiform tablets it was described as the "plant of happiness". In 1500 BC. opium is mentioned in the Ebers Papyrus along with other medicinal plants. In 1100-800 B.C. Arab physicians created many opium-containing drugs to treat mental problems, epilepsy, flu, and eye fatigue. The poppy entered Greek mythology and took the name of morphine in honor of Morpheus, the god of dreams. In 800 BC Homer describes Helen and Presephone in the Odyssey as using a wine drugged with opium to relieve pain and sadness. In 500 BC Hippocrates knew its excellent narcotic and analgesic action and used it against coughs and for some gynecological condition. During Hellenism the poppy had spread to India, Persia, China and other countries (Brook et al., 2017). In 300 BC. the Chinese surgeon Hua T'o used an anesthetic based on wine and opium. In the 1st century AD, at the time of Emperor Nero, the physician Andromachus used opium in a medical preparation which he called Theriaca andromaci and Dioscorides in De Materia Medica was the first to provide a written description of how to obtain opium from the poppy. In the Middle Ages, between the 5th and 14th centuries AD, opium was produced by apothecaries. In the 14th-17th centuries AD. Paracelsus produced the opium tincture called Laudanum. In the 17th century Thomas Sydenham created what became a staple in European medicine, his alcohol-based opium tincture called laudanum, mentioned in Shakespeare's Othello. Between the 18th and 19th centuries, the use of laudanum for medical or recreational purposes was common. Benjamin Franklin, Napoleon, and Egar Allan Poe, among other notable figures of the time, were opium users. From the beginning of the 19th century opium was widely used. Opium is obtained from the juice collected from the shells of poppy heads. From 1805 to 1950, a series of discoveries followed that led to further advances.. In 1805 Serturner isolated morphine, in 1819 Meissner classified morphine as the first compound of the alkaloid class, in 1847 Lauren determined the chemical formula of morphine. Robinson managed to determine the structural formula of morphine and won the Nobel Prize for Chemistry in 1947, while in 1952 Gates and Tschudi synthesized morphine for the first time in the laboratory (Brook et al., 2017). To date, although it is possible to artificially synthesize morphine, it is still manly obtained from the plant using techniques that have remained substantially unchanged in eight millennia. Morphine is an iconic example of a phytochemical that has stood the test of time and remains one of the most used medications for the control of severe pain. Even today, despite advances in chemical production capabilities, humans cannot compete with extraction from plants, which is much cheaper and more efficient than synthetic production. Regarding the pharmacodynamics of morphine, it determines most of its analgesic effects by binding to the mu-opioid receptor in the central nervous system (CNS) and peripheral nervous system (PNS). (Murphy et al., 2023). Morphine is currently included in the WHO list of essential drugs (WHO, 2023a) and is indicated in the treatment of moderate to severe pain resistant to other painkillers, particularly in cancer-associated pain, myocardial infarction, and postoperative pain. It is also indicated in acute pulmonary edema, general and local anesthesia, and epidural analgesia during childbirth (Murphy et al., 2023; Šantić et al., 2017).

Acetylsalicylic acid – At some point in life, most people have taken a pain or fever control medication derived from willow bark, namely acetyl salicylic acid derived from salicyline. Salyciline is a glycoside contained in the willow bark of Salix alba and Salix fragilis, plants belonging to the Salicaceae family (Fig. 2). To date, acetylsalicylic acid is the most used drug in the world and among the oldest remedies still in use (Mann, 2000). Acetylsalicylic acid inhibit the activity of the enzyme called cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever (Vane & Botting, 2003). This is another example of how nature and traditional knowledge have contributed to modern medicine. The first written evidence of the use of willow bark dates back to over 3,500 years ago, when it was used as an analgesic, anti-inflammatory and anti-rheumatic by the Sumerians and Egyptians (Montinari et al., 2019). The ancient Egyptians in the Ebers Papyrus describe the use of decoctions of myrtle and willow leaves, to be applied on the abdomen and back to relieve local inflammatory and painful conditions (Bryan, 1930).

Hippocrates, about 1000 years later, and subsequently Galen, were aware of the medicinal properties of Salicaceae plants. Willow bark was prescribed by Hippocrates to treat inflammatory pain and to relieve the pain of childbirth (Montinari et al., 2019). In De Materia Medica, Dioscorides describes willow decoctions for the treatment of gout, colic and earache. In Natural History, Pliny the Elder also reports the use of of preparations of these plants as antipyretic and analgesic remedies (Jeffreys, 2008). Populations from different parts of the world, who had no contact with each other, such as the indigenous populations of the Hottentots of South Africa or the American Indians used willow extracts to treat fever, arthrosis and headaches. The use of willow bark spread throughout the Western world from 216 AD, as military and commercial maritime contacts intensified. Thus, it then arrived in China and other eastern countries. Decoctions of plants containing salicylates were used to treat wounds, rheumatic pain, ulcers, headaches and dysmenorrhea during the Middle Ages, the Renaissance and up to the 18th century (Jeffreys, 2008). In the following centuries, starting from the 18th and 19th centuries, a series of notable advances occurred in the characterization of the active ingredient contained in willow bark. In 1758 Edward Stone of Chipping Norton, Oxfordshire, England, looking for a valid and economical remedy compared to expensive cinchona bark to cure "fever" (i.e. malaria), studied the healing properties of willow (Wood, 2015). He administered the aqueous extract of Salix alba bark to treat 50 febrile patients and discovered that the administration of these extracts had a marked antipyretic action. This study by Stone is now recognized as the first to demonstrate with scientific rigor the effectiveness of willow bark in the treatment of fever. The first extraction of the active component of willow

Figure 2. Illustration of Salix Fragilis, from the Museum's Botany Library Plate Collection.

bark appears to have been carried out in 1824 by two Italian pharmacists from Verona, Francesco Fontana (Fontana, 1824) and Bartolommeo Rigatelli (Rigatelli, 1824). Rigatelli called the drug "indigenous substitute for quinine sulphate" (Rigatelli, 1824) and "very bitter antipyretic saline solution" (Rigatelli, 1826) , while Fontana (Fontana, 1824) used the name salicin, the same term that a few years later would be adopted by others.

