UNIVERSITY OF GENOA

Department of Earth, Environment and Life Sciences - DISTAV

PhD Course in:

Sciences and Technologies for the Earth and Environment - STAT

Curriculum:

Biology Applied to Agriculture and Environment

By-products from medicinal and aromatic plants: from tradition to innovation

by

Cristina Danna

Thesis submitted for the degree of Doctor of Philosophy (36° cycle)

20 March 2024

Tutor: Prof. Laura Cornara

Coordinator of the PhD course: Prof. Mauro Mariotti

Coordinator of the PhD school: Prof. Marco Scambelluri

Table of Contents

Abstract

The Ph.D. project focused on characterizing selected species of medicinal and aromatic plants (MAPs) and valorisation of their bioactive compounds for health or environmental applications. Specifically, the objective was to utilize parts of plants typically considered waste: 1) the aerial part of a medicinal plant (*Peucedanum*), traditionally known for its roots/rhizomes' medicinal properties; 2) the less valuable fronds of an aromatic plant (*Eucalyptus*), a species recently cultivated for ornamental purposes as well. The goal was to bridge traditional historical uses with new applications, emphasizing the recovery of waste material that remains a rich source of compounds with significant biological activities and potential uses across various sectors.

1) The first topic of the project was the study of the species *Peucedaum ostruthium* (L.) W.D.J. Koch (Apiaceae), selected among the alpine medicinal plants documented in ethnobotanical research conducted in the Aosta Valley side of the Gran Paradiso National Park. It was investigated from micromorphological, phytochemical, and pharmacological perspectives. The antioxidant and anti-inflammatory properties, inhibitory activities against enzymes involved in the skin's extracellular matrix degradation, and cicatrizing abilities of hydroalcoholic extracts obtained from both rhizomes and leaves were evaluated. Tests, conducted through cell-free enzymatic techniques and *in vitro* cell cultures, were aimed at scientifically validating the medicinal properties reported in traditional medicine. The rhizomes of *P. ostruthium* have a long tradition of medicinal use and have been known as "*Divinum remedium*" since the 18th century, considered a panacea for various ailments. They were included in the BELFRIT list (2018) for regulating digestive function, bronchial secretion fluidity, and as a tonic for relieving physical and mental fatigue. Additionally, in the Aosta Valley (Italy), leaves are claimed to treat skin diseases (such as wounds, infections, and insect bites), muscular inflammations, and hematomas. Scientific validation of the traditional uses of both parts of the plant is crucial for incorporating their extracts into new herbal medicinal products. Moreover, collected data suggest the potential dermatologic use of the leaves of this species, which represent a considerable biomass currently underexploited or not exploited at all.

2) The second main topic of this project concerned the valorisation of by-products obtained from several *Eucalyptus* species (Myrtaceae). The cultivation of *Eucalyptus* is widespread globally and has been increasingly extended to Italy. Particularly in Liguria, several *Eucalyptus* species are cultivated for their foliage, which is cut and sold in Northern Europe for floral compositions. *Eucalyptus* plantations undergo constant pruning to maintain the production of juvenile branches that are more suitable for the market. Consequently, floriculture generates large volumes of waste biomass annually, mainly consisting of pruned fronds that are typically burned or buried for disposal. However, this by-product retains rich bioactive compounds that could be recovered and used in the organic farming sector. The leaves of *E. gunnii* Hook.f., *E. pulverulenta* Sims cv "Baby blue", *E. cinerea* F. Muell. Ex Benth, and *E. nicholii* Maiden & Blakely were micromorphologically characterized, and their essential oils (EOs) were phytochemically evaluated. Subsequently, these EOs were tested for their phytotoxic and antimicrobial activities to evaluate their potential use as bioherbicides and biocontrollers. In addition, a review was conducted to evaluate the potential activities of the *Eucalyptus* EOs as repellents, insecticides, and acaricides, with applications in human and animal health, food storage, and crop protection sectors. Essential oils represent valuable alternatives to synthetic herbicides and pest controllers, being biodegradable and safe for humans and the environment. From this perspective, utilizing these waste biomasses aligns with the principles of the circular economy.

General introduction

Background and Objectives

Since the beginning of human evolution, nature has provided humans with all its resources. Plants have always played a crucial role in human sustenance, providing shelter, building materials, heat, food, and medicines. The knowledge of plants derives from a long tradition of investigations and uses that has evolved and consolidated in all the environments where humans have managed to survive. Different civilizations and populations have discovered, albeit with effort, how to exploit and use the precious resources provided by plants. This knowledge has been partly handed down and is constantly evolving according to human needs to cope with emergent problems. While knowledge was once linked to specific communities and adapted to particular environments, nowadays, due to globalization, modern humans can draw on the knowledge of numerous cultures and the resources of the most disparate environments. However, modern man has to cope with the loss of traditions, natural habitats, and biodiversity.

The idea in this thesis originates from an ethnobotanical point of view. Ethnobotanical research in the Italian Alps, particularly on the Aosta Valley side of the Gran Paradiso National Park, helped recover valuable traditional knowledge preserved in this bio-cultural haven. Among the most quoted species in alpine traditional medicine, we selected an important medicinal plant, *Peucedanum ostruthium* (L.) W.D.J. Koch, to characterize the plant from micromorphological, phytochemical, and pharmacological perspectives. This species is harvested in the wild or subjected to small-scale cultivation. Precisely, this study aims to highlight the medicinal properties of both the rhizomes and leaves of *P. ostruthium*, drawing on traditional medicine while seeking scientific validation of the virtues associated with the plant. To achieve this goal, we separately investigated the hydroalcoholic extracts obtained from rhizomes and leaves to evaluate their beneficial effects in treating skin diseases (e.g., wounds) and inflammations. Some results obtained by our research group have already been published (see Published articles below), while other new data concerning phytochemistry and bioactivities of the hydroalcoholic extracts are presented in this thesis. Furthermore, we are proceeding with the study of *P. ostruthium* carrying forward the characterization of EO and its bioactivity (ongoing research).

For the second main topic of this thesis, several species of the *Eucalyptus* genus, widespread worldwide with a long history of traditional uses, were selected for further investigations. In this case, the research focused on recovering waste biomasses derived from the processing procedure. The biological properties of the EOs recovered from this plant material were then evaluated for potential environmentally friendly applications. Four *Eucalyptus* species were studied: initially *E. gunnii* Hook.f. and *E. pulverulenta* Sims cv "Baby blue;" secondly *E. cinerea* F. Muell. ex Benth and *E. nicholii* Maiden and Blakely, cultivated in the hinterland of Western Liguria for cut foliage production for the European floriculture market. To preserve their quality, careful branch selection is essential, generating a significant amount of waste biomass. Managing these *Eucalyptus* by-products could be crucial in developing strategies for recovery and enhancing value-added products. In two different studies, we provided data on micromorphology, phytochemical profiles, and biological properties, including phytotoxic, antibacterial, and antifungal activities exhibited by the EOs. The characteristics of the different EOs highlighted in these studies will guide their suitable use in organic farming applications.

Additionally, alongside the aforementioned studies, an extensive review was conducted on the applications of *Eucalyptus* EOs as repellents, insecticides, and acaricides. The objective here is to offer alternatives to synthetic products used in pest control that are applicable in the medicinal/veterinary, food storage, and plant protection sectors.

The idea of recovering discharged biomasses, on a small scale in the case of *Peucedanum ostruthium* and a bigger scale in the case of *Eucalyptus* species, comes from an important principle that the past teaches us. In the past, there was no concept of waste since every discharged biomass and by-product was considered renewable raw material and applied in different sectors meeting human needs. The industrialization of the agricultural sector brought with it the problem of huge waste biomass production, and the need to eliminate and treat it correctly has grown exponentially. Nowadays, recovering biomass and treating it as a resource is part of a circular economy perspective that is aligned with sustainability.

In the period of this research, I also tried to explore plants' microscopic world. It was wonderful to discover the patterns that nature repeats on small and large scales. For this reason, I have provided at the end of this thesis some tables on science-art microscopy photographs obtained during the study of the above-mentioned species.

> *"Can we ever expect to understand existence? Clues we have, and work to do, to make headway on that issue. Surely someday, we can believe, we will grasp the central idea of it all as so simple, so beautiful, so compelling that we will all say to each other: Oh, how could it have been otherwise! How could we all have been so blind so long!"* [1]

Published articles

The research briefly described above have led to the publication of the following scientific articles:

- Danna, C.; Poggio, L.; Smeriglio, A.; Mariotti, M.; Cornara, L. Ethnomedicinal and Ethnobotanical Survey in the Aosta Valley Side of the Gran Paradiso National Park (Western Alps, Italy). ***Plants*** 2022, 11, 170. https://doi.org/ 10.3390/plants11020170

- Danna, C.; Bazzicalupo, M.; Ingegneri, M.; Smeriglio, A.; Trombetta, D.; Burlando, B.; Cornara, L. Anti-Inflammatory and Wound Healing Properties of Leaf and Rhizome Extracts from the Medicinal Plant *Peucedanum ostruthium* (L.) W. D. J. Koch. ***Molecules*** 2022, 27, 4271. https://doi.org/10.3390/ molecules27134271

- Danna, C.; Cornara, L.; Smeriglio, A.; Trombetta, D.; Amato, G.; Aicardi, P.; De Martino, L.; De Feo, V.; Caputo, L. *Eucalyptus gunnii* and *Eucalyptus pulverulenta* 'Baby Blue' Essential Oils as Potential Natural Herbicides. ***Molecules*** 2021, 26, 6749. https://doi.org/10.3390/ molecules26216749

- Malaspina, P.; Papaianni, M.; Ranesi, M.; Polito, F.; Danna, C.; Aicardi, P.; Cornara, L.; Woo, S.L.; De Feo, V. *Eucalyptus cinerea* and *E. nicholii* by-Products as Source of Bioactive Compounds for Agricultural Applications. ***Plants*** 2022, 11, 2777. https://doi.org/ 10.3390/plants11202777

- Danna, C.; Malaspina, P.; Cornara, L.; Smeriglio, A.; Trombetta, D.; De Feo, V.; Vanin, S. *Eucalyptus* essential oils in pest control: a review of chemical composition and applications against insects and mites. ***Crop protection*** 2024, 176(June 2023). https://doi.org/10.1016/j.cropro.2023.106319

The thesis, divided into two parts as previously indicated, will be preceded by short paragraphs that act as a background to contextualize the research subsequently treated.

Conceptual Introduction

Ethnobotany, Circular economy, and Sustainability

Ethnobotany

Since time immemorial, man has depended on Mother Nature for all his basic needs. Plants had always attracted the curiosity of man, offering him food, shelter, and protection, and then also providing him with remedies for injuries and diseases. Ethnobotany is a multidisciplinary science describing the direct interactions between man and plants [2]. It can also be defined as the science of survival, documenting plants used for food, construction, and medicinal purposes [3]. The term ethnobotany was used for the first time in 1895 by the botanist Dr. John William Harshberger in a lecture held in Philadelphia to describe his research among indigenous peoples [4]. Harshberger, in an article published in 1896 in the Botanical Gazette entitled "The Purposes of Ethnobotany" proposes the birth of a new field of study to describe people–plant relationships. The discipline is primarily a fusion of botany and anthropology, but many other disciplines are involved, such as taxonomy, nutrition, pharmacognosy, phytochemistry, palynology, ecology, conservation biology, and other social sciences and humanities, namely political science, geography, environmental studies, economics, psychology, linguistics, and philosophy [5]. Before Harshberger, we were not familiar with the term "ethnobotany." In any case, the idea of a branch of science examining the relationship between people and plants has a long history [6].

> *"This idea, I do not accept because we advocate that the traditional knowledge on the human-plant relationship has an antiquity of about 4000 B.C, since there are documents from this age which broach the theme, especially in agronomy and medicinal plants. I am also against the idea that ethnobotany comes from the definition of Harshberger (…)"* [7] *cited by* [8]

We can affirm that ethnobotany was not previously present because we define this discipline using our own actual parameters based on Western science linked to academic institutions [9]. But in terms of colloquial language, ethnobotany has existed since humans and plants have been in contact. We can, therefore, define *ethnobotanical knowledge* as the scientific knowledge produced investigating the "traditional" knowledge systems [6].

In the 20th century, anthropology as a discipline was maturing, and with it also ethnobotany expanded its field of interest to include other aspects such as the role of plants in folklore, literature, and ceremonies. In 1994, Richard Ford published a schematic representation of the evolution of ethnobotany as a discipline since its inception [10]. A more inclusive, effective, and realistic approach to ethnobotany is seen in the works of Richard Evans Schultes (1915–2001), which is considered the father of modern ethnobotany [5]. For many centuries, ethnobotany has also played an important role in the development of new drugs and in collecting data regarding health practices [11].

Ethnobotany can be considered a branch of the Ethnosciences, particularly linked to ethnoecology since traditional ecological knowledge (TEK) can be defined as "a body of knowledge built by a group of people through generations living in close contact with nature. It includes a system of classification, a set of empirical observations about the local environment, and a system of self-management that governs resource use" [12,13].

Ethnoecological research can help to understand the dynamic relations between biodiversity and socio-cultural systems. In addition, TEK can help to achieve sustainable development and implement natural resource conservation [13].

The research methodologies applied in ethnobotany have considerably evolved and been implemented over time, also gaining different methods of analysis from other disciplines [13]. Modern ethnobotany is based on observations and interviews with native people to document the TEK and describe the interrelationship between humans and plants in a specific temporal and spatial context. The selection of the informants is a key point for ethnobotanists: key informants and community elders are frequently the repositories of the largest amounts of native plant knowledge [5]. The collection of plant specimens and herbarium preparation is an important part of ethnobotanical research in the field. The scientific rigor of ethnobotanical research has largely increased in recent decades, also due to the adoption of quantitative methods [14].