In 1828 the pharmacologist Joseph Buchner also isolated the active ingredient of willow "Salicin" while the following year Henri Leroux perfected the salicin extraction process allowing the extraction of a significant quantity of pure salicin (Montinari et al., 2019). Raffaele Pirìa, a 19th century Italian chemist, extracted salicylic acid from salicin and determined its molecular formula (Montinari et al., 2019). In 1852 the French chemist Charles Gerhardt synthesized acetylsalicylic acid, without realizing it, but in an impure and unstable form (Gerhardt, 1853). In 1855 Cesare Bertagnini, a student of Raffaele Piria, first described the ototoxicity that occurs following high ingestions of the drug (Montinari et al., 2019). In 1859 Hermann Kolbe synthesized salicylic acid in the laboratory, and in the following years his students began producing it on an industrial scale at a price 10 times lower than that of willow bark (Sneader, 2005). In 1876 Franz Stricker and Thomas MacLagan published on the efficacy of sodium salicylate and salicin, extending their indications not only as an antipyretic but also as an antirheumatic and analgesic (Maclagan T., 1876; Montinari et al., 2019). In 1877 the effectiveness of sodium salicylate was described by Germain See in chronic rheumatism and gout (Sée G., 1877). In 1877 and 1878 Noemisio Bosisio and Giovanni Brugnoli described the effectiveness of salicylic acid also in the treatment of rheumatic fever (Montinari et al., 2019). In those years the pharmaceutical company Bayer set to work to produce a salicylate derivative that did not cause the negative effects of sodium salicylate, namely nausea, gastric irritation and tinnitus. This task was accomplished by Felix Hoffman, a chemist who synthesized acetylsalicylic acid on August 10, 1897 (Zündorf, 1997). In 1899 Bayer registered the new compound as "Aspirin", a name derived from acetyl and Spiraea ulmaria, the tree from which salicylic acid was extracted (Montinari et al., 2019). In 1948 and 1953 respectively Paul Gibson proposed salicylic acid in the treatment of coronary thrombosis (Gibson, 1949) and Lawrence Craven used aspirin in primary cardiovascular prevention (Craven, 1953). In 1971 Vane discovered the mechanism of action of aspirin for which he later received the Nobel Prize. In 1974, the first randomized trial on the use of aspirin in the secondary prevention of coronary thrombosis and myocardial infarction was conducted (Montinari et al., 2019). Between 1975 and 1988 it was discovered that aspirin: reduces the synthesis of thromboxane A2, inhibits cyclooxygenase, is effective in the secondary prevention of stroke, can be used in the prophylaxis of unstable angina and that early treatment with aspirin is effective in reducing mortality from myocardial infarction (Montinari et al., 2019). In addition to recent developments in secondary and primary cardiovascular prevention, regular aspirin use has been found to have another important benefit: decreasing the risk of developing cancer (National Cancer Institute, 2014). Finally, low-dose acetylsalicylic acid is now prescribed to pregnant women at high risk of developing preeclampsia to reduce their risk of developing it and also to prevent preterm delivery and intrauterine growth restriction (ACOG, 2018).

Curare (d-Tubocurarine) – Curare is obtained from various plants, including Chondrodendron tomentosum of the Menispermaceae family and Strychnos toxifera of the Loganiaceae family (Burr & Leung, 2014). d-Tubocurarine acts as a non-depolarizing competitive antagonist at nicotinic acetylcholine receptors on the motor end plate of the neuromuscular junction, causing the relaxation of skeletal muscle (Burr & Leung, 2014). For centuries, Curare has been used by indigenous South American tribes to hunt (Raghavendra, 2002; Šantić et al., 2017). Tales of the mysterious "flying death" were brought to the Old World by Spanish conquerors and thus began the characterization of the active ingredient and its evolution into today's synthetic drugs in Europe. A chronicler of the Spanish court, Peter Martyr d'Anghera, in his book De Orbe Novo first described arrows poisoned with Curare in 1516 (Sykes, 1993). The description he made was composed of a mixture of reality and fantasy and contributed significantly to starting the search for Curare. In 1594, in the book Discovery of the Large, Rich and Beautiful Empire of Guyan, Sir Walter Raleigh mentions the use of arrows poisoned with Curare, a name given to the poison by one of his lieutenants, during his visit to Venezuela (Birmingham, 1999; Crul, 1982; Rowbotham, 1948). Further exploration of South America, until the 18th century, was prevented by wars between the English, Spanish and Portuguese. Until the physician Edward Bancroft spent five years in South America and brought samples of raw Curare back to Europe. Sir Benjamin Brodie, thanks to the use of these samples, demonstrated by injecting curare into small animals that they could be kept alive thanks to artificial respiration with bellows (Brodie, 1968). Charles Waterton, an explorer, obtained several samples of curare in South America and later tested them on large animals in 1814. In particular, he demonstrated to an audience, which included Sir Benjamin Brodie among others, the effects of curare on three donkeys, showing that if the animals injected with curare were ventilated, they survived. Furthermore, he demonstrated that if curare was injected into a limb and allowed to act only on that limb through the action of a tourniquet, the animal survived (Raghavendra, 2002). This was an early indication that Curare act on the neuromuscular junction. Claude Bernard in 1846 with experiments on frogs definitively demonstrated that the Curare acted on the neuromuscular junction (Raghavendra, 2002). He demonstrated that the drug injected into a limb blocked muscle contraction, but that this could still be induced by external nervous stimulation.In the 1800s, a series of discoveries laid the foundation for the subsequent use of neuromuscular blocking drugs in anesthesia, which actually began after World War II. One of these events is linked to the chemical activity of alkaloids (Bynum, 1970). At the end of the 19th century, the role of acetylcholine, underlying neuromuscular transmission, was established in Great Britain by Sir Henry Dale and colleagues (Dale, 1934). Harold King isolated d-tubocurarine from a sample of Curare (National Library of Medicine, 1968). Richard Gill, an American living in Ecuador, became ill with multiple sclerosis, and his neurologist in the United States, Walter

Freeman, suggested that he might benefit from curare therapy. From Ecuador Gill brought back Curare in crude form and samples of the plants from which Curare was obtained (Humble, 1982). These samples were analyzed and recognized as plants of two families: Menispermaceae, which includes the genus Chondrodendron; and Loganiaceae, which includes the genus Strychnos. Oscar Wintersteiner and James Dutcher in 1942 were the first to isolate the alkaloid d-tubocurarine from samples of Chondrodendron tomentosum (Wintersteiner & Dutcher, 1943). A H Holladay standardized the commercial preparation of curare by naming the drug Intocostrin. A neuropsychiatrist AE Bennett, who used convulsive electroshock in the therapy of his patients due to the high incidence of vertebral fractures, was on the verge of abandoning this type of intervention when he decided to try to also include in the technique the use of curare (Raghavendra, 2002). In 1940, Bennett presented a film on the use of the curare at the 91st annual session of the American Medical Association at which Lewis Wright was among the attendees. Wright hypothesized that Curare could be useful in anesthesia and donated Intocostrin to EA Rovenstine of New York University so that he could experiment with the drug during surgical practice. Rovenstine gave it to one of his residents, EM Papper. Pepper tested it on two patients undergoing ether anesthesia who developed apnea and were then manually ventilated throughout the night (Betcher, 1977). At the time, indeed, endotracheal intubation was not commonly used. In the 1940s, a Montreal hospital anesthesiologist and cyclopropane enthusiast, Harold Randall Griffith, was specialized in endotracheal intubation to overcome occasional episodes of apnea induced by cyclopropane. He started to use curare. Together with his resident Enid Johnson in 1942 they administered curarization to a young patient undergoing appendectomy (Gillies & Wynands, 1986). Griffith is considered to be the one who introduced curarization into anesthesia (Kyle & Shampo, 1992). World War II interrupted work on curare derived drugs. Until John Halton, an anesthetist from Liverpool, had Intocostrin brought from the United States by an American soldier friend (Raghavendra, 2002). The experiences of Halton and Cecil Gray with the use of the cure on patients were successful and were reported by the two in 1946 (Gray & Halton, 1946). This laid the foundations of what became known as the Liverpool technique: a triad of narcosis, analgesia and muscle relaxation. This technique is still used today for all surgical procedures by all anesthetists. The study of Curare as a neuromuscular blocking drug has revolutionized the practice of anesthesia, surgery and medicine in general, allowing physicians to achieve results never seen before in terms of treatment. Before the discovery of neuromuscular blockers, anesthesia was induced and maintained using intravenous or inhaled agents, tracheal intubation was rare, and muscle relaxation, if necessary, was provided by deep inhalation anesthesia with the attendant risks of respiratory or cardiac depression (Raghavendra, 2002). With the introduction of neuromuscular blockers, anesthesia has undergone a conceptual change, being redefined as a triad of narcosis, analgesia and muscle relaxation. To produce each of these effects, specific drugs are used, of which neuromuscular blockers are one of the three essential drug categories. To date, tubocurarine is no longer used in anesthesia but has been replaced by a series of neuromuscular blockers (depolarizing such as succinylcholine and non-depolarizing such as rocuronium, vecuronium, atracurium, cisatracurium, mivacurium) (Cook & Simons, 2024; Raghavendra, 2002). Most of these are of synthetic origin and were created taking inspiration from Curare. The synthesis of neuromuscular blocking molecules has become necessary because some of the existing drugs, including tubocurarine, present a series of side effects and pharmacokinetics that do not adapt perfectly to all clinical scenarios. Neuromuscular blocking drugs belong to two groups, depolarizing and non-depolarizing (Cook & Simons, 2024). Depolarizers act on the neuromuscular junction by imitating the effect of acetylcholine, first causing muscle contractions (fasciculations) and then paralysis. Suxamethonium, a drug that belongs to this class, has the advantage of acting within 60 seconds. It induces muscle relaxation lasting less than 5 minutes. It does not respond to anticholinesterases such as neostigmine, but plasma cholinesterase causes the effect to wear off quickly. Among the side effects it induces are life-threatening hyperkalemia, malignant hyperpyrexia, increased intraocular pressure. A large number of deaths attributable to hyperkalemic cardiac arrest in children with undiagnosed muscular dystrophies have occurred following the use of suxamethonium (Raghavendra, 2002). There is now a clinical need for a safer drug that works just as quickly. As for non-depolarizing drugs, they are characterized by a slower onset of action (2-3 minutes) and are therefore unsuitable for rapid control of the airways. Neostigmine reverses their action and works through competitive blockade of the neuromuscular junction and does not cause initial muscle fasciculation. Given the side effects described so far, the search for the "ideal neuromuscular blocking drug" continues today (Prabhakar et al., 2016; Shah et al., 2021) (i.e. with short, non-cumulative and non-depolarizing neuromuscular action, with rapid onset and recovery, reversible with an appropriate antagonist and free of clinical side effects).