> *"The assertion of more than twenty years ago remains valid: "ethnobotany as a discipline, according to the criteria of authors that have undertaken its theoretical aspects, may be considered in different ways, often overlapping with the goals of other disciplines, but presenting the analysis of the link, interaction, relationship and contact between people and plants as common factor, whatever it be the sense addressed when studying this link"* [15]*. Now, we should take a further step in this discipline."* [6]

Circular economy and Sustainability

The core defining element of circular economy (CE) is the restorative use of resources, proposing that raw materials shall not become discarded waste. Born in opposition to the linear economy, the roots of the concept of a circular economy date back many decades. Circular systems are unavoidable to guarantee human life on earth in the long term [16]. The traditional linear economy without recycling elements cannot be sustainable and must be replaced by a circular system [17,18]. The high pressure that humans have exerted and continue to exert on natural environments through the extraction of materials and the generation of waste is widely recognized. The circular economy has, therefore, emerged as a potential solution to make better use of resources and to guarantee the durability of human sustenance on Earth [19].

One of the most recognized definitions of the CE is given by the Ellen MacArthur Foundation [20]: "A circular economy is restorative and regenerative by design and aims to keep products, components, and materials at their highest utility and value at all times." [21] Another definition is the one reported from the EU Action Plan for the Circular Economy:

> "*In a circular economy the value of products and materials is maintained for as long as possible; waste and resource use are minimised, and resources are kept within the economy when a product has reached the end of its life, to be used again and again to create further value.*" [22]

Prominent principles such as the 3 Rs (reduce, reuse, and recycle) were often quoted to summarize the approach of the CE. In this sense, CE incorporates aspects of sustainable development [23]; however, concerns have been raised regarding some purported circular economy practices being promoted as "sustainable" yet resulting in detrimental impacts on the environment and society [19]. This is surely the result of linguistic deception since terms related to sustainability have often been used even when there were no principles or foundations for such use.

Nature is built on circularity principles, without waste, time pressure, and financial constraints [24]. To build a circular economy, it is fundamental to copy and follow the laws underlying the natural systems, gaining an eco-systemic point of view.

To solve the urgent and pressing sustainability concerns, a circular economy must be fully integrated with sustainable development [19].

Sustainable development, such as ethnobotany and circular economy, are fluid concepts that are still evolving. All these disciplines have their roots in the systems ecology literature of the 1960s–70s, achieving a joint evolution around 1990. Sustainable development dates from the 1960s when the environmental risks associated with economic and societal development began to become evident. Sustainability science is rooted in concerns around resource overexploitation and environmental decline during times of economic growth [19,25]. The criticism of the development model of continuous economic growth had already been considered by the economist Thomas Robert Malthus (1766–1834), who with his theory of "environmental limits" may be considered a precursor to the concept of sustainable development [26].

In the 1980s, the new paradigm of sustainable development was popularized and became more widely used. The World Commission on Environment and Development (WCED) submitted its report, entitled *Our common future,* in which it stated that "Humanity can make development sustainable to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs" [27]. The report expressed the belief that social equity, economic growth, and environmental maintenance are simultaneously possible. Indeed, sustainable development requires simultaneous improvement of environmental, social, and economic outcomes, known as the triple bottom line [28,29]. The WCED's definition of sustainable development also highly encourages a "global view" with respect to the future of our planet [26]. A change in social values is fundamental to balancing the economy with the environment. The concept of sustainable development represents a compromise between growth and conservation, between anthropocentric and eco-centric points of view [25]: "Protecting human life is the main reason anthropocentric humans seek environmental sustainability" [29]. Growth simply implies quantitative increase while development implies qualitative improvement by expanding potentialities. Development is defined as "an evolutionary process in which the human capacity increases in terms of initiating new structures, coping with problems, adapting to continuous change, and striving purposefully and creatively to attain new goals." [30]

Brief overview on plant secondary metabolites

Plant primary metabolites refer to the compounds such as proteins, carbohydrates, and lipids that are essential for plant growth, development, and reproduction. Secondary metabolites, which are non-essential to life, are important for the survival and reproductive fitness of the plant, playing a role in mediating the ecological interaction with the environment and in protecting plants from biotic or abiotic stresses. Secondary metabolites are usually minor compounds, generally present in the organism in low concentrations, but their production can be highly inducible in response to stresses [31]. Secondary metabolites accumulate in different organs of the plant, e.g., roots, stems, leaves, flowers, fruits, or seeds, and their presence and levels can vary from individual to individual of the same species, depending on the variety and the conditions of growth [32]. Thousands of structurally different secondary metabolites have been produced during plant evolution. They play an important role in defending plants against herbivores, bacteria, fungi, and viruses. They also serve as signal compounds to attract pollinating and seed-dispersing animals, as agents of plant-plant competition/cooperation and plant-microbe symbioses, or as antioxidants and UV protectants [33,34]. The ability of plants to compete and survive is, therefore, profoundly influenced by the ecological functions guaranteed by their secondary metabolites [31].

Plant secondary metabolites (SM) can be chemically divided into three groups: terpenes, phenolics, and nitrogen- and sulfur-containing compounds [35]. Principally three pathways are involved in secondary metabolites production: the shikimate, the isoprenoid, and the polyketide pathways [31]. Terpenes comprise the biggest group of secondary metabolites, sometimes also defined in the classification of secondary metabolites with the term terpenoids. However, it is not an interchangeable term, as terpenoids are a modified class of terpenes with a different functional oxidized methyl group. Terpenes come from a common starting point called isopentenyl diphosphate and its allylic isomer, dimethylallyl diphosphate. They are usually grouped according to the number of isoprenes (C_5H_8) into monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), tetraterpenes ($C_{40}H_{64}$), and polyterpenes [36]. Among terpenes/terpenoids are aromatic oils, resins, waxes, steroids, saponins, carotenoids, and rubbers. Phenolic compounds are secondary metabolites that contain a hydroxyl functional group on an aromatic ring, a defined phenol group. They are a heterogeneous group including phenylpropanoids, coumarins and furanocoumarins, lignans and lignin, flavonoids and anthocyanins, catechins, and tannins. Sulfur-containing secondary metabolites include glutathione, glucosinolate, thionins, defensins, and alliin. Nitrogen-containing secondary metabolites include alkaloids, cyanogenic glucosides, and non-protein amino acids.

Plant SMs are responsible for herbivory avoidance, relying principally on their toxicity and carcinogenic activity [37]. They can also interfere with the physiology, metabolism, and reproduction of microorganisms. For instance, toxins and antimicrobial substances can protect against threats from bacteria, viruses, and other pathogens. Meanwhile, some secondary compounds help assemble a healthy microbial community, supporting the plant's well-being.[38]. SMs are also involved in pollinators, seed dispersal attraction, and/or in their food rewards, relying on the presence of compounds such as flavonoids, anthocyanins, carotenoids, terpenoids, amines, and phenylpropanoids [39]. On the other hand, they are also responsible for deterrent and insecticidal actions [40], as well as allelopathy mechanisms with negative or positive effects on the surrounding plants [41].

Plant SMs represent a rich spectrum of bioactive compounds filtered by natural selection that can be used by humans for different purposes. Knowing and deepening the mechanisms of action and the effects of bioactive compounds in plants allows their application in many fields of interest. Bioactive compounds can be applied in the medicinal sector, with benefits for human health; in the food industry as natural preservatives and dyes; and in environmental applications, for example, as environmentally friendly herbicides and pesticides.

Applications for human health

Phytotherapy and Cosmetic Focuses

Phytotherapy is the science that deals with the treatment and prevention of diseases using medicinal plants and herbal products [42]. Throughout history, people all over the world have used herbs to ensure health, and we can affirm that in the art of healing, the role of plants is central. An ongoing process of searching and verifying in all cultures resulted in an empirical science on the treatment of diseases using medicinal plants [43]. These traditional practices date back to the first human cultures and over time, several documented uses of plants have been handed down, mainly orally but also in written documents. Modern phytotherapy started with the advent of organic chemistry when a process of isolation of active compounds. The research in the isolation of bioactive compounds, their characterization, and the study of their effects led to the replacement of medicines containing whole plant extracts with medicines containing only one or a few selected pharmacologically active compounds. However, nowadays the importance of phytocomplex has been re-evaluated, since their use rather than that of single molecules has demonstrated synergic effects, mainly in terms of bioavailability. Different plant materials are applied for therapeutic, cosmetic, and nutrition-related purposes, e.g., herbal drugs, herbal cosmetics, and herbal spices, respectively. All aspects related to the history of herbal medicine document the difficult but extremely fruitful development of human civilization [44]. The applications of herbs in medicines vary as the diseases that can affect humans. The following paragraphs consider their applications in the skin-care field.

Skin-care sector

The skin represents the first layer of contact between the body and the external environment. It provides the first barrier of protection against external physical, chemical, and biological attacks; it prevents the dehydration of the body and participates in processes of thermoregulation, excretion, and tactile perception. The skin consists of three overlapping tissues, beginning outwardly from the epidermis, the dermis, and the hypodermis, all in a relation of contiguity with the rest of the body [45,46].

The epidermis consists of keratinized, stratified squamous epithelial tissue, lacking a vascular system. The layers of the epidermis include the *stratum corneum*, *s. lucidum, s. granulosum*, *s. spinosum*, and the *s. basale* which is the deepest portion containing stem cells. At least 80% of cells in the epidermis consist of keratinocytes responsible for synthesizing keratin, a long protein with a protective role. The differentiation/maturation process occurs during the migration of the cells from the basal layer to the surface, and it is known as keratinization, resulting in cell death as a form of terminal differentiation. In the epidermis, other non-keratinocytes cells are present: melanocytes, consisting of dendritic, pigment-synthesizing cells producing melanin and transferring it to keratinocytes; Merkel cells, mechanoreceptors in sites of high tactile sensitivity; Langerhans cells, involved in T-cell responses. The interface between the epidermis and dermis consists of a basement membrane that allows the exchange of cells and fluids and holds the two layers together. The dermis is an integrated system of fibrous, filamentous, and amorphous

connective tissue, containing a network of nerves and vascular bundles, epidermally derived appendages (including eccrine and apocrine glands, ducts, and pilosebaceous units), fibroblasts, macrophages, and mast cells. Additionally, lymphocytes, plasma cells, and other leukocytes enter the dermis in response to various stimuli. The dermis consists of two regions, *papillary dermis* and *reticular dermis.* The principal component of the dermis is collagen, a fibrous structural protein present in the dermis. Fibroblasts are responsible for the secretion of collagen, reticulum, and elastic fibers. The dermis provides the skin with its pliability, elasticity, and tensile strength. It protects the body from mechanical injuries, includes water in its network, is involved in thermoregulation, and includes sensory receptors. The hypodermis consists of adipocytes, large blood vessels, and collagen; it provides an energy reserve to the body and is involved in hormone regulation.

The term cosmeceutical is commonly used to define cosmetic products containing bioactive compounds promoting beneficial topical actions and providing protection against degenerative skin conditions [47]. Botanical extracts are common ingredients used as cosmeceuticals, as rich sources of vitamins, antioxidants, essential oils, hydrocolloids, proteins, terpenoids, and other bioactive compounds [48]. Many pharmaceuticals copy and integrate the activity of natural constituents of the dermis due to the high content of phospholipids (useful for hydration and protection from UV rays), emollient mucilage (which prevents skin dryness and exfoliation), astringent tannins, and anti-inflammatory flavonoids.

Skin care includes the treatment of dry skin and reducing trans epidermal water loss through an external film such as natural vegetable oils. Another important goal is the prevention of ultraviolet radiation damage and skin aging by exploiting the free-radical scavenging effects of plant extracts that contain phenolic derivatives, such as tannin and flavonoids [49]. In cases of tissue damage, an inflammatory response occurs to direct the healing process and achieve tissue homeostasis. A complex network of leukocyte cells and pro- and anti-inflammatory mediators governs the healing process, while the dysregulation of these interactions results in pathologic and chronic inflammatory disease states [50]. During inflammation, reactive oxygen species are produced by leukocytes, while some enzymes (e.g., collagenase, elastase, and hyaluronidase) involved in the degradation of the extracellular matrix (a network of collagen, fibronectin, and elastin) are activated to allow the arrival of macrophages at the site of damage. In addition, they are also involved in the release of growth factors to implement the repair process. In chronic inflammation status, at high exposure to UV rays and/or other oxidant factors, in the aging process, as well as in some pathological conditions, there is an anomalous increase in the level of free radicals (e.g., ROS concentration) and/or in the activity of proteases. However, several natural compounds may help counteract the inflammatory process. In the search for new botanical products, the chemical characterization of plant extracts is essential, as are biological tests useful to assess their antioxidant potential and their influence on proteases. Preliminary in vitro tests are carried out on several cell lines to attest to the safety and beneficial effects of these bioactive compounds, indicating the concentrations that can be used without having a cytotoxic effect. By analysing the data as a whole, it is, therefore, possible to confirm the suitability of a plant extract in the skin care sector.

Environmental Applications

Agricultural Sector

The use of synthetic products, such as herbicides and pesticides, known to afflict biodiversity and cause resistance phenomena in target species, has caused huge phenomena of environmental degradation [51,52]. Biocontrol consists of using natural interactions that drive inter-species relationships for the control of weeds and pest populations [53]. The search for natural bioactive compounds to replace synthetic products is a growing sector aimed at mitigating the environmental impact and obtaining effective and environmentally friendly alternative formulations. It is well known that secondary metabolites are produced in plants for

interacting with the environment, and humans can make use of such bioactive compounds which, being biodegradable, present low side-effects with respect to the synthetic products. In this regard, interest in essential oils has recently grown. Essential oils (EOs) are complex mixtures of monoterpenes, sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones that exert allelopathic and attraction/repellent effects and are useful for the control of weeds and pests [54,55]. Terpenes and terpenoids, with additional phenolic compounds, are usually the major constituents of volatile organic compounds (VOCs) released by plants, which can exert phytotoxic and repellent activities [56,57]. In terms of sustainability, the selection of the species from which to obtain essential oils is of paramount importance. For this purpose, species producing high biomasses with a high oil yield are preferable for these applications. Following the circular economy criteria, waste biomasses and by-products from the agricultural and forestry sectors should be preferred sources of bioactive compounds [58]. Another fundamental aspect of the research on bioactive compounds is the identification of their mechanisms of action on the target species. Additionally, prior to assessing the applicability of the bioactive compounds as bio-controllers, attention must be given to the subject of selectivity [59]. The research on natural alternative pesticides cannot ignore the study of possible side effects on non-target species through tests that evaluate the eco-compatibility and health safety of the selected compounds. [60]. Additionally, despite the promising potential shown by bioherbicides and biopesticides, to obtain long-term commercial success, laboratory investigations must be integrated with studies on performance in field conditions. This approach is indispensable before moving on to the application of new biopesticides in integrated pest management programs [53,61].