Quinine and (its stereoisomer) Quinidine – Cinchona succirubra of the Rubiaceae family contains two important alkaloids: quinine, which became famous as the first antimalarial, and quinidine, still used today against cardiac arrhythmias (Petrovska, 2012). To further underline how deeply medicine and botany are intertwined, in 1735 Linnaeus graduated in medicine from the University of Harderwijk with a thesis on malaria (Linnaeus, 1735). In his thesis two treatments derived from plants effective against malaria are cited: cinchona bark and Artemisia Annua . It is speculated that he was aware of the use of the latter, probably because travelers such as Marco Polo had introduced knowledge of Chinese pharmacopoeia to Europe. (Aydin-Schmidt et al., 2010). Quinine is certainly one of the greatest discoveries of all time in the field of herbal medicine. While we don't know exactly who discovered the use of cinchona to treat malaria, there is no doubt that the drug was used by indigenous Andean people for medical purposes (Miller et al., 2023). It is also known that the Jesuits brought the knowledge of quinine to Europe in the 1600s to cure intermittent fever (as malaria was called at the time). In 1631, for example, the vice king of Peru fell ill with malaria in Lima and recovered thanks to the administration of cinchona bark powder by a Jesuit (Miller et al., 2023). In fact, it would seem that Andean healers used cinchona as a fever medicine in general (Crawford, 2016). This knowledge would have developed in the Loja region of Ecuador, where there was an important center of traditional medical knowledge. It should in fact be underlined that Plasmodium spp. (one of four protozoa of the genus Plasmodium that cause malaria) it is not native to South America but seems to have arrived in this region with the African slave trade to South America started by the Portuguese in the first decades of the 16th century who transferred malaria parasites to the New World. It is thought that local healers were therefore confronted, from that moment on, with at least more than a century of circulation of Plasmodium spp., experimented with the effects of cinchona bark in the area in which they practiced, and taught its use to Europeans, in particular to the Jesuits, whose presence in the region dates back to 1568 (Klaiber, 2004). Molecular studies confirm that malaria parasites began arriving in the Americas from Africa in the mid-16th century. (Rodrigues et al., 2018). Quinine bark was introduced to Spain in 1638 (Achan et al., 2011). Until 1820 the antimalarial drug was produced by drying cinchona bark, grinding it into a powder which was then dissolved in a liquid and drunk. Pierre Joseph Pelletier and Joseph Caventou isolated quinine from the bark in 1820 which later became the standard treatment against malaria (Achan et al., 2011). The phytochemical alkaloids of cinchona are different and in addition to quinine they include quinidine, quinine and cinchonidine, all effective against malaria. Quinidine is also one of the first antiarrhythmics discovered (Hellgren et al., 2014). However, after 1890 quinine became the predominantly used alkaloid to treat malaria (Achan et al., 2011). Until the 1920s, when more effective synthetic antimalarials became available, quinine remained the mainstay of malaria treatment. Chlorquine is the most important of these synthetic drugs and has been widely used especially since the 1940s (Hellgren et al., 2014). However, starting in the late 1950s, due to the intense use of chloroquine, chloroquine-resistant Plasmodium falciparum developed. This initially occurred in Southeast Asia and South America in the late 1950s, then spread to nearly all areas affected by falciparum malaria in the 1980s. As we described in the part about artemisinin, in this period other very effective drugs were developed against chloroquine-resistant malaria. Moreover, with the development of increasing resistance to chloroquine, quinine was rediscovered especially in the treatment of severe malaria (Hellgren et al., 2014) and still plays a significant role in the management of malaria today. Quinidine is a stereoisomer of quinine, so it can be derived from the bark of the cinchona tree or prepared from quinine (Jain & Sisodia, 2024). Quinidine, classified as a class IA antiarrhythmic, was first described in 1848 by Van Heymingen and named by Pasteur in 1853 and has a long history as an antiarrhythmic (Yang et al., 2009). It works by prolonging the effective refractory period and reducing automaticity in the heart, and has been used in the treatment of nearly all cardiac arrhythmias, particularly atrial fibrillation (AF), since the beginning of the 20th century (Vitali Serdoz et al., 2019). As an antimalarial, quinidine accumulates in the food vacuole of the parasite and forms a complex with heme, inhibiting the activity of heme polymerase leading to the accumulation of cytotoxic-free heme (Jain & Sisodia, 2024).Over the past two decades, primarily due to concerns about side effects, such as proarrhythmia, and the new availability of therapies such as new antiarrhythmic drugs and the availability of catheter ablation procedures, there has been a decline in quinidine prescriptions.. Despite this, quinidine still remains one of the oldest cardiac drugs available in the modern era of antiarrhythmic therapy (Vitali Serdoz et al., 2019). Quinidine is currently indicated for: the treatment of Plasmodium falciparum malaria in cases of severe and complicated malaria, both as an independent therapy and in association with exchange transfusion, as pharmacological conversion therapy of atrial fibrillation/flutter to sinus rhythm, in atrial fibrillation/flutter to reduce the frequency of relapses, for the suppression of ventricular arrhythmia and, finally, for the treatment of pseudobulbar syndrome in association with dextromethorphan. (Jain & Sisodia, 2024). The mechanism of action of quinine, instead, has not yet been fully elucidated. The drug could intercalate between DNA chains, blocking their activity, or it could interfere with oxygen uptake or carbohydrate metabolism. Quinine could also increase intracellular pH.