Bioherbicides (weeds management)

The degree of weed spread is of primary importance in crops, causing a reduction in the yield of agricultural production. Plant extracts acting as allelochemicals are an emergent method for weed control in sustainable agriculture [53,62]. Synthetic herbicides have been over-employed on herbicide-resistant crops, particularly glyphosate-resistant crops such as corn, soybean, and cotton, causing glyphosate resistance also in weed populations. Nowadays, it is essential to diversify weed management and discover herbicides with different mechanisms of action. This is pivotal, considering the well-known harmful environmental and health effects of glyphosate and other synthetic compounds [63,64]. Plants produce various metabolites such as water-soluble organic acids, alcohols, aldehydes, ketones, lactones, fatty acids, polyacetylenes, quinones, phenolics, cinnamic acid, coumarins, flavonoids, tannins, terpenoids, and steroids that can act as allelopathic compounds [54,65]. Plant extracts can be applied as bioherbicides as they inhibit germination and growth of noxious weeds. Plant bioherbicides can affect plant morphology and physiology by several mechanisms, inhibiting the germination and growth of weeds. These mechanisms include a change in membrane structure and interference with the signal transduction pathway; interference with DNA and protein synthesis, cell cycle, and mitosis; interference with amylase activity and other biochemical processes delaying or inhibiting seed germination. Additionally, the growth of weeds can be retarded, affecting root–cell division, nutrient uptake, photosynthesis, and plant growth hormone synthesis, or inducing the production of reactive oxygen species or stress hormones [66,67]. Preliminary laboratory investigations include the evaluation of allelopathic effects of herbal extracts of both selected crops and weeds to assess their selectivity and efficacy. Finally, further field experiments are needed to understand the effectiveness of bioherbicides in weed management and elucidate their interaction with the environment.

Biopesticides (Pests management)

Pest control is of primary importance in crop protection and food storage; however, the indiscriminate use of synthetic pesticides has given rise to many environmental and health problems. The potential use of plant bioactive compounds as deterrents against crop pests and also during the post-harvest period opens the way to effective and environmentally friendly solutions [40,68]. Biochemical pesticides, or herbal pesticides, are substances derived from plant material and applied to control crop pests [61]. Secondary metabolites are especially useful as biopesticides, having behavioral and physiological effects on agriculturally important pests [69]. Botanical pesticides are in particular applied to control insects, acari, fungi, bacteria, nematodes, and viruses [69]. Insects are reported to be the main target of biopesticides, as they cause serious damage to agricultural plant crops and bring about losses occurring both during the pre-and post-harvest periods [70]. Botanical insecticides can have different modes of action, surely depending on the phytochemical composition of the extract and the target species analysed, and also considering the different developmental stages [70,71]. Against insects, botanical pesticides are applied as repellents, deterrents in feeding and oviposition, and as insecticides. Many laboratory investigations have been carried out to assess the effects on the targets, considering contact and topical toxicity, fumigant toxicity, fecundity tests, ovicidal activity tests, repellent activity, antifeedant activity, acetylcholinesterase inhibition, and antennal response [72]. Field tests are needed to ensure the development of strategies applicable in the territory, ensuring the management of the plant pests in the plantations or the post-harvest period.

PART I

Ethnobotanical journey into Alpine environments. From traditions to scientific validation.

PART IA

Ethnobotanical Survey in the Aosta Valley Side of the Gran Paradiso National Park (Western Alps, Italy).

Introduction

The traditional uses of plants express the symbiotic relationship between human communities and their environment. This cultural heritage, accumulated and evolved by living in close contact with nature, is rapidly disappearing due to socio-economic changes and different land uses over time[73]. In areas historically exposed to very few external influences and characterized by a subsistent economy, such as alpine regions, this trend is less evident. These areas represent, therefore, important BioRefugia for the conservation of plant and animal biodiversity as well as cultural differences [74–76].

In recent years, several ethnobotanical investigations have focused on different North Italian alpine regions, such as Piedmont [77–79], Lombardy [80–84], Liguria [85], and Trentino [86,87]. Moreover, the ethnobotanical traditions of Valle Orco, located on the Piedmont side of Gran Paradiso National Park, were previously investigated in a master's thesis [88]. Regarding the Aosta Valley, previous ethnobotanical studies are limited and dated, e.g., the scientific studies on the traditional use of some local species [89] and the medicinal plants used in Valtournanche [90]. However, there are several informative books concerning the same topic [91–95].

The Aosta Valley, located at the north-western end of Italy, is the smallest region of the country, with an extension of 3620 km^2. The region is surrounded by mountains (Gran Paradiso, Cervino, Monte Rosa, and Monte Bianco) that separate it from Piedmont, Switzerland, and France. The Aosta Valley has been inhabited since the Neolithic period and, starting in 25 BC, it was annexed to the Roman Empire due to its strategic position. After the fall of the Roman Empire, the region suffered various invasions and dominations and, in 575 AD, it fell under the Franco-Burgundian kingdom, which marked the passage from a Celtic-Ligurian-Latin culture to a Franco-Roman one [96]. After the dissolution of the Carolingian empire and various dominations from the 11th century, the region was annexed to the Savoy dominium. French was adopted in the Aosta Valley in the 16th century and was adopted as an official language. Even after the Italian unification (1861), the Aosta Valley tried to preserve its peculiar linguistic and cultural traditions, which made it an autonomous bilingual region with a special statute (from 1948). The original population of the Aosta Valley commonly uses the patois dialect, which is influenced by the Franco-provençal language [97]. The originality of the patois lies in its variety since there are many dialectal inflections according to the valleys, neighboring municipalities, and villages.

Aosta Valley, due to its partial geographical and socio-cultural isolation, represents an ideal breeding ground for ethnobotanical research. Typical traditions, representative of the original population, are still preserved in these areas, being scarcely affected by external influences.

We collected data concerning the TEK of the three valleys (Cogne, Valsavarenche, and Rhêmes) included in the Gran Paradiso National Park, an area of high naturalistic interest. This study aimed to preserve the traditional uses of the local flora to revitalize the strong cultural identity of the alpine valleys. The recovery of traditional knowledge about plants has an intrinsic cultural value and mainly provides some new opportunities for sustainable land management and the valorisation of local products [98]. In addition, data obtained on the traditional use of natural products could be useful for the rational development of new medicines for the health community.

From the examination of the medicinal plants used, some cases also emerged in which the portion considered most precious and, therefore, collected is represented by the root. The aerial biomass, although quite substantial, is generally discarded. From these observations, we took the cue to continue with a subsequent work of recovery and valorisation of this plant material, which will be described later.

Materials and Methods

Study area and People

The investigated area includes the three valleys, namely Cogne, Valsavarenche, Rhêmes, of the Gran Paradiso National Park (PNGP) located in the Aosta Valley, Western Alps, Northern Italy (*Figure 1*).

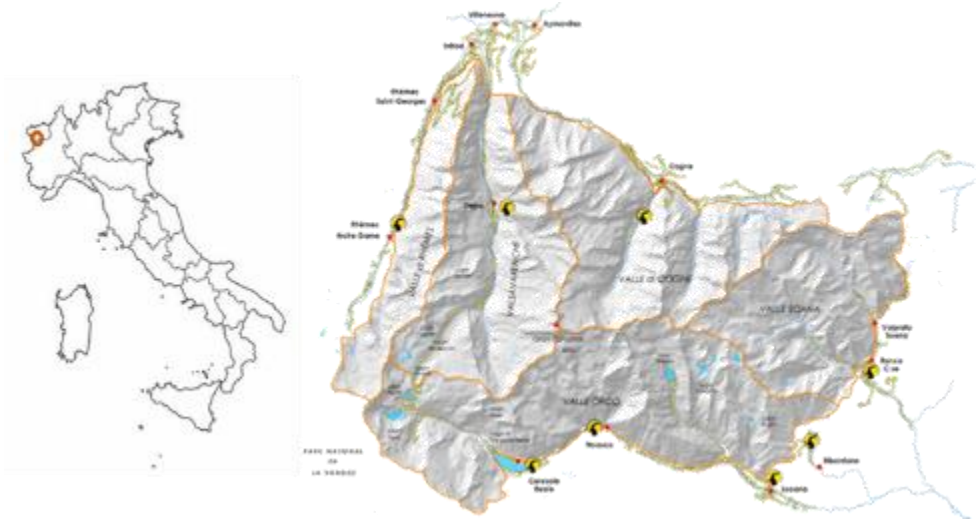


Figure 1 Map of the Gran Paradiso National Park (Aosta Valley and Piedmont sides) [99].

The territory of the park, formerly a royal hunting reserve ceded to the state at the beginning of the 20th century by the King Vittorio Emanuele III of Savoy, is a protected area established in 1922. It is the first National Park in Italy, established to preserve fauna, flora, and conserve the natural beauty of the landscape. The PNGP covers 71.044 ha between Aosta Valley (52%) and Piedmont (48%) and includes five valleys (Cogne, Valsavarenche, Rhêmes, Orco, Soana) surrounding the Gran Paradiso peak (4061 m). The lithology, geomorphological and climatic features strongly influence vegetation and flora. The great biodiversity of the park is due - in addition to the considerable extension of its territories - to the presence of two slopes, the Aosta Valley and the Piedmont ones, very different in lithology and climatic characteristics. Furthermore, the landscape is characterized by several micro-environments due to the significant differences in altitude (from 800 m to 4061 m a.s.l.). The protected area has an average altitude of about 2.400 m and the subalpine, alpine, and snowy vegetation types are the most represented natural environments. Forest formation mainly consists of *Larix decidua* and *Picea abies*, with *Pinus cembra* at the higher altitudes; in alpine grasslands *Festuca sp*., *Carex curvula* and *Sesleria caerulea* are the most represented species. The flora of prairies and pastures is mainly composed of acidophilous species, due to the wide dominance of the siliceous substrates; however, in calcareous outcrops also basophil species are present.

Until today, about 1160 taxa [100], including Lycopods, Horsetails, Pteridophytes, Gymnosperms and Angiosperms, have been listed within the PNGP, 82 of which are endemic to the Alps. Native species account for 99% of the total (1127 species).

In the 13 Municipalities of the Park, including 6 municipalities in Piedmont and 7 in Aosta Valley, live about 8.300 people. Our research has been focused on the municipalities of Cogne (1370 inhabitants), Valsavarenche (169 inhabitants) and Rhêmes (251 inhabitants), representing the Aosta Valley area belonging to the PNGP (*Figure 2*). The Valdôtain dialect used by all the residents of the three valleys is commonly known as *patois*.

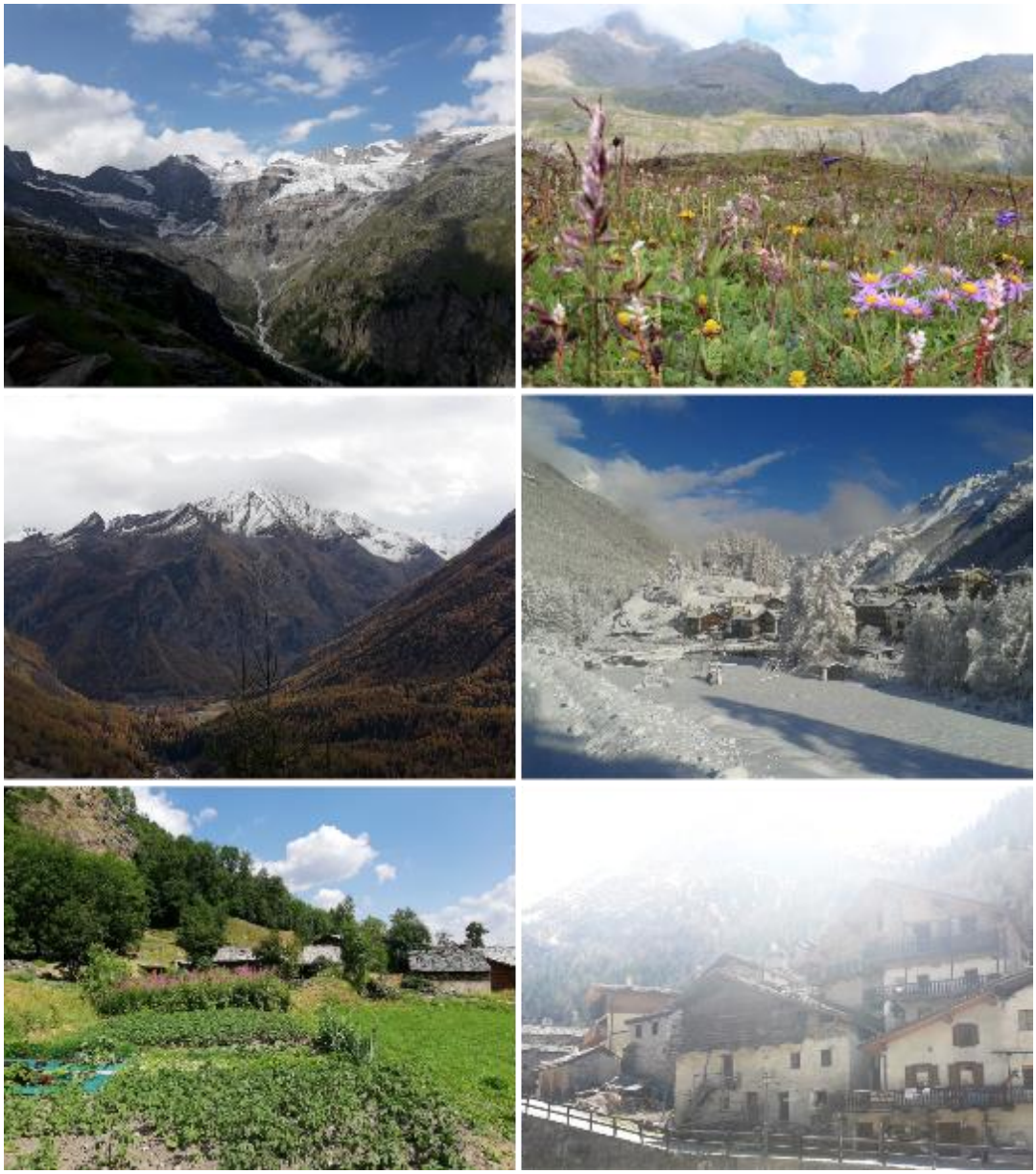


Figure 2 Natural and anthropic environments in different seasons.