Metformin and Biguanides from Galega Officinalis – The fascinating history of metformin for the treatment of type 2 diabetes begins in medieval Europe (Zhao H. et al., 2021). Galega officinalis (also known as goat's rue, French lilac, Italian fitch, Spanish sainfoin, or professor's herb) of the Fabaceae family, from which metformin is derived, was already used by medieval herbalists to treat polyuria (excessive urination), a key symptom in patients with diabetes (so much so that the name of the disease, diabetes, which is derived from the Greek diabaínō, means excessive emission of urine) (Bailey & Day, 2004; Bailey & Day, 1989; Bailey & Turner, 1996). At that time, nothing was known about the causes of diabetes and the active ingredient of the plant was unknown (Pollak, 2010). However, Galega owes its name ("Galega" comes from the Greek and means "stimulating milk") to the fact that it was a plant with widely recognized galactagogue power in Europe in ancient times (an indication for which Galega is still prescribed today as a phytotherapy and is widely used internationally for this purpose) (Drugs and Lactation Database (LactMed®), 2006). Furthermore, around 1600-1700 Galega was also used and described to treat helminth infections, epilepsy, fever and plague (Bailey, 2017). In the mid-1800s, chemical analyzes were conducted on G. officinalis which demonstrated that the plant, particularly the immature pods, was rich in guanidine (metformin – dimethylbiguanide – is a derivative of guanidine) and related compounds (Bailey, 2017). Around the 1920s, guanidine was discovered to reduce blood glucose in animals. In particular, several mono-guanidine derivatives have proven effective for this purpose, in particular galegin (isoamylene guanidine) and diguanidines, such as syntylin (two guanidines separated by a methylene chain) (Bailey, 2017). However, around the 1930s, a toxicity of these substances was described which reduced their use and, at the same time, the newly discovered insulin became more widely available thus contributing to the reduction in the use of guanidine derivatives (Bailey, 2017). Metformin was synthesized in 1922 and was used in animal experiments to reduce glycaemia (Werner & Bell, 1922). Although metformin has been found to be much less toxic than mono- and diguanidines (Slotta & Tschesche, 1929) due to the high doses required to achieve modest hypoglycemic effects in nondiabetic animals, the true potential of this agent has been underestimated. Therefore, for the reasons described above, neither biguanides nor other guanidine-based agents were developed for the treatment of diabetes and were forgotten in the following decade. Subsequently, however, the usefulness of metformin in type 2 diabetes was rediscovered thanks to studies on antimalarial research (development of a guanidine-based antimalarial, proguanil – Paludrine which was modified into metformin); metformin in the 1940s, both in clinical studies and in clinical practice, proved useful in the treatment of influenza (at the time it was marketed under the name of flumamine), also demonstrating a hypoglycemic effect (Chen & Anderson, 1947; Curd et al., 1945; Garcia, 1950). The hypoglycemic properties of flumamine were then studied and explored in animal studies and clinical models by Jean Sterne, a French physician, who first reported the use of metformin to treat diabetes in 1957 (Sterne, 1959). Despite Stern's studies, metformin had received at that time limited attention because compared to other biguanides such as phenformin and buformin it was less potent in its oral hypoglycemic activity (McKendry et al., 1959; Ungar et al., 1957). However, the use of phenformin and buformin was discontinued in the late 1970s due to the high risk of lactic acidosis they caused (Bailey, 2017). The reputation and reliability of metformin, belonging to the biguanide class, was thus questioned even though there was evidence demonstrating considerable tolerability of metformin compared to other biguanides. Despite this, pharmacokinetic and pharmacodynamic studies conducted in Europe in the 1980s highlighted metformin's ability to counteract insulin resistance and address hyperglycemia in adulthood without causing weight gain or increased risk of hypoglycemia (Bailey, 2017; Pollak, 2010; Zhao H. et al., 2021). In the United States, the FDA, mindful of the negative experiences with other biguanides, did not look favorably on the use of metformin. Nonetheless, at the insistence of Dr. Gerard Daniel, the FDA initiated a thorough review of the literature and after some clinical trials metformin was considered available for clinical use in the United States starting in 1995 (DeFronzo & Goodman, 1995; Bailey et al., 2007). In 1998, new reasons for the adoption of metformin as initial therapy to manage hyperglycemia in type 2 diabetes were highlighted, i.e. the long-term cardiovascular benefits of metformin were described. Sixty years after its introduction in the treatment of diabetes, metformin (dimethylbiguanide derived from guanidine contained in galega officinalis) has become the first-line and most prescribed oral hypoglycemic drug in the world for the management of type 2 diabetes, with potential to further therapeutic applications (for example in cancer treatment) (Bailey, 2017; Pollak, 2010; Zhao H. et al., 2021). The mechanism of action of metformin is now known: it works by lowering blood glucose levels, decreasing glucose production in the liver, reducing intestinal glucose absorption, and improving insulin sensitivity (Corcoran & Jacobs, 2024). Today metformin is still the most prescribed anti-diabetic worldwide despite the revolutionary discovery of SGLT2 inhibitors and GLP1 receptor agonists (Drzewoski & Hanefeld, 2021) and is included in the World Health Organization's (WHO's) essential medicines list (WHO, 2023a) . Currently, as of 2022, $4,028 billion has been spent on metformin worldwide, a figure that is expected to reach $6,420 billion by 2030 (DBMR – Data Bridge Market Research, 2023).

Ephedrina – Ephedra sinica (called Ma huang in Chinese) or guarana of the family Ephedraceae, known and used for at least 5000 years millennia in TCM, is the plant from which ephedrine is derived (Statler et al., 2023; Torpy et al., 2003). Around 2,700 BC it was described in the famous work of the Chinese emperor Shen Nung, who cataloged 365 herbs based on their bitterness (Lee, 2011). Li Shih-Chen in his work Pents'ao Kang Mu, in the latter part of the 16th century, clearly described the plant. In TCM it was used as a circulatory stimulant, diaphoretic and antipyretic. It has also been used for over a millennium as a remedy for respiratory illnesses, which is why the stem has become an important ingredient in many cough preparations and the basis of many herbal medicines for managing respiratory disease outbreaks. It has also recently been used in China to manage the symptoms of Covid-19. (Li et al., 2022; Tian et al., 2022). A fundamental impulse in the discovery of the alkaloid ephedrine came from Japan, a country where the dried stems of the plant were exported from China at the end of the 16th century. In fact, about 300 years later, the pure alkaloid ephedrine was isolated and characterized for the first time by the Japanese scientist Nagayoshi Nagai (1844-1929) in 1885. It was then forgotten until it was rediscovered by Chen and Schmidt in the early 1920s (Lee, 2011). Ephedrine became a very popular and effective treatment for asthma, especially because, unlike adrenaline (until then the standard therapy), it could be administered orally. Ephedrine as a treatment for asthma reached its peak in the late 1950s, since then there has been a gradual and inevitable decline in its therapeutic use. In the 1990s and 2000s, ephedra and ephedrine were then used for weight loss, a use that had never been contemplated in traditional medicine (International Agency for Research on Cancer, 2002; Shaw, 1998). Finally, ephedrine has also become one of the street drugs as ephedrine represent a major source of methamphetamine production and is therefore currently banned in many countries. At the pharmacodynamic level, ephedrine acts by directly activating alpha- and beta-adrenergic receptors and stimulating the release of norepinephrine from nerve endings. The pharmacological actions of ephedrine are akin to those of catecholamines and include: bronchial smooth muscle relaxation, bladder smooth muscle relaxation, sphincter contraction and detrusor muscle relaxation, intestinal muscle relaxation, increased hepatic glycogenolysis, and oxygen consumption, relaxation of the uterine muscles , stimulation of the respiratory center, cyclopegia without loss of accommodation, cardiac stimulation and increased systolic and diastolic blood pressure. Due to these effects, ephedrine is indicated for the treatment of clinically significant hypotension during spinal anesthesia, as a bronchodilator and finally as a nasal decongestant (Statler et al., 2023). In most countries worldwide it is no longer indicated as a weight loss drug.

In conclusion, there are numerous other herbal drugs that are used or have been used effectively for clinical therapy. But because there are so many, it is impossible to mention them all, so below we will mention only a few in a short list:

Atropine – derived from various plants including Atropa belladonna of the Solanaceae family, is the first-line therapy (Class IIa) for symptomatic bradycardia in the absence of reversible causes, it is also used as a pretreatment in Rapid Sequence Intubation (RSI) (McLendon & Preuss, 2023).