Field study on traditional uses of plants

Ethnobotanical data were collected during summer over two consecutive years (2017–2019), through extensive dialogues and semi-structured interviews with 68 inhabitants. Informants were native or long-time residents in the area and had strong links with the traditional human activities of the territory. We selected informants using snowball techniques and we tried to ensure that all key informants were interviewed. We obtained oral prior informed consent from all informants, according to the ISE (International Society of Ethnobiology) Code of Ethics. During the individual or groups interviews, we tried to build a relationship of confidence with informants, to facilitate the dialogue. The age, gender, origin, level of education and occupation of all informants have been recorded.

The questions were intended to document the use of plants as food and medicine for humans and animals. In addition, liquor, domestic, cosmetic and others uses have been also noted. The informants were asked to provide local name, parts used, period of gathering, association with other plants, preparation and use, related recipes, and further indications. We have thus reported the plant uses derived from the oral tradition in the local community. During the interviews, we collect several fresh plants and dried samples representative of the local officinal flora.

The identification of the plants was performed through digital sources and books [101,102]. The nomenclature followed "*Plants of the world Online*" [103] and the corresponding synonymous, according to "*Flora d'Italia*" [104,105]**,** were added. "*Index fungorum*" [106] was used for the nomenclature of fungal species. Voucher specimens of the wild cited plant species were prepared and deposited at the Ethnobotanical Herbarium of the PNGP in the Paradisia Alpine Botanic Garden (Valnontey, Cogne).

Data analysis and quantitative indices

All the ethnobotanical data were organized in spreadsheets of Microsoft Excel, to process the survey results. Data were evaluated by quantitative parameters and Ethnobotanical indexes (Ethnobotanicity index, Ethnophytonomic index, Relative frequency of citation, Factor informant consensus, Fidelity level). Ethnobotanicity index (EI) [107] allows to estimate the importance of the useful plants in a defined area. Ethnophytonomic index (EPI) [108] allows to estimate people's knowledge about the local plant species vernacular names. The Relative Frequency of Citation (RFC) estimates the local importance of each cited species [109]. The Factor informant consensus (FIC) [110] identify the main categories of diseases reported. The Fidelity Level index (FL) [111,112] indicates the informants' choice for a potential plant species to treat a given disease.

Results

A total of 68 informants (30 men and 38 women) with age ranging from 36 to 92 years (mean age 70 years) were interviewed in the valleys of Cogne, Valsavarenche and Rhêmes, respectively (*Table 1*) and (*Figure 3*).

Table 1 Distribution of the informants according to their gender, and residence in the municipalities of Cogne (1.544 m), Valsavarenche (1.541 m), Rhêmes (R. Saint-Georges 1.218 m and R. Notre-Dame 1.725 m) [99].

Gender	Number of informants and residence valley		
	Cogne (1370 residents)	Valsavarenche (169 residents)	Rhêmes (251 residents)
men	19	4	7
women	21	9	8
total	40	13	15

Almost all informants (97%) have always been residents in their respective valleys. As can be assumed from the advanced average age of the informants, 66.2% of them was retired at the time of the interviews. Regarding their currently or previously employments the informants were housewives or employed as farmers, breeders, cheesemaker, veterinary, teachers, park guards, nature guides, restaurateurs, public servants etc.

Figure 3 Interviews with native informants.

Plants Diversity analysis

The data collected refer to 220 plants (69 families), 10 mushrooms (6 families), 1 lichen. Wild species (149) cultivated (41) and purchased (29) plants were considered. As reported in previous ethnobotanical studies concerning the Italian Alps [78,80,81,85], the most quoted families (>10 species) were Asteraceae, with 26 species, followed by Rosaceae (15) and Lamiaceae (11). In this study, Poaceae and Apiaceae, with 10 species, followed by Fabaceae (9) were also reported. The final database included 3918 reports concerning 231 taxa. The most representative uses were medicinal (42%), and food (33%) followed by other categories as reported in *Figure 4*.

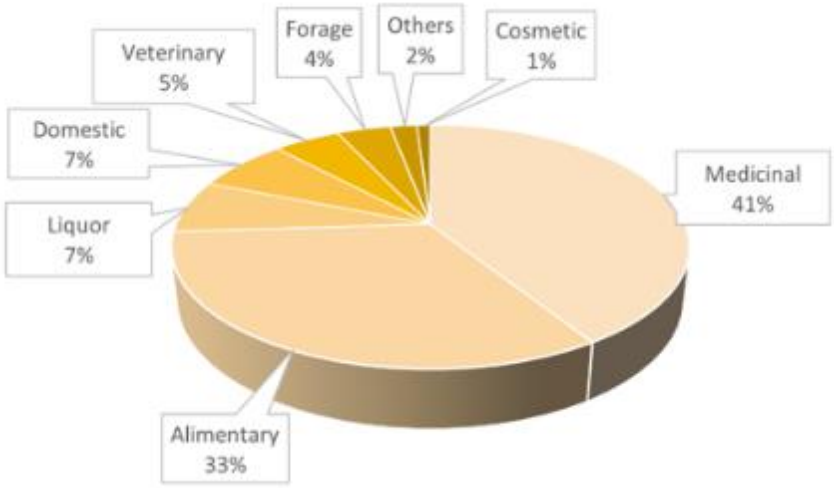


Figure 4 Percentage of citation related to the categories of use [99].

The general importance of the useful plants in the investigated area was evaluated calculating EI and EPI indices. Ethnobotanicity Index (EI) was calculated as the ratio between the number of the wild taxa cited in the medicinal, cosmetic, veterinary, and food (alimentary and liquor) sectors and the estimated number of taxa in the wild flora of the area [81]. The resulting value of EI obtained (12,8%) falls above the range of values (5.37–10.75%) reported for different Italian regions [113], and above those referred in different Italian Alpine areas, e.g. 6.2% for Val San Giacomo [81], 9.7% for Stelvio National Park [82], 11% for North-Western Ligurian Alps [85] and 12% for South Tyrol [114].

The richness of popular knowledge about the wild species was verified by EPI Ethnophytonomic index, higher (EPI 0.11) than the EPI values previously reported in Italy and for another alpine areas (EPI 0.06) [81]. Nevertheless, this value shows an erosion of the linguistic heritage associated with plants, suggesting that only 11% of the wild taxa have a vernacular name.

In *Table 2* are listed the taxa cited in the ethnobotanical research carried out in the Gran Paradiso National Park. For information about detailed uses of the cited plants see Danna et al. [99].

Table 2 Taxa reported in the ethnobotanical survey.

Family Scientific Name	Origin
PLANTAE	
Amaranthaceae	
Amaranthus retroflexus L.	W
Beta vulgaris L. subsp. *vulgaris* syn *Beta vulgaris* L. subsp. *cicla* (L.) Schübl. and G.Martens	C
Beta vulgaris L. subsp. *vulgaris* var. *vulgaris*	C
Blitum bonus-henricus (L.) Rchb. syn *Chenopodium bonus-henricus* L.	W
Chenopodium album L.	W
Amaryllidaceae	
Allium ampeloprasum L.	C
Allium cepa L.	C
Allium sativum L.	C
Allium schoenoprasum L.	W
Apiaceae	
Angelica sylvestris L.	W
Bunium bulbocastanum L.	W
Carum carvi L.	W
Daucus carota L.	C
Foeniculum vulgare Mill.	P
Heracleum sphondylium L.	W
Levisticum officinale W.D.J. Koch	C/W
Petroselinum crispum (Mill.) Fuss	C
Peucedanum ostruthium (L.) W.D.J.Koch syn *Imperatoria ostruthium* L.	W
Pimpinella anisum L.	W
Araliaceae	
Hedera helix L.	W
Aristolochiaceae	
Aristolochia clematitis L.	W
Asparagaceae	
Paradisea liliastrum (L.) Bertol.	W
Ruscus aculeatus L.	P
Asteraceae	
Achillea erba-rotta All.	W
Achillea moschata Wulfen	W
Achillea millefolium L.	W
Arctium lappa L.	W
Arnica montana L.	W
Artemisia absinthium L. A.abs.HBPNGP_ETN	W
Artemisia campestris L. subsp. *borealis* (Pall.) H.M.Hall and Clem.	W
Artemisia glacialis L.	W
Artemisia genipi Stechm.	W
Artemisia umbelliformis Lam.	W
Artemisia pontica L.	W
Artemisia vulgaris L.	W
Calendula officinalis L.	C/W
Carduus defloratus L.	W
Carlina acaulis L.	W
Centarea cyanus L.	C/W
Cirsium eriophorum (L.) Scop.	W
Doronicum grandiflorum Lam. subsp. *grandiflorum*	W
Leontopodium nivale (Ten.) È. Huet and A.Huet ex Hand.-Mazz. subsp. *alpinum* (Cass.) Greuter	W
Matricaria chamomilla L.	P
Matricaria discoidea DC	W
Petasites hybridus (L.) G. Gaertn., B.Mey. and Scherb.	W
Tanacetum vulgare L.	C
Taraxacum officinale aggr.	W
Tragopogon pratensis L.	W
Tussilago farfara L.	W
Berberidaceae	
Berberis vulgaris L.	W
Betulaceae	
Alnus viridis (Chaix) DC	W
Betula pendula Roth	W
Corylus avellana L.	P
Boraginaceae	
Borago officinalis L.	C
Myosotis alpestris F.W. Schmidt	W
Brassicaceae	
Brassica nigra (L.) W.D.J. Koch	P
Brassica oleracea L.	C
Brassica rapa L.	C
Brassica rapa L. subsp. *rapa*	C
Capsella bursa-pastoris (L.) Medik	W
Campanulaceae	
Phyteuma ovatum Honck.	W
Cannabaceae	
Cannabis sativa L.	P
Humulus lupulus L.	P
Caprifoliaceae	
Lonicera caerulea L. subsp. *caerulea*	W
Valeriana celtica L. subsp. *celtica*	W
Valeriana officinalis L.	P

Family Scientific Name	Origin
Caryophyllaceae	
Agrostemma githago L.	W
Silene vulgaris (Moench) Garcke	W
Colchicaceae	
Colchicum autumnale L.	W
Colchicum bulbocodium Ker Gawl.	W
Convolvulaceae	
Convolvulus arvensis L.	W
Cornaceae	
Cornus sanguinea L.	W
Crassulaceae	
Hylotelephium maximum (L.) Holub syn *Sedum telephium* L.	C/W
Sedum album L.	W
Cucurbitaceae	
Cucurbita maxima Duchesne	C
Cupressaceae	
Juniperus communis L.	W
Juniperus sabina L.	W
Elaeagnaceae	
Hippophae rhamnoides L.	W
Equisetaceae	
Equisetum arvense L.	W
Ericaceae	
Arctous alpina (L.) Nied.	W
Arctostaphylos uva-ursi (L.) Spreng.	W
Rhododendron ferrugineum L.	W
Vaccinium myrtillus L.	W
Vaccinium uliginosum L.	W
Vaccinium vitis-idaea L.	W
Euphorbiaceae	
Euphorbia seguieriana Neck	W
Euphorbia helioscopia L.	W
Fabaceae	
Astragalus alopecurus Pall.	W
Lotus corniculatus L.	W
Medicago sativa L.	C/W
Onobrychis viciifolia Scop.	C/W
Phaseolus vulgaris L.	C
Trifolium alpinum L.	W
Trifolium pratense L.	W
Trifolium repens L.	W
Vicia faba L.	C
Gentianaceae	
Gentiana acaulis L.	W
Gentiana verna L.	W
Gentiana lutea L.	W
Gentiana punctata L.	W

Family Scientific Name	Origin
Geraniaceae	
Geranium robertianum L.	W
Pelargonium sp.	C
Grossulariaceae	
Ribes alpinum L.	W
Ribes nigrum L.	C
Ribes petraeum Wulfen	W
Ribes rubrum L.	C
Ribes uva-crispa L.	W
Hypericaceae	
Hypericum perforatum L.	W
Iridaceae	
Crocus vernus (L.) Hill	W
Juglandaceae	
Juglans regia L.	P
Juncaceae	
Juncus jacquinii L.	W
Lamiaceae	
Hyssopus officinalis L.	P
Lavandula angustifolia Miller	P
Melissa officinalis L.	P
Mentha longifolia L.	C/W
Nepeta cataria L.	P
Salvia officinalis L.	P
Salvia pratensis L.	W
Salvia rosmarinus Spenn. syn *Rosmarinus officinalis* L	P
Satureja montana L.	P
Teucrium chamaedrys L.	W
Thymus pulegioides L. syn *T. serpyllum* Auct.	W
Lauraceae	
Laurus nobilis L.	P
Lentibulariaceae	
Pinguicula sp.	W
Liliaceae	
Lilium candidum L.	C
Linaceae	
Linum usitatissimum L.	C
Malvaceae	
Malva neglecta Wallr.	W
Malva sylvestris L.	W
Tilia platyphyllos Scop.	P
Melanthiaceae	
Veratrum album L.	W
Myristicaceae	
Myristica fragrans Houtt.	P*

Family Scientific Name	Origin
Myrtaceae	
Syzygium aromaticum (L.) Merr. and L.M.Perry	P*
Oleaceae	
Fraxinus excelsior L.	W
Syringa vulgaris L.	C
Olea europaea L.	P
Onagraceae	
Epilobium angustifolium L.	W
Ophioglossaceae	
Botrychium lunaria (L.) Sw.	W
Orchidaceae	
Gymnadenia nigra (L.) Rchb. f. Syn *Nigritella nigra* (L.) Rchb f.	W
Orobanchaceae	
Euphrasia officinalis L. subsp. *rostkoviana* (Hayne) Towns.	W
Rhinanthus alectorolophus (Scop.) Pollich	W
Oxalidaceae	
Oxalis acetosella L.	W
Papaveraceae	
Chelidonium majus L.	W
Papaver somniferum L.	C
Pinaceae	
Abies alba Mill.	W
Larix decidua Mill.	W
Picea abies (L.) H. Karst.	W
Pinus cembra L.	W
Pinus mugo Turra	W
Pinus sylvestris L.	W
Plantaginaceae	
Plantago afra L. syn *P. psyllium* L.	P
Plantago lanceolata L.	W
Plantago major L.	W
Plantago media L.	W
Veronica fruticans Jacq.	W
Poaceae	
Arrhenatherum elatius (L.) P.Beauv. ex J.Presl and C.Presl.	W
Avena sativa L.	C
Cynodon dactylon (L.) Pers.	W
Festuca ovina L.	W
Hordeum vulgare L.	C
Oryza sativa L.	P*
Secale cereale L.	C
Stipa pennata L.	W
Triticum sp.	C
Zea mays L.	P*

Family Scientific Name	Origin
Polygonaceae	
Bistorta officinalis Delarbre syn *Persicaria bistorta* (L.) Samp.	W
Polygonum aviculare L.	W
Rheum rhabarbarum L.	C
Rumex acetosa L.	W
Rumex acetosella L.	W
Rumex alpinus L.	W
Polypodiaceae	
Dryopteris filix-mas (L.) Schott	W
Polypodium vulgare L.	W
Primulaceae	
Primula pedemontana E.Thomas ex Gaudin	
Primula veris L.	W
Ranunculaceae	
Clematis vitalba L.	W
Pulsatilla alpina (L.) Delarbre	W
Ranunculus kuepferi Greuter and Burdet	W
Ranunculus montanus Willd.	W
Trollius europaeus L.	W
Rosaceae	
Alchemilla xanthochlora Rothm. syn *A. vulgaris* L.	W
Amelanchier ovalis Medik.	W
Aria edulis (Willd.) M.Roem. syn *Sorbus aria* (L.) Crantz	W
Aruncus dioicus (Walter) Fernald	W
Filipendula ulmaria (L.) Maxim.	W
Fragaria vesca L.	W
Malus domestica (Suckow) Borkh.	P
Prunus avium L.	C
Prunus amygdalus Batsch syn *Prunus dulcis* (Mill.) D.A.Webb	P
Rosa × *alba* L.	C
Rosa canina L.	W
Rosa × *centifolia* L.	C
Rubus idaeus L.	W
Rubus saxatilis L.	W
Sorbus aucuparia L.	W
Rubiaceae	
Coffea sp.	P*
Galium verum L.	W
Galium lucidum All.	W
Galium mollugo L.	W
Rubia tinctorum L.	P
Salicaceae	
Salix babylonica L.	P
Salix caprea L.	W
Salix purpurea L.	W

Family Scientific Name	Origin
Sapindaceae	
Acer pseudoplatanus L.	W
Scrophulariaceae	
Scrophularia nodosa L.	W
Verbascum thapsus L.	W
Solanaceae	
Solanum lycopersicum L.	C/P
Solanum tuberosum L.	C
Thymelaceae	
Daphne mezereum L.	W
Tropaeolaceae	
Tropaeolum majus L.	C
Urticaceae	
Parietaria officinalis L.	W
Urtica dioica L.	W
Verbenaceae	
Aloysia citrodora Palau	P
Verbena officinalis L.	P
Viburnaceae	
Sambucus nigra L.	W
Sambucus racemosa L.	W
Viburnum lantana L.	W
Violaceae	
Viola calcarata L.	W
Viola odorata L.	W
Viola tricolor L.	W
Vitaceae	
Vitis vinifera L.	P
NON-PLANT-BASED FOODS AND REMEDIES	
LICHENES	
Parmeliaceae	
Cetraria islandica (L.) Ach.	W
FUNGI	
Agaricaceae	
Calvatia gigantea Lloyd	W
Lycoperdon perlatum Pers.	W
Macrolepiota procera Singer	W
Boletaceae	
Boletus edulis Bull.	W
Leccinum scabrum (Bull.) Gray	W
Cantharellaceae	
Cantharellus cibarius Fr.	W

Family Scientific Name	Origin
Clavariaceae	
Ramaria botrytis (Pers.) Bourdot	W
Fomitopsidaceae	
Fomitopsis officinalis (Vill.) Bondartsev and Singer	W
Suillaceae	
Suillus granulatus (L.) Roussel	W
Suillus grevillei (Klotzsch) Singer	W
OTHERS	
Insect galls	
Ibex marrow	
Marmot fat	
Quinine	
Theriaca	
Honey	
Snake skin	
Breast milk	
Mud	
raw wool	

W, wild;

C, cultivated;

P, purchased from adjacent areas at lower altitude (from Aosta Valley (e.g., Aymavilles, Introd or from Piedmont)).

P*, commercially purchased from distant areas.

The local importance of each species was calculated by using the Relative Frequency of Citation (RFC). In *Table 3* the species that obtained RFC values > 0.50 were reported.

Table 3 Species with a hight value of Relative frequency of Citation (RFC>0.50) [99].

Species	FC	NC	RFC	Species	FC	NC	RFC
Peucedanum ostruthium	66	4	0.97	*Artemisia genipi.*	50	4	0.74
Urtica dioica	62	7	0.91	*Artemisia absinthium*	49	5	0.72
Blitum bonus-henricus	61	1	0.90	*Achillea erba-rotta*	48	3	0.71
Juniperus communis	60	5	0.88	*Fragaria vesca*	47	2	0.69
Bistorta officinalis	59	2	0.87	*Linum usitatissimum*	46	2	0.68
Vaccinium myrtillus	58	3	0.85	*Pinus cembra*	44	4	0.65
Taraxacum officinale aggr.	55	4	0.81	*Tussilago farfara*	41	1	0.60
Rubus idaeus	55	2	0.81	*Picea abies*	40	4	0.59
Malva neglecta	55	3	0.81	*Gentiana punctata*	38	5	0.56
Arnica montana	53	3	0.78	*Carum carvi*	38	3	0.56
Rosa canina	52	4	0.77	*Larix decidua*	37	4	0.54
Berberis vulgaris	52	5	0.77	*Rumex acetosa*	34	2	0.50
Viola calcarata	51	3	0.75	*Polypodium vulgare*	34	3	0.50

FC, Number of informants mentioning the species.
NC, Number of categories of use
RFC, Relative Frequency of Citation.

Human disorders were classified in 14 categories based on the International Statistical Classification of Diseases and Related Health Problems (ICD-10) by the World Health Organization [115], as reported in *Table 4*.

Table 4 Factor informant consensus (Fic) index related to disease subcategories [99]

Disease subcategories	Number of citations	Number of species	FIC
Respiratory tract	352	36	0.90
Musculoskeletal system and connective tissue	156	25	0.85
Skin and subcutaneous tissues	219	36	0.84
Digestive system	315	52	0.84
Genitourinary tract	169	32	0.82
Sensory system (eye and adnexa; ear and mastoid process)	62	13	0.80
Dental and oral	33	9	0.75
Circulatory system	79	23	0.72
Pregnancy, childbirth, and the puerperium	50	15	0.71
Infections and parasitosis	50	16	0.69
Nervous system	73	23	0.69
Symptoms and signs not elsewhere classified	40	19	0.54

Considering the use of plants in relation to specific disease categories, the Fidelity Level (FL) was calculated and the species with a FL > 70% and with at least 10 citations in the disease category for which the highest FL has been obtained were selected (*Table 5*). *Figure 5* shows images of some important medicinal plants mentioned.

Table 5 Fidelity level (FL) value of medicinal plants against a given disease subcategory [99].

Species	FL	NI	NC	Disease subcategories
Cetraria islandica	100%	28	30	Respiratory tract
Pinus cembra	100%	19	19	Respiratory tract
Arctostaphylos uva-ursi	100%	18	18	Genitourinary tract
Chelidonium majus	100%	10	11	Skin and subcutaneous tissues
Tanacetum vulgare	100%	10	11	Infections and parasitosis
Pinus sylvestris	100%	9	10	Respiratory trat
Viola calcarata	98,30%	49	58	Respiratory tract
Pinus mugo	95,80%	24	24	Respiratory tract
Tussilago farfara	91,70%	41	48	Respiratory tract
Allium sativum	85,70%	20	21	Infections and parasitosis
Plantago major	84,60%	13	13	Skin and subcutaneous tissues
Gentiana punctata	82,80%	22	29	Digestive system
Plantago media	78,40%	24	37	Skin and subcutaneous tissues
Arnica montana	76,40%	53	72	Musculoskeletal system
Juniperus communis	72,50%	50	80	Digestive system

NI number of informants reporting the species for a given disease subcategory
NC number of citations of the species for a given disease subcategory

Figure 5 Medicinal plants: (A) *Peucedanum ostruthium;* (B) *Arnica montana;* (C) *Salvia officinalis;* (D) *Achillea erba-rotta;* (E) *Malva sylvestris;* (F) *Tussilago farfara;* (G) *Plantago afra;* (H) *Viola calcarata;* (I) *Juniperus communis;* (J) *Pinus sylvestris* syrup; (K) *Pinus cembra* syrup.

Since *Peucedanum ostruthium* is the species with the higher value of RFC index and having been the subject of further subsequent investigations during the PhD, its documented uses have been reported in full in the *Table 6*.

Table 6. Documented uses of *Peucedanum ostruthium* in the Gran Paradiso National Park (Aosta Valley) [99].

Family Scientific Name Voucher Number	Vernacular and Dialectal Names	Origin and Parts Used		Ethnobotanical Uses
Apiaceae *Peucedanum ostruthium* (L.) W.D.J.Koch Syn. *Imperatoria ostruthium* L. P.ost.HBPNGP_ETN	Imperatoria Agrù	Wild	Rhizomes Leaves Flowers (fresh or dried)	**Dom/Hand:** roots fumigations as disinfectants for the stables. **Liq**: roots flavouring in liqueur and grappa as digestive. **Med**: to treat skin problems: leaves, sometimes sprinkled of hot oil or butter, or directly applied; leaf or root decoction used as compress (e.g., against wounds, burns, thorns, insect bites, infections, etc.) (**skin**) (**cip**); compress against muscle inflammation and contusions, hematomas and rheumatic pains; decoction for foot baths and compresses against leg and knees pain (**musc**); roots or leaves directly applied against caries or mouth ulcers and abscesses (**dent**); decoction for vaginal washings in case of infections or after delivery (**uro-gen**) (**pcp**); ointment of chopped roots mixed with marmot fat applied to the chest against respiratory problems (**resp**); flowers infusion to be ingested against inflammations and fever (**abn**); chopped root added in the beaten egg yolk and ingested as invigorator (**enm**). **Vet**: chopped root placed in a butter ball given to livestock against digestive problems; decoction or minced root mixed with fat or butter used to treat hoof problems; leaves and roots decoction used as external and internal disinfectant post-partum for cows.

Use Categories: **Dom/hand**: Domestic and handicraft; **Liq**: Liquoristic; **Med**: Medicinal; **Vet**: Veterinary. **Medicinal subcategories**: (**abn**) abnormal symptoms, signs not elsewhere classified (including fever); (**cip**) certain infections and parasitosis; (**dent**) dental and oral; (**dig**) digestive tract; (**enm**) endocrine, nutritional and metabolic; (**gen-uri**) genitourinary system; (**musc-skel**) musculoskeletal system and connective tissue; (**nerv**) nervous system; (**pcp**) pregnancy, childbirth and puerperium; (**resp**) respiratory tract; (**skin**) skin and subcutaneous tissues.

Discussion

Several uses regarding the alimentary, medicinal, veterinary, and domestic sectors, as well as liquors, were recorded. The present results have been compared and found to be partly similar to ethnobotanical data from other Italian alpine areas [77–87,89,90].

The medicinal plants used are applied especially as a remedy for respiratory diseases; e.g., the use of different Pinaceae species was reported for the preparation of antitussive syrups with expectorant and decongestant properties. Phenolic acids, flavonoids, proanthocyanidins, terpenoids, and resin acids with antimicrobial and anti-inflammatory activities can contribute to their healthy effect on the respiratory system [116–119]. Among the species quoted for the treatment of cough, cold, and flu, *Viola calcarata,* as well *as Cetraria islandica*, *Salvia officinalis,* and *Artemisia genepi* were recorded. *Viola calcarata*, scarcely referred to in other ethnobotanical studies, contains different bioactive compounds such as flavonoids, rutin, and mucilages that could be related to its activity against airway problems [120]. Indeed, the effectiveness of quercetin-type flavonols such as rutin against viral infections of the lower respiratory tract has been recently demonstrated [121]. The traditional use of lichen *C. islandica* for the treatment of respiratory diseases is probably due to the presence of polysaccharides with antiviral [122] and anti-inflammatory properties [123]. Regarding *Artemisia* species, the bioactive compounds are mainly sesquiterpene lactones, which are probably involved in the activation of bitter receptors via stimulation of ciliary motion and in the relaxation of bronchial tissues [124]. In this way, these compounds prevent infections and improve ventilation; the absorption path of these volatile compounds explains the use of inhalations in addition to herbal tea, the main preparation traditionally used.

Digestive diseases are also very common in the studied area, and the use of different species of *Gentiana* (mainly *G. punctata*), *Achillea,* and *Artemisia*, as well as *J. communis* and *C. carvi*, was recorded. Skin and musculoskeletal problems often affected the population involved in manual and fieldwork and, consequently, several plants were cited for the treatment of these pathologies. The use of *Arnica montana* for musculoskeletal diseases was related to the presence of helenalin and dihidrohelenalin-type sesquiterpene lactones, flavonoids, and phenolic acids with anti-inflammatory activity [125–127]. *Plantago* spp. was widely used, being rich in polysaccharides and flavonoids with anti-inflammatory effects, for the treatment of wounds and other skin problems [128]. Some species were also indicated against infections and parasitosis, such as *Allium sativum* and *Tanacetum vulgare*. *A. sativum,* which, thanks to the presence of sulfur-containing phytoconstituents and flavonoids, showed antibacterial, antiviral, antifungal, antiprotozoal, antioxidant, and anti-inflammatory properties [129], whereas *T. vulgare*, rich in β-thujone, was responsible for the anthelmintic activity [130].

Various liqueurs and grappas were prepared as digestives, e.g., using the roots of *Gentiana punctata* and *G. lutea*, as well as the flowers of *G. acaulis* and *G. verna*. The aerial parts of different species, such as *Achillea erba-rotta* and *A. moschata,* were added to the Fernet liqueur, as were *Artemisia genipi*, *A. glacialis*, and *A. umbelliformis* in the Genepì liqueur. The Kummel liqueur was made by using the seeds of *C. carvi,* while the Arquébùse used the leaves of *T. vulgare*. In addition, several species belonging to the Pinaceae were quoted to flavor grappas and liqueurs (e.g., *Larix decidua*, *Picea abies*, *Pinus cembra*, *P. mugo,* and *P. sylvestris*). Even wild berries were used to flavor grappas (e.g., *Rubus idaeus*, *Vaccinium myrtillus,* and *Rosa canina*). Several liquors were also used for the treatment of diseases, especially respiratory and digestive problems.