Oseltamivir – derived from the Shikimic acid contained in the Illicium verum of the Schisandraceae family, is an antiviral medication used to manage acute, uncomplicated influenza A or B in adult and pediatric patients, including neonates older than 2 weeks and off label for the treatment avian influenza strains, including the highly pathogenic avian influenza A (H5N1) (Sur et al., 2024).

Digoxin – from Digitalis lanata of the Scrophulariaceae family (Šantić et al., 2017), a cardioactive glycoside used to manage and treat heart failure , certain types of arrhythmia and in inducing fetal demise prior to abortion (David & Shetty, 2023).

Galantamine – from Galanthus nivalis of the Amaryllidaceae family an acetylcholinesterase inhibitor used to manage Alzheimer disease by elevating acetylcholine levels in the brain, thereby improving cognitive function and memory (Kalola et al., 2024).

Bearberry leaf – from the plant Arctostaphylos uva-ursi is a species of the Ericaceae family introduced in therapy by Galen as an uroantiseptic and a mild diuretic, (Petrovska, 2012) is actually available as a treatment of uncomplicated cystitis in several European countries (Tóth et al., 2022).

Senna – from the plant Senna alexandrina belonging to the Fabaceae family introduced by the ancient Arabs as a laxative (Petrovska, 2012), it is still used today for this purpose and is part of the class of stimulant laxatives (Bashir & Sizar, 2024) **.**

Hypericum perforatum, commonly known as St John's wort, of the family Hypericaceae described by Paracelsus (Petrovska, 2012) acts as Serotonin and norepinephrine reuptake inhibitors (SNRI) i.e. as an atypical antidepressant and is prescribed for mild to moderate depression (Peterson & Nguyen, 2023).

Scopolamine – from the Hyoscyamus niger of the Solanaceae family Scopolamine used to treat postoperative nausea, vomiting and motion sickness. With off-label indication for the treatment of chemotherapy-induced nausea, asthma, depression, as a smoking cessation therapy, for excessive sweating and for the treatment of gastrointestinal spasms (Riad & Hithe, 2023).

Conclusion and future directions

Most people in the world are being treated or have been treated with phytochemicals or drugs derived from phytochemicals.

Furthermore, many of these are included in the WHO list of essential medicines (WHO, 2023a). Plant-based drugs have changed human history, providing a fundamental contribution to the survival and improvement of life expectancy of our species (Dar et al., 2017). Excellent examples are chemotherapy drugs (vinblastine, vincristine, etoposide, teniposide, paclitaxel, irinotecan, topotecan) antibiotics and antivirals (cephalosporins and oseltamivir), antiparasitic drugs (quinine and artemisinin), ICU drugs (morphine, neuromuscular blockers, ephedrine), cardiovascular drugs (digoxin, quinidine, atropine, reserpine) antidiabetics (metformin) derived from plants and fungi (Raghavendra, 2002).

Plants often contain more than one phytochemical useful for different diseases and indications. Examples include Galega officinalis (the phytotherapeutic is useful as a galactagogue, while metformin was subsequently synthesized from the guanidine contained in the plant (Bailey, 2017)), the Cinchona succirubra (from which quinine and quinidine are derived (Hellgren et al., 2014)) or the Catharanthus roseus (from which vinblastine and vincristine were extracted (Martino et al., 2018)). However, there are many other plants being studied to search for phytochemical substances useful for therapy. An example is Fibraurea tinctoria of the Menispermaceae family which has antidiabetic, antimicrobial, antiprotozoal, antitumor, lipid-lowering properties and which is already used in numerous traditional Asian medicines (Laumer et al., 2024).

Some lessons can be drawn from the history of the use of phytotherapeutics and phytochemicals:

- plants have developed secondary metabolites as phytochemicals over thousands of years, creating a very diverse number of compounds with numerous pharmacological activities over time (Dar et al., 2017; Khan, 2014). Often, in fact, numerous phytochemicals with different pharmacological activities are present in the same plant. It is therefore the task of scientists to identify useful compounds (see vincristine, etoposide, metformin to name just a few examples) and use them directly as phytochemicals or to create active derivatives as treatments. Furthermore, as happened with vincristine, vinblastine, morphine and artemisinin, plant phytochemicals, once discovered, are not always immediately replicable through laboratory synthesis. We must therefore continue to draw on this practical "knowledge" of defense and interaction with the environment developed by plants over thousands of years and passed down throughout the plant kingdom. The history of the use of plants in therapy demonstrates how current knowledge of pharmacotherapy is based on plant products. Perhaps it would have been impossible for humans to discover some essential drugs for medicine, independently of phytochemicals of plant origin.
- Ancient manuscripts on medicinal plants and also the Western and non-Western traditional medicines should not be looked upon with superiority and disillusionment (see, for example, the discovery of artemisinin as an antimalarial derived from TCM or that Linnaeus knew of the use of Artemisia Annua to treat malaria (Martin et al., 2003; Nature Portfolio, 2024; Zhang et al., 2022; Zhao L. et al., 2022). Rather they need to be studied with a critical eye as potential sources of contemporary pharmacotherapy, given the recent fundamental discoveries in the treatment of diseases derived from it. A prime example of ancient practices borrowed from traditional medicine (unrelated to the use of plants in medicine) that have allowed modern medicine to definitively eradicate devastating and deadly human diseases is the practice of variolation. In 1721, Lady Mary Wortley Montagu observed the popular Turkish practice of vaccination among Greek and Armenian women (the practice involved transferring material from smallpox sores to healthy people, causing milder forms of the disease) and successfully vaccinated her son against smallpox. This practice was also used in Persia, China, India and Africa. The development of the smallpox vaccine was inspired by this experience (WHO, 2023b). We must draw on the knowledge acquired since immemorial time that humans have discovered by researching the cure of diseases in the plant kingdom. Our ancestors identified the healing properties of plants through practical experience and passed them on to subsequent generations. In this way, knowledge was refined from generation to generation, transferring it from one generation to another who modernized old properties and discovered new ones, up to the present day (Petrovska, 2012). For this reason, ethnobotanical studies could help in the discovery of new drugs (Aziz et al., 2018). The properties of periwinkle in the treatment of cancer were known, for example, in TCM (Efferth et al., 2007; Wang et al., 2012). Therefore, knowing that this plant was already used in traditional medicine might have allowed its anticancer activities to be identified earlier.