Among the species collected as food, several plants were included; e.g., the leaves of *Bistorta officinalis*, *Blitum bonus-henricus*, *Urtica dioica*, and *Taraxacum officinale* were used as ingredients in soups, omelets, or stir-fried with butter and eaten as a side dish. Other wild species less frequently reported as ingredients for soups were *Silene vulgaris*, *Tragopogon pratensis*, *Primula veris*, *Phyteuma* spp., and *Rumex acetosa*.

Botrychium lunaria is a very valuable fern used in summer soups, especially in mountain pastures. Various wild or cultivated fruits were eaten fresh or used for jelly, jams, or syrups. The most quoted included *Fragaria vesca*, *Ribes nigrum, R. rubrum*, *Rubus idaeus, Vaccinium myrtillus*, followed by *Amelanchier ovalis*, *Berberis vulgaris*, *Hippophae rhamnoides*, *R. canina*, *Arctous alpina*, *Ribes uva-crispa, R. petraeum*, *Rubus saxatilis*, *Sambucus nigra*, *Vaccinium uliginosum,* and *V. vitis-idaea*. Several species were mainly eaten in the past as a snack, e.g., the bulbs of *Bunium bulbocastanum* for their chestnut flavor. Many plants were reported as rennet used in the past in cheesemaking, e.g., *Ranunculus* sp.pl., *Galium* sp.pl., *Lotus corniculatus*, *Urtica dioica*, *Bistorta officinalis,* and *A. genipi*.

Several remedies were cited as veterinary prescriptions, as in the case of cow pregnancy, where some emollient plants were given in the form of decoction, such as *Linum usitatissimum* and *Malva neglecta* or *M. sylvestris*. Extensive knowledge of the best plants for milk production has been documented, and among these, one of the most quoted was *Trifolium alpinum*.

Several species were used for the manufacture of small tools and as construction wood. *Larix decidua* and *Picea abies* were used for construction purposes such as to build roofs and perimeter walls, respectively. *Pinus cembra* was considered the most valuable wood for building furniture because of its resinous smell and its mothproof power. *Fraxinus excelsior* was used to make blades and pickaxes. The branches of *Sambucus nigra*, *Viburnum lantana*, *Clematis vitalba*, *Salix caprea,* and *S. purpurea* were used for making baskets such as the traditional “gerle.” Other uses were mentioned, such as religious and magic-ritual ones.

It seems important to underline the fact that non-vegetable remedies have also been documented as they are closely linked to the resources provided by the territory; e.g., marmot fat and the ibex bone marrow were indicated to treat respiratory and musculoskeletal diseases. Additionally, the presence of local healers called *Rabeilleurs* showing therapeutic ability in massage cases for musculoskeletal problems was also documented. In Aosta Valley also persists the practice of *Sécrèts*, which consists of the treatment of several diseases (e.g., warts, worms, burns, pains, and others) by using prayers and rituals. The healing formulas were kept secret and handed down mainly orally, usually within the family or village [131].

Although the patois dialect in the Aosta Valley, as already mentioned, is very widespread, several differences in the plant dialectal names among the three valleys were found. The documented dialectal names used in Aosta Valley were similar to those used in Lower and Central Valais (Switzerland) (e.g., *J. communis* called *tsénèvro* in Aosta Valley and *dzenièvro* in Switzerland, *Alchemilla vulgaris* reported as *porta rusò* and *porta-rozò* and *V. vitis-idea* known as *gravelòn* and *gravèlong*, respectively) [132]. This linguistic knowledge is nowadays disappearing because of the linguistic homologation that characterizes modern societies and because of the lack of knowledge of plants in younger generations. The documentation of these local names is also crucial to preserving TEK handed down orally from one generation to the next.

Conclusion

Our survey has provided an exhaustive prospect of the ethnobotanical traditional knowledge in the territory of the Gran Paradiso National Park, located in the Aosta Valley, an area so far scarcely studied from the perspective of plant folk traditions. Data collected confirmed that this knowledge mainly remains in the memories of the eldest population and enhanced the importance of the mountain areas as biocultural refugia. However, TEK is fast disappearing among the new generations and, therefore, documenting and preserving such information is crucial in reducing the loss of biocultural diversity. The results of our study contribute to the goal of Article 8(j) of the Convention on Biological Diversity (CBD), which recognizes the importance of preserving the traditional knowledge of indigenous communities to conserve biological diversity and ensure the sustainable use of natural resources.

We observed that the use of wild plants to prepare typical dishes and liqueurs, as well as the use of medicinal plants to cure the most common diseases of humans and livestock, are still well preserved by the local population.

We noted that local people attributed a great healing value to *Peucedanum ostruthium.* In fact, in these valleys, in addition to the underground root/rhizome portion, which was mostly used and whose use has been renowned since ancient times, leaves and inflorescences were also employed to treat some diseases. However, throughout the Alps, the tendency remains to use the root/rhizome and discard the rest of the plant.

For this reason, we decided to continue with the study, carrying out the phytochemical characterization of the extract obtained from the leaves of this species and analysing its biological activity. Leaves constitute, in fact, a significant biomass that is generally discarded during wild harvesting. This aspect also takes on importance because projects are underway for the sustainable cultivation of this plant in the Alpine area [133–135].

Scientific contribution

Data have also been presented in Scientific congress receiving the Taddei Prize:

- **Danna C.**, Poggio L., Cornara L., Master degree dissertation. Ethnobotanical research in the Gran Paradiso National Park (Aosta Valley, Western Alps, Italy). XVIII Congresso Nazionale di Fitoterapia (S.I.Fit), XI SYRP Conference, 21–23 May 2021 (Online)

Detailed and complete data have been published and extensively discussed in the following article published in Open Access by the Editor MDPI under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/):

plants

MDPI

Article

Ethnomedicinal and Ethnobotanical Survey in the Aosta Valley Side of the Gran Paradiso National Park (Western Alps, Italy)

Cristina Danna [1,*], Laura Poggio [2], Antonella Smeriglio [3], Mauro Mariotti [1] and Laura Cornara [1,*]

[1] Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genoa, Italy; m.mariotti@unige.it
[2] Forest Botanical Conservation Office, PNGP, Paradisia Alpine Botanic Garden, Valnontey 44, 11012 Aosta, Italy; laura.poggio@pngp.it
[3] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Acontres 31, 98166 Messina, Italy; antonella.smeriglio@unime.it
* Correspondence: cristina.danna@edu.unige.it (C.D.); laura.cornara@unige.it (L.C.)

Abstract: Most of traditional knowledge about plants and their uses is fast disappearing because of socio-economic and land use changes. This trend is also occurring in bio-cultural refugia, such as mountain areas. New data on Traditional Ethnobotanical Knowledge (TEK) of Italian alpine regions were collected relating to three valleys (Cogne, Valsavarenche, Rhêmes) of the Gran Paradiso National Park. Extensive dialogues and semi-structured interviews with 68 native informants (30 men, 38 women; mean age 70) were carried out between 2017 and 2019. A total of 3918 reports were collected, concerning 217 taxa (including 10 mushrooms, 1 lichen) mainly used for medicinal (42%) and food (33%) purposes. Minor uses were related to liquor making (7%), domestic (7%), veterinary (5%), forage (4%), cosmetic (1%) and other (2%). Medicinal plants were used to treat 14 ailment categories, of which the most important were respiratory (22%), digestive (19%), skin (13%), musculoskeletal (10%) and genitourinary (10%) diseases. Data were also evaluated by quantitative ethnobotanical indexes. The results show a rich and alive traditional knowledge concerning plants uses in the Gran Paradiso National Park. Plants resources may provide new opportunities from the scientific point of view, for the valorization of local products for health community and for sustainable land management.

Keywords: Cogne valley; Valsavarenche valley; Rhêmes valley; BioRefugia; cultural heritage; traditional knowledge; medicinal plants; human well-being

Citation: Danna, C.; Poggio, L.; Smeriglio, A.; Mariotti, M.; Cornara, L. Ethnomedicinal and Ethnobotanical Survey in the Aosta Valley Side of the Gran Paradiso National Park (Western Alps, Italy). *Plants* **2022**, *11*, 170. https://doi.org/10.3390/plants11020170

PART IB

Micromorphological, Phytochemical and Pharmacological analysis of *Peucedanum ostruthium* (L.) W. D. J. Koch

Introduction

Peucedanum ostruthium (L.) W. D. J. Koch (syn. *Imperatoria ostruthium* L.), commonly known as masterwort, is a rhizomatous perennial species belonging to the Apiaceae family. Native to the mountains of Central and Southern Europe, it is widespread around the world and generally grows in riverbanks and wet grassy and anthropic areas (*Figure 1*).

Figure 1 Plants of *P. ostruthium* in their natural environment, and freshly sampled plants [136].

The rhizome has a long tradition for liqueur production and as a popular medicine, to such an extent that during the 19th century, the plant was known as "*Divinum remedium*" (divine remedy) [137]. Records from historical and modern ethnobotanical studies have reported a long list of popular uses of the plant [87,99,114,132]. More specifically, it has been employed as a stimulant, stomachic, and diuretic for rheumatic, chronic inflammatory, and musculoskeletal diseases, as well as for skin problems, typhoid fever, paralytic conditions, and delirium tremens [138,139]. Some experimental studies have been carried out investigating the phytochemical profiles of EOs and extracts of *P. ostruthium*, also focusing on some bioactivities such as anti-inflammatory and antibacterial activities [138,140–145].

The rhizome is the part of the plant officially recognized in traditional medicine, as shown by its inclusion in the European BELFRIT list [146]. Among its different uses, the rhizome is known as a remedy for superficial injuries and wounds, but similar use of the leaves has been reported among the local population in Aosta Valley [99] (*Figure 2*). This suggests new possibilities of exploitation for medicinal purposes and a re-evaluation of the leaves of this plant, which are generally discharged during rhizome harvesting for medicinal purposes.

Figure 2 Leaves and rhizomes of *P. ostruthium* used in Aosta Valley as traditional medicines.

In this study, we provide experimental confirmations of the therapeutic properties of the *P. ostruthium* rhizome extract, as suggested by traditional usage. Moreover, we confirm the indications of empirical evidence suggesting that the leaves can also be used as a remedy, thereby opening the way to more intensive exploitation of the plant. We examined the effects of polyphenol-rich leaf and rhizome hydroalcoholic extracts on various activities like antioxidants, anti-inflammatory responses, and wound healing using in vitro tests without cells and with cells.

Materials and Methods

Plant Material

Plant material was collected at the Gran Paradiso National Park, Buthier, Cogne, Aosta Valley, Italy (alt: 1555 m a.s.l.; lat. 45.603671; long. 7.3494468) (*Figure 3*). Permissions for plant sampling were obtained from the National Park Authority (Ente Parco Nazionale Gran Paradiso, Torino, Italy) n. 1884, 6 September 2020; n. 2432/2020, 7 October 2020; n. 2959/2020 8 October 2020. Voucher specimens were deposited at the Ethnobotanical Herbarium of the Gran Paradiso National Park, Paradisia Alpine Botanic Garden (Valnontey, Cogne-AO, Italy) (P.ost. HBPNGP_ETN). The nomenclature follows the Plants of the World Online, Kew Science classification, available at http://www.plantsoftheworldonline.org/ (accessed on 28 June 2022), and the corresponding synonymous was added, according to the IPFI: Index Plantarum, available online at https://www.actaplantarum.org/flora/flora.php (accessed on 28 June 2022).

Figure 3 Leaves and rhizomes of *P. ostruthium* collected in Aosta Valley.

Chemicals

Reagents were purchased from Sigma-Aldrich (Milan, Italy) unless otherwise specified. Reference standards (purity ≥ 98%) of compounds reported in Table 2 were purchased from Extrasynthase (Genay, France) and Merck (Darmstadt, Germany).

Micromorphological Analyses

Leaves and rhizomes micromorphological features were elucidated by LM and SEM. For LM analyses, fresh leaves and rhizomes were hand-cut with a razor blade, and cross sections were observed using a transmission light Leica DM 2000 microscope equipped with a DFC 320 camera (Leica Microsystems, Wetzlar, Germany). Phloroglucinol-HCl was used as dye for lignin, while toluidine blue O (TBO) was used for highlighting phenolic substances [147]. For SEM analyses, small pieces of leaves and rhizomes (~2 cm^2) were incubated overnight at 4 ◦C in 70% ethanol/FineFIX solution (Milestone s.r.l., Bergamo, Italy) [148]. Samples were then subjected to dehydration through ethanol series, critical point dried (K850CPD 2 M Strumenti s.r.l., Roma, Italy), disposed on aluminium stubs, and sputter-coated with 20 nm gold. Observations were carried out under a Vega3-Tescan LMU SEM microscope (TescanUSA Inc., Cranberry Twp, PA, USA) at an accelerating voltage of 20 kV.

Extraction

Fresh leaves and rhizomes were powdered using a blade mill (IKA® A11, IKA®-Werke GmbH & Co. KG, Staufen, Germany) with liquid nitrogen. A sample preparation procedure was developed and optimized to maximize the extraction of all classes of bioactive compounds (coumarins, phenolic acids, and flavonoids), using food-grade solvents. Maximum yield w/w of dried extracts was obtained with 80:20 (EtOH/H_2O), v/v, for 6 h, repeated three times. Ten grams of rhizome and leaf frozen powders were extracted under continuous stirring in the dark at room temperature. Extracts were then centrifuged at 3000× for 15 min at 4 ◦C. Thereafter, the supernatants were collected and evaporated until dry by a rotary evaporator (Büchi R-205, Cornaredo, Italy) at 37 ◦C. Yields of 31.70% and 32.50% were obtained for the leaf (LE) and rhizome extract (RE), respectively. Extracts were then suspended and properly diluted in a hydroalcoholic mixture for phytochemical characterization and subsequent analyses.