Today it is estimated that more than 20,000 medicinal plants have been inventoried by the World Health Organization (WHO) and only a few have been analyzed to identify their phytochemical components. It can therefore be hypothesized that there are probably many plants with medicinal properties that are still waiting to be discovered (Domingo-Fernández et al., 2023; International Agency for Research on Caner, 2002). For these reasons about 250 pharmaceutical companies worldwide are currently involved in the study of medicinal plants in scientific laboratories (Pelkonen et al., 2014; Šantić et al., 2017). Pfizer, for example, owns a laboratory in the New York Botanical Garden to study the pharmacological potential of North American flora (NYBG, 2024).

Nowadays it is possible to accelerate drug discovery from plants by combining knowledge gained in automation, biotechnology and chemistry through machine learning, high-pressure liquid chromatography (HPLC), gas chromatography (GC) ,mass spectrometry (MS) and high-throughput mass spectrometry revealing the chemical structures of plant compounds, thus discovering potentially useful phytochemicals more quickly and easily (Kaur et al., 2019 (Nature Portfolio, 2024). Identifying the chemical structures of molecules produced by plants and fungi allows to radically accelerate the selection process for studying drug candidates. So, through virtual screening, coupling small molecule libraries of plant-derived bioactive components with protein structures that are more likely to bind to specific drug targets found in databases allows for notable progress as well as great time savings. These isolated substances can also be exploited as a starting point for the synthesis of new compounds effective in therapy. In addition, a revolutionary breakthrough is the use of artificial intelligence (AI) applied to the study of vast traditional medical knowledge. This technology makes possible the mapping of evidence, patterns and trends once elusive in traditional systems of care. Given the history of plant-based drugs useful to humans, since there are numerous medicinal plants in the world, most of which have not yet been thoroughly studied for their properties, and because it has been estimated that phytochemicals are often more effective drugs than those developed in the laboratory (Khan, 2014) it can be speculated that plants may still be a source of useful drugs in the treatment of disease (Kaur et al., 2019).

However, it should not be underestimated that the study of phytochemicals in plants can be frustrating and arduous. Tu Youyou gives an exemplary vision of it. When she received the Nobel Prize for Physiology and Medicine, she stated that the study of plants is challenging because it involves numerous difficulties ranging from the management of extraction and purification technologies to the variables at play in the study of different species, to the collection and use of the different parts of the plant (et al., 2015). Furthermore, given the great dependence on environmental factors, even cloned plants can produce roots, leaves and fruits of varying quantity and quality. This can contribute to hindering attempts to characterize the numerous botanical extracts such as those used for example in traditional medicines.

But molecules of plant origin still hold promise and can lead to exceptional discoveries. In fact, among other peculiarities, molecules of plant origin also have particular pharmacokinetic characteristics: some, for example, such as colchicine, an alkaloid used for the treatment of gout and extracted from plants of the Colchicum autumnale of the Colchicaceae family, have a short half-life which however corresponds to a local action in the liver (Nature Portfolio, 2024). Molecules of plant origin possess these characteristics which are difficult to obtain with synthetic drugs, which are often instead absorbed systemically.

In the end we suggest that, since there are numerous technical and taxonomic challenges in plant research, it would be useful to deepen taxonomic knowledge in this field by accelerating knowledge of taxonomy with machine learning and research on genomic, chemical, morphological and ecological traits. An example in this sense is the Commercial Innovation Unit of the Royal Botanical Gardens, Kew, London, one of the largest botanical collections in the world where research on plants aimed at pharmaceutical products is monitored and where a research group of 300 people currently operates focused on discovering potential plant-derived drugs (Nature Portfolio, 2024). A project has also been launched at Kew to improve the replication of studies on plants used in TCM, through more controlled global standards. To this end, the Good Practice in TCM Research Association was founded in 2012. This association involves 112 institutions and 24 countries, working to create better guidelines (Corporate Member/ Institutional Member Showcase | ASSOCIATION, 2024).

Furthermore, since today phytotherapeutic products are increasingly prescribed independently and in combination with synthetic drugs, it would be useful to develop a separate herbal pharmacopoeia and develop a rational phytotherapy, in which the effectiveness of the preparations is verified through clinical trials and which is based on the application of preparations whose effectiveness depends on the dose applied and the active components identified (Falzon & Balabanova, 2017; Fürst & Zündorf, 2015). This could also serve to further reduce the possibility of human poisoning by herbal drugs (Farzaei et al., 2020; Halberstein, 2005). This is even more important in developing countries, i.e. 75-80% of the world's population, which rely heavily on medicinal plant medicine, and which in recent decades have invested significantly in ethnobotanical research and the use of medicinal herbs (Prasathkumar et al., 2021).

Unfortunately, in recent years a series of trends have begun to threaten biodiversity and consequently also phytochemical resources. Fortunately, however, a countertrend is underway to preserve natural botanical resources through the implementation of ecological laws and social movements but also the creation of botanical gardens, arboretums, greenhouses, herbariums, tissue cultures, propagation laboratories and seed banks. The history of phytotherapy and phytochemicals further highlights and justifies the importance of the scientific perspective of "One Health," based on the recognition that human health, animal health and ecosystem health are inextricably linked. (Pitt & Gunn, 2024). The preservation of biodiversity, in fact, represents the opportunity to draw on one of the major sources of curative medicines for humans (Halberstein, 2005; Prasathkumar et al., 2021). By following the "One Health" approach of preserving nature, in addition to impacting human health by reducing pollutants, reducing the emergence of multidrug-resistant pathogens, and positively affecting mental health, it could also foster the discovery of revolutionary new phytochemicals.