Phytochemical Screening

TLC

Preliminary chemical qualitative aspects of the *P. ostruthium*' leaves and rhizomes extracts were determined using a TLC system (CAMAG, Muttenz, Switzerland). Extracts solutions were prepared for concentration 10 mg/mL in methanol. The samples of the extracts were applied into 6.5 x 10 cm TLC plates (silica gel 60, F254, Merck, Darmstadt, Germany) under the control of the software platform vision CATS with the following standard settings: 5 tracks with 5 mm bands, 1 cm distance from the lower edge, 1,3 cm from the left and right edges, and 3 mm between the different tracks. The samples were applied in different volume on the plates: both 5 and 10 µl for LE and RE. The plates were developed inside a glass jar (containing the mobile phase at the base) until the extract solutions reached 9 cm, then the plates were removed and dried. For investigating the phytochemical profile several mobile phases were tested: in normal phase plates: Ethyl acetate, methanol, water, formic acid (55:7:5:1; *v*/*v*/*v*/*v*); Dichloromethane, methanol (70:30; v/v); Dichloromethane, methanol (97:3; v/v); in reverse phase plates: water, acetonitrile (80:20; v/v). The plates were documented under UV 254 and 366 nm and after spraying with sulfuric vanillin (i.e., 5% *w*/*v* vanillin in MeOH/5% *v*/*v* H_2SO_4 in MeOH 1:1 *v*/*v*) in the visible spectrum and under UV 366 nm using the TLC Visualizer.

Total Phenols, Flavonoids, Vanillin Index and Proanthocyanidins

The methodologies applied are extensively reported by Danna et al. [136].

^{1}H-NMR analysis

For ^{1}H-NMR measurement, 10 mg of hydroalcoholic extracts were dissolved both in 1 mL of methanol-d4 (MeOD) and 1 mL of Dimethyl sulfoxide (DMSO). After sonication (5 min) on an Ultra Sonic bath (Elma Schmidbauer GmbH, Germany) 600 µL of the solutions were transferred to 5 mm NMR tubes (LabScape, Bruker, Germany). The ^{1}H-NMR spectra were acquired at 298 K on a Bruker Avance Neo 400MHz NMR spectrometer equipped with a 5 mm PABBI 1H/D-BB inverse detection probe. Data acquisition and processing were 209 done with Bruker TopSpin 4.1.4. Chemical shift values were referenced to the residual solvent signal (3.31 for MeOD and 2.5 for DMSO).

RP-LC-DAD-ESI-MS Analysis

Polyphenol characterization of LE and RE was carried out by RP-LC-DAD-ESI-MS analysis. Chromatographic elution was carried out by a Luna Omega PS C18 column (150 mm × 2.1 mm, 5 µm; Phenomenex, Torrance, CA, USA) at 25 ◦C by using mobile phase 0.1% formic acid (Solvent A) and methanol (Solvent B). The UV–Vis spectra were recorded ranging from 190 to 600 nm, and chromatograms were acquired at different wavelengths (220, 260, 292, 330, and 370 nm) to identify all polyphenol classes. A mass spectrometer (ion trap, model 6320, Agilent Technologies, Santa Clara, CA, USA) operating in the negative (ESI−) and positive (ESI+) ionization mode was used. Data were acquired by Agilent ChemStation software version B.01.03 and Agilent trap control software version 6.2. Detailed information regarding the chromatographic elution program and the experimental parameters of the mass spectrometer are extensively reported by Danna et al. [136].

Antioxidant Activity

The antioxidant and free-radical scavenging activity of *P. ostruthium* LE and RE was evaluated by several *in vitro* colorimetric assays based on different mechanisms and reaction environments. The results were expressed as the inhibition percentage (%) of the oxidative/radical activity, calculating the IC50 with the respective C.L. at 95% by Litchfield and Wilcoxon's test using PHARM/PCS software version 4 (MCS Consulting, Wynnewood, PA, USA).

DPPH, TEAC, FRAP and ORAC Assays

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated according to Smeriglio et al. [149]. The Trolox equivalent antioxidant capacity (TEAC) was evaluated according to Monforte et al. [150]. Ferric reducing antioxidant power (FRAP) was evaluated according to Smeriglio et al. [151]. Oxygen radical absorbance capacity (ORAC) was evaluated according to Smeriglio et al. [152]. The methodologies applied are also briefly described by Danna et al. [136].

Anti-Inflammatory Activity

The anti-inflammatory activity of *P. ostruthium* LE and RE was evaluated by in vitro enzymatic and non-enzymatic assays.

Bovine Serum Albumin (BSA) Denaturation Assay and Protease Inhibition Assay

The ability of LE and RE to inhibit the heat-induced BSA denaturation was evaluated according to Smeriglio et al. [153]. The protease inhibitory activity was evaluated according to Smeriglio et al. [154]. Absorbance was recorded by a multi-well plate reader (Multiskan GO; Thermo Scientific, Waltham, MA, USA). The methodologies applied are also briefly described by Danna et al. [136].

Lipoxygenase (LOX) and Cyclooxygenase (COX-2) Inhibition Assays

The effects of extracts on LOX were evaluated using the Cayman's Lipoxygenase Inhibitor Screening Assay Kit Reagents (soybean lipoxygenase, item n. 760700, Cayman Chemical, Ann Arbor, MI, USA). The effects of extracts on COX were evaluated using the Cayman's COX inhibitor Screening Assay (item n. 560131, Cayman Chemical) on human recombinant COX-2 (item n. 460121, Cayman Chemical). The methodologies applied are briefly described by Danna et al. [136].

Enzymes inhibition

The inhibition of enzymes involved in skin extracellular matrix degradation (e.g. collagenase) and in the control of melanin production (e.g. tyrosinase) were evaluated by in vitro enzymatic assays.

Collagenase inhibitory activity

Collagenase inhibitory activity was determined using a slightly modified method according to the literature [155–157]. Briefly, the test samples (dissolved in DMSO and then diluted ensuring a final DMSO concentration ≤ 1%), enzyme solution (collagenase from *Clostridium histolyticum* SIGMA C9263 diluted in Buffer obtaining a concentration of 100 µg/mL) and Tris-HCl buffer 10mM (pH 7.3) were added in equal amounts (25µl) to 96-well microtiter plate and preincubated for 10 min at 37 °C. Afterwards, the substrate solution (fluorogenic peptide substrate MMP2 (MCA-Pro-Leu-Ala-Nva-DNP-Dap-Ala-Arg-NH2) SIGMA SCP0192 diluted in buffer obtaining a concentration of 55.5 µg/mL) was added (25µl) to initiate the reaction. The fluorescence values were measured at an excitation of 320 nm and an emission of 405 nm after 30 min incubation at 37 °C. These assays were performed in triplicate using phosphoramidon as a positive control (Phosphoramidon disodium salt SIGMA R7385, well concentration 3.75 µg/mL ∽50% inhibition). The inhibition ratio of the samples was calculated by comparing the absorbance values produced by the sample with that of the negative control.

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined using a slightly modified method according to the literature (Masuda et al., 2005), investigating for the ability of the extracts to inhibit the oxidation of L-DOPA (L-3,4-dihydroxyphenylalanine) to dopaquinone and subsequently to dopachrome by the enzyme tyrosinase. The extracts were initially dissolved in DMSO (10 mg/mL) and subsequently diluted in the proper concentration in phosphate buffer (PBS) 1/15 M (NaH2PO4/Na2HPO4), pH=6.7. Final concentrations of DMSO in the well did not exceed 2%. In 96-well plates, 80 µL of PBS buffer, 40 µL of sample in buffer and 40 µL mushroom tyrosinase SIGMA T3824 (92 Units/mL) in buffer, were mixed. The plate was incubated for 10 min at 25 °C, before 40 µL of 2.5 mM l-DOPA (3,4-Dihydroxy-L-phenylalanine) SIGMA diluted in buffer were added. After incubation at 25 °C for 5 min, the absorbance at 475 nm of each well was measured. Extracts were evaluated at 100 µg/mL and 300 µg/mL, in triplicate, blank samples for every fraction were also measured, whereas kojic acid (well concentration 2 µg/mL) and *Glycyrrhiza glabra* root methanolic extract (well concentration 5 µg/mL) were used as positive controls.

Cell Viability and Wound Healing Assays

The HaCaT human keratinocyte cell line was obtained from DKFZ, Deutsches Krebsforschungszentrum, Heidelberg, Germany [158], while L929 cells were from the Tissue Bank of the IRCCS San Martino Hospital (Genova, Italy). The cell lines were used at passage levels around 50 (HaCaT) and 130 (L929). Cell culture methodology is extensively described by Danna et al. [136].

Cell viability was evaluated by the 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) test in both cell lines (HaCaT and L929) exposed to increasing concentrations of extracts for 24 or 48 h. Absorbance data were used to obtain dose–response curves and derive IC_{05} and IC_{50} values.

Wound healing activity was evaluated with scratch wound assay as described by Bazzicalupo et al. [159] in both cell lines (HaCaT and L929) exposed to increasing concentrations of extracts for 24 h. Positive controls were obtained by incubation with 50 µg/mL allantoin (Sigma-Aldrich, 05670-25G). Wounded cell layers were photographed using a Leica M205 C stereomicroscope coupled to a Leica EZ 2.1.5 camera, wound widths and the number of migrated cells were measured by the ImageJ software (https://imagej.nih.gov/ij/, accessed on 28 June 2022). Wound closure was expressed as a percentage of control closure [160]. Cell migration was expressed as cell density within the wound space (number of cells per unit area).

Detailed methodologies are described by Danna et al. [136].

Statistical analysis

The statistical analysis was based on one-way analysis of variance (ANOVA) Student t with Bonferroni post-hoc tests for multiple pairwise mean comparisons, using the Data Analysis Tool Pack in Excel. Results were considered statistically significant for $p < 0.05$.

Results

Micromorphological Analysis

Peucedanum ostruthium' leaves and rhizomes collected in the wild were used for micromorphological analyses. Micromorphological characteristics of the leaves and rhizomes using LM and SEM were presented in *Figure 4* and *Figure 5*, respectively.

Light microscopy observations of leaf transversal sections (*Figure 4*) show the midvein vascular bundles surrounded by secretory channels (sec), in which the essential oil is produced. The channels are protected by collenchyma protrusions (*Figure 4*A). Polyphenol/tannin cells in the parenchyma are highlighted by TBO staining appearing in blue (*Figure 4*B). In scanning electron microscopy (SEM) micrographs non-glandular trichomes emerging on the leaf surface in correspondence with collenchyma protrusions are well visible (*Figure 4*C). In addition, in a transversal section of the dorsoventral leaf anatomy, the palisade and spongy parenchyma can be seen (*Figure 4*D).

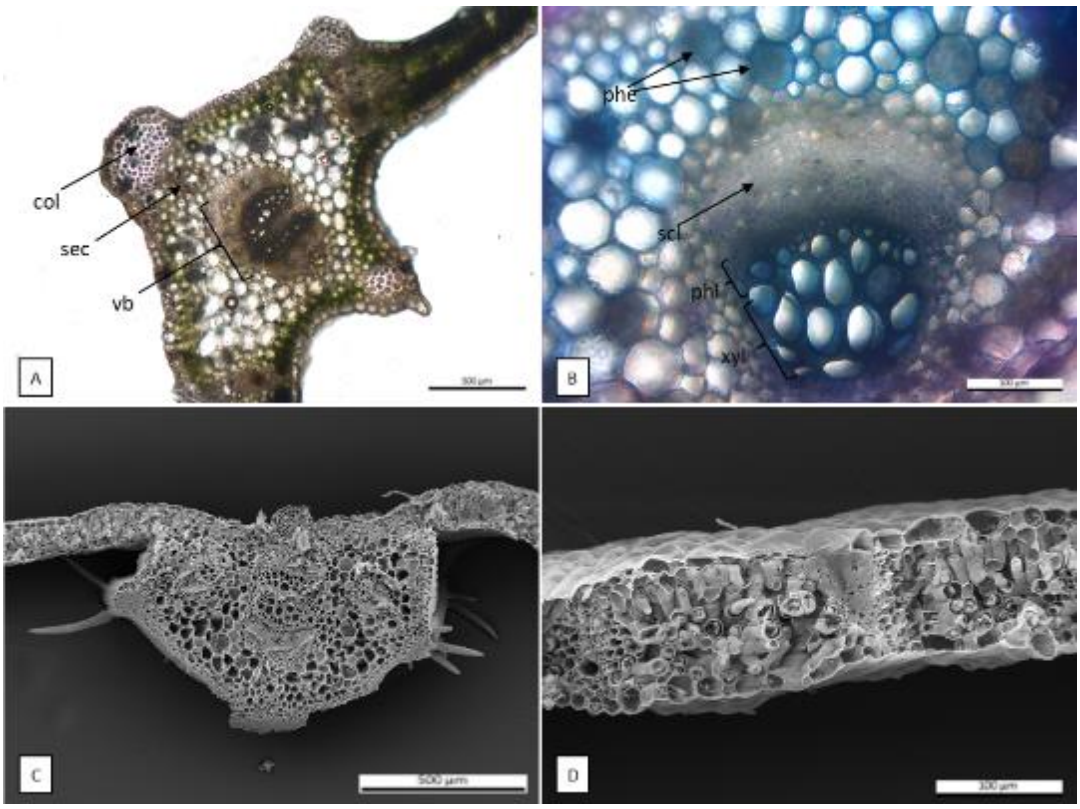


Figure 4 Micromorphological features of the *P. ostruthium* leaf. LM (**A**) cross section at the midvein level. col: collenchyma; sec: secretory channel; vb: vascular bundle. (**B**) vascular bundle after staining with TBO: several parenchymatous cells rich in polyphenols appear blue/green (phe). scl: sclerenchyma; phl: phloem; xyl: xylem. SEM (**C**) cross section at the midvein level showing typical trichomes located on the collenchyma protrusions. (**D**) leaf cross section showing the mesophyll structure [136].

Light microscope analysis of the rhizome reveals a structure consisting of different vascular bundles and numerous secretory channels (*Figure 5*A). The stratified phloem shows little or no lignified fibers, while the xylem is interspersed with sclerenchyma fibers with extremely thick and lignified walls (*Figure 5*B). SEM micrographs highlight the circular shape of the rhizome with a secondary structure of the fascicular type, in which a ring of 30–70 collateral vascular bundles surround the central medulla and are separated by multiseriate medullary rays (*Figure 5*C). Large secretory ducts are located both in the cortex and in the peripheral zone of the medulla. The rhizome is rich in amiliferous parenchyma and around the secretory channels the parenchymatous cells are oriented in concentric layers (*Figure 5*D). Externally, the rhizome is covered by cork layers and a multi-layered phelloderm.