In conclusion, the study of medicinal plants and phytotherapy are among the ancient healing practices that have changed and continue to change human history. Examples of medical practices, similar in effectiveness and very old, are variolation (Ellenberger, 1970; WHO, Retrieved May 20, 2024), psychotherapy (Ellenberger, 1970), the interpretation of dreams (Kurland, 1972; Palagini & Rosenlicht, 2011; Scarpelli et al., 2022), and body-mind therapies (WHO, 2023b). Some of these approaches, viewed with suspicion and considered folkloric until recently, are being rediscovered in this historical period. Indeed, as our time is characterized by the burden of chronic diseases, which are currently the leading cause of death, these practices, which are proving to be revolutionary in the fight against chronic diseases (Claro et al., 2024) and at the same time in the implementation of the "One Health" approach, should be increasingly implemented. Finally, the WHO has also recognized the importance of the ancient medical practices and their effectiveness in current medical treatments (WHO, 2023b)

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INSTRUCTIONS TO AUTHORS

Types of Papers

Research articles: substantial, original research contributions on any aspect of coenology and plant ecology. The main body of the text (excluding tables and references) should not exceed 6000 words, and eight figures and tables.

Notes: short papers, including for examples preliminary reports on new findings of significant results that do not require a full-length paper. The main body of the text (excluding tables and references) should not exceed 3000 words, and four figures and tables.

Manuscripts

Manuscripts must be written in English, and should conform to standard rules of English grammar and style. Text should be written in MS Word, double-spaced with settings for A4 (210 x 297 mm) paper with wide margins. Use Times New Roman font, pt-size 12 (symbol palette for additional characters). Lines and pages should be consecutively numbered.

Please organize your manuscript in a single file, as follows:
First page
Abstract
Keywords
Main Text
Acknowledgements
References
Tables
Figure captions

Figures should be submitted in separate files.
First page: includes a concise and informative title, a running head (shortened title), authors and addresses. Where authors have different addresses, use numbered superscripts to refer to each address provided. State the author for correspondence and include their telephone and e-mail details.

Abstract: up to 200 words. It should include (1) aims, (2) methods, (3) key results and (4) the main conclusion, including key points of discussion. It should not contain citations of other papers.

Keywords: five to eight keywords must be given at the end of the Abstract.

Main text: Concise, well-organized submissions are strongly encouraged. Wordiness, ambiguity, vagueness, run-on sentences and passive voices should be avoided. Please note the correct use of periods and commas for presentation of numbers and dates. Latin and Greek words or expressions are italicized. All taxonomic names should be subjected to the International Code of Botanical Nomenclature. In phytosociological manuscripts, all names of syntaxonomical units should be subjected to the International Code of Phytosociological Nomenclature (www.iavs.org/ResourcesClassification.aspx). Syntaxonomical schemes, reporting Author's names for each unit, should be included after the Coclusions. Avoid footnotes. The first line of text in each section is NOT indented. Arrange the papers under the headings: Introduction (including a clear statement of objectives), Materials and Methods (including study area), Results, Discussion (Results and Discussion sections should be presented separately), Conclusions (summarizing the main achievements of the paper). Headings and Sub-heading hierarchy: Level one, headers typed in bold font, small capitals letters, lowercase except for first letter of first word, left justified, followed by one blank line; Level two, headers typed in bold font, lowercase except for the first letter of the first word, left justified, followed by one blank line. Do NOT number headings and subheadings.

Acknowledgements: Brief list of individuals who provided help during the research. References to research projects/funds can be quoted here.

References: Citations in the text should take the following format: Single author (Manes, 2007); two-author (Smith & Jones, 2008); and three or more authors (Spada et al., 2007). Where different references would appear identical when cited in this manner, use letters after the date in the citations and reference list (Rossi et al., 2008a,b). Order lists of references in date order (oldest first) and alphabetically when of the same date: (Thompson et al., 2003; Larcher et al., 2007; Loreto et al., 2007). Cite references 'in press' only if accepted by a named journal. Personal communications must be cited in the text as follows: (S. Pignatti, pers. comm.).

All publications cited in the text must be in the Roman alphabet, and listed alphabetically by the surname of the first authors, in the APA form from Google Scholar. For example:

ApOSTOLovA, I. V. A., & MESHINEV, T. (2006). Classification of semi-natural grasslands in north-eastern Bulgaria. Annali di Botanica, 6, 29-52.

Manes, F., De Santis, F., Giannini, M. A., Vazzana, C., Capogna, F., & Allegrini, I. (2003). Integrated ambient ozone evaluation by passive samplers and clover biomonitoring mini-stations. Science of the Total Environment, 308(1-3), 133-141.

Pignatti, S. (1982). Reprint 1997. Flora d'Italia. Edagricole, Bologna (IT), 3Vols.

Barbour, M. G., & Billings, W. D. (Eds.). (2000). North American terrestrial vegetation. Cambridge University Press.

Bennett, K.D., (2005). Documentation for psimpoll 4.25 and pscomb 1.03: C programs for plotting pollen diagrams and analysing pollen data. Retrieved from http://chrono.qub.ac.uk/psimpoll/psimpoll.htmlTitles of journals should be given in full.

Tables: should be cited consecutively in the text, should be self-explanatory, each presented on a separate page, and included in the file after the references. Following a concise, informative heading, each table should be fully understandable through column headings.

Figure captions: All illustrations (including diagrams, photographs and maps) are classified as figures and they should be numbered consecutively as first cited in the text. Figure captions should be inserted at the end of the paper. Figure captions should make the material completely understandable and abbreviations should be defined. Panels should be labelled (a), (b), (c), etc. and referred to in the text as, for example, Fig. 1a.

Abbreviations: The SI system should be used for all scientific data. All non-standard abbreviations must first appear in parentheses following their meaning written in full at first mention. Avoid abbreviations if possible in the title, headings and Abstract.

Figures

Figures should be sent as separate files, in TIFF format at 300 dpi. No illustration (including caption) will be given more space than 224 mm x 177 mm, that is the text area of the journal page. Where possible, the figures should be drawn to fit either the page width or a column width (84.5 mm). Along with photographs, include any scale bars on the picture. On maps, scale information should be provided, preferably as a scale bar within the figure. Maps should also include adequate geo-referencing information. Colour figures will be accepted only if necessary, and the printing of colour figures will be subjected to the Editor decision.

ANNOTATIONS