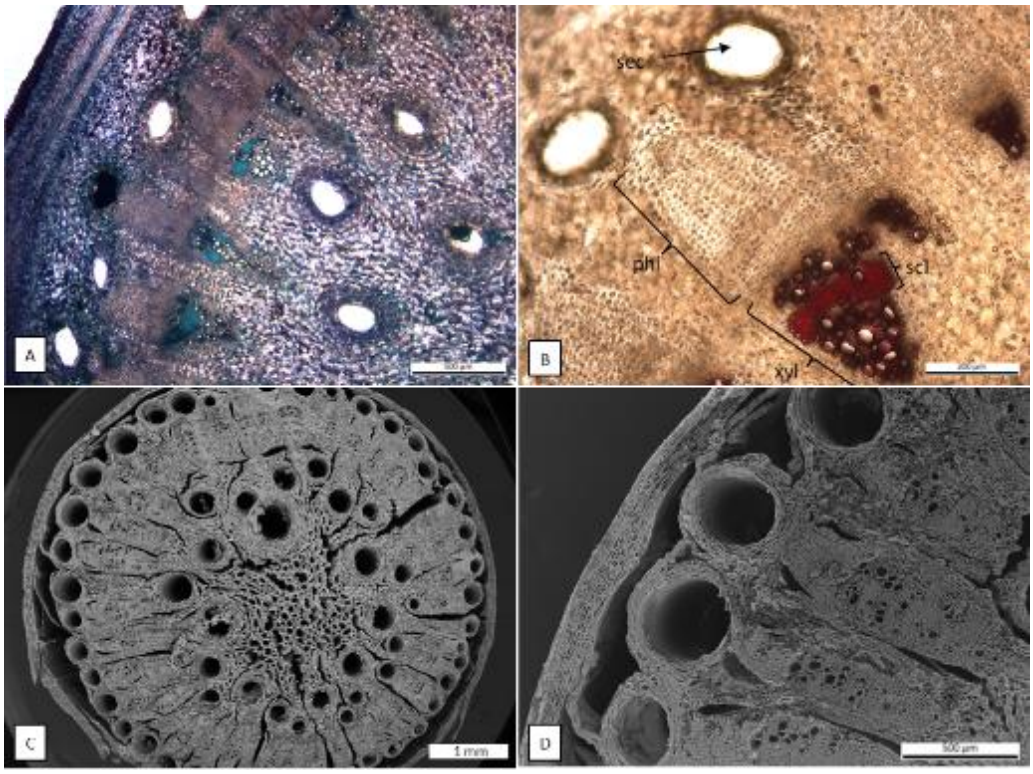


Figure 5 Micromorphological features of the *P. ostruthium* rhizome. LM (**A**) cross section stained with TBO; (**B**) cross-section after phloroglucinol-HCl staining, showing xylem vessels (xyl) interspersed with sclerenchyma fibers stained in purple/red (scl). sec: secretory channel; phl: stratified phloem. SEM (**C**) cross section of an old rhizome; (**D**) Detail of at higher magnification [136].

Phytochemical Analyses

TLC

Preliminary chemical qualitative aspects of the *Peucedanum ostruthium*' leaves and rhizomes extracts have been determined using a TLC system and several mobile phases. TLC chromatogram developed using a normal phase plate and ethyl acetate, methanol, water, formic acid (55:7:5:1; *v*/*v*/*v*/*v*) as mobile phase is presented in *Figure 6*; TLC chromatogram developed using a normal phase plate and dichloromethane, methanol (70:30; v/v) as mobile phase is presented in *Figure 7*; TLC chromatogram developed using a normal phase plate and dichloromethane, methanol (97:3; v/v) as mobile phase is presented in *Figure 8*; TLC chromatogram developed using a reverse phase plate and water, acetonitrile (80:20; v/v) as mobile phase is presented in *Figure 9*.

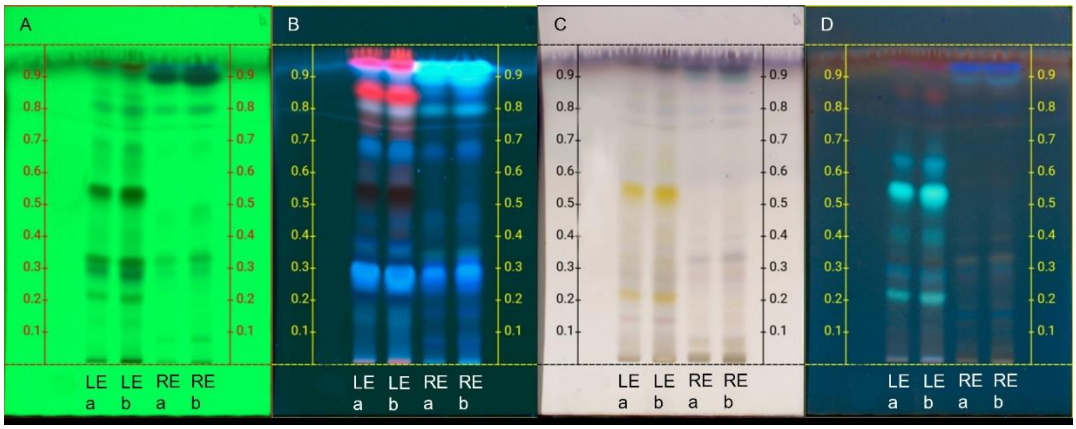


Figure 6 TLC chromatogram developed using a normal phase plate and ethyl acetate, methanol, water, formic acid (55:7:5:1; v/v/v/v) as mobile phase. A: 254 nm; B: 366 nm; C: visible spectrum after vanillin staining; D: 366 nm after vanillin staining. LE a: leaf extract (5 µl of 10 mg/ml); LE b leaf extract (10 µl of 10 mg/ml); RE a: rhizome extract (5 µl of 10 mg/ml); RE b rhizome extract (10 µl of 10 mg/ml).

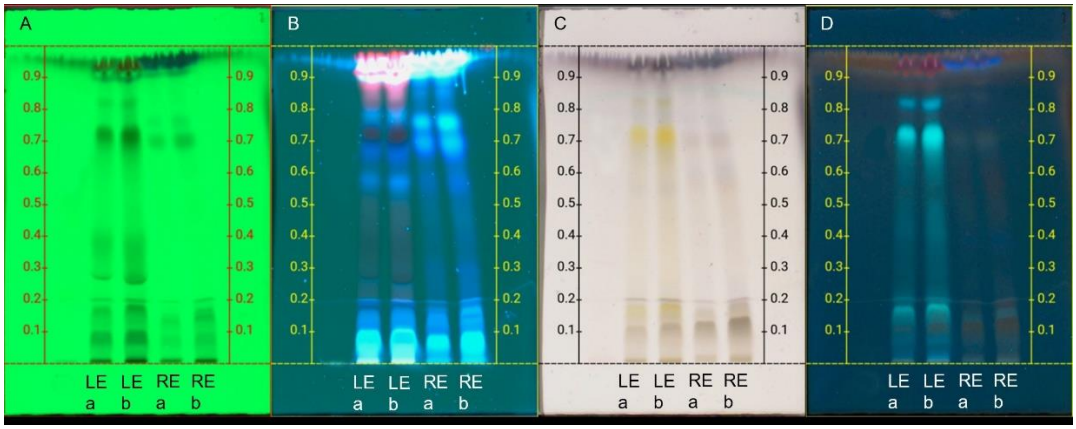


Figure 7 TLC chromatogram developed using a normal phase plate and dichloromethane, methanol (70:30; v/v) as mobile phase. A: 254 nm; B: 366 nm; C: visible spectrum after vanillin staining; D: 366 nm after vanillin staining. LE a: leaf extract (5 µl of 10 mg/ml); LE b leaf extract (10 µl of 10 mg/ml); RE a: rhizome extract (5 µl of 10 mg/ml); RE b rhizome extract (10 µl of 10 mg/ml).

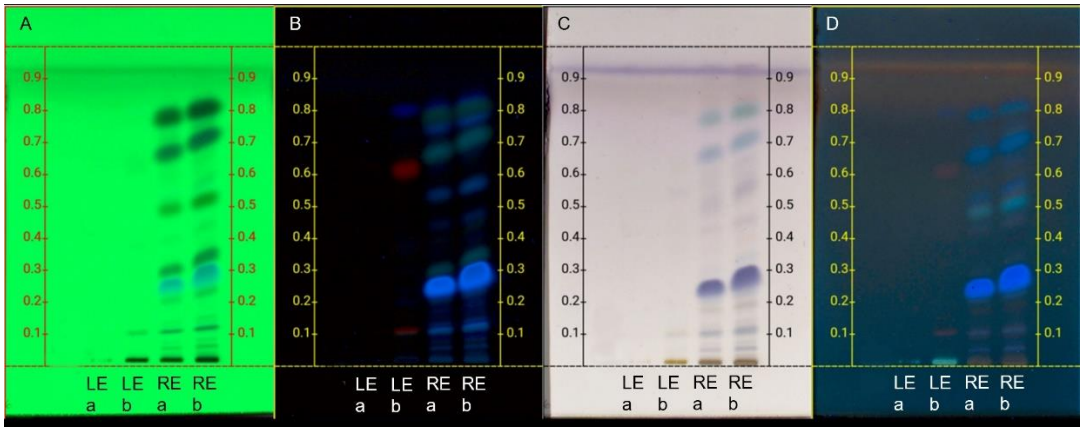


Figure 8 TLC chromatogram developed using a normal phase plate and dichloromethane, methanol (97:3; v/v) as mobile phase. A: 254 nm; B: 366 nm; C: visible spectrum after vanillin staining; D: 366 nm after vanillin staining. LE a: leaf extract (5 µl of 10 mg/ml); LE b leaf extract (10 µl of 10 mg/ml); RE a: rhizome extract (5 µl of 10 mg/ml); RE b rhizome extract (10 µl of 10 mg/ml).

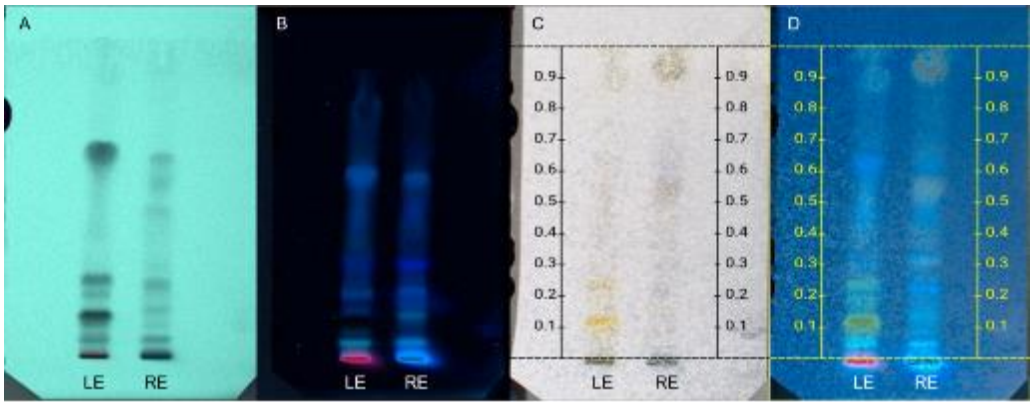


Figure 9 TLC chromatogram developed using a reverse phase plate and water, acetonitrile (80:20; v/v) as mobile phase. A: 254 nm; B: 366 nm; C: visible spectrum after vanillin staining; D: 366 nm after vanillin staining. LE leaf extract (10 µl of 10 mg/ml); RE rhizome extract (10 µl of 10 mg/ml).

The TLC chromatograms highlight the presence of polyphenol compounds in both extracts, and more specifically the presence of flavonoids especially in the LE (appearing in yellow after staining with sulfuric vanillin) (*Figure 6* and *Figure 7*). The separation of the compounds in the RE was better obtained with a more apolar system allowing the separation and visualization of the coumarins (*Figure 8*).

Total phenols, flavonoids, flavanols, and proanthocyanidin

The phytochemical screening investigated the leaf hydroalcoholic extract (LE) and the rhizome hydroalcoholic extract (RE) investigated in terms of total phenols, flavonoids, flavanols, and proanthocyanidin content; the last two parameters are also useful to calculate the polymerization index. Data are presented in *Table 1*.

Table 1 Phytochemical screening of *P. ostruthium* leaf and rhizome hydroalcoholic extracts. Results are the mean ± standard deviation (S.D.) of three independent experiments in triplicate (n = 3) [136].

Assay	LE	RE
Total phenols (mg GAE [a]/100 g DE [b])	10668.30 ± 581.55	9538.00 ± 622.24
Flavonoids (mg RE [c]/100 g DE)	52914.94 ± 384.84 *	13694.83 ± 561.33
Flavan-3-ols (mg CE [d]/100 g DE)	200.19 ± 1.58 *	334.89 ± 12.66
Proanthocyanidins (mg CyE [e]/100 g DE)	0.078 ± 0.00 *	0.003 ± 0.00
Polimerization index [f]	2575.46 *	111.630

[a] GAE, Gallic acid equivalents; [b] DE, Dry extract; [c] RE, Rutin equivalents; [d] CE, Catechin equivalents, [e] CyE, Cyanidin equivalents; [f] Polymerization index = Flavonols/Proanthocyanidins. * $p < 0.005$ vs. RE.

^{1}H-NMR profiling

^{1}H-NMR spectra of total extracts revealed the presence of different phytochemical categories of compounds based on peaks in specific regions of NMR spectra (*Figures 10-13*). The red-framed region (8.5-6.0 ppm) corresponds to flavonoids, phenolic compounds, as well as other compounds with aromatic protons. The blue and green-framed regions correspond to sugars. The blue one (5.9-4.3 ppm) shows the anomeric protons of the sugars, while the green one (4.2-2.8 ppm) shows the rest of the sugars' protons. Lastly, the yellow-framed region (2.6-0.6 ppm) corresponds to terpenoids and aliphatic protons.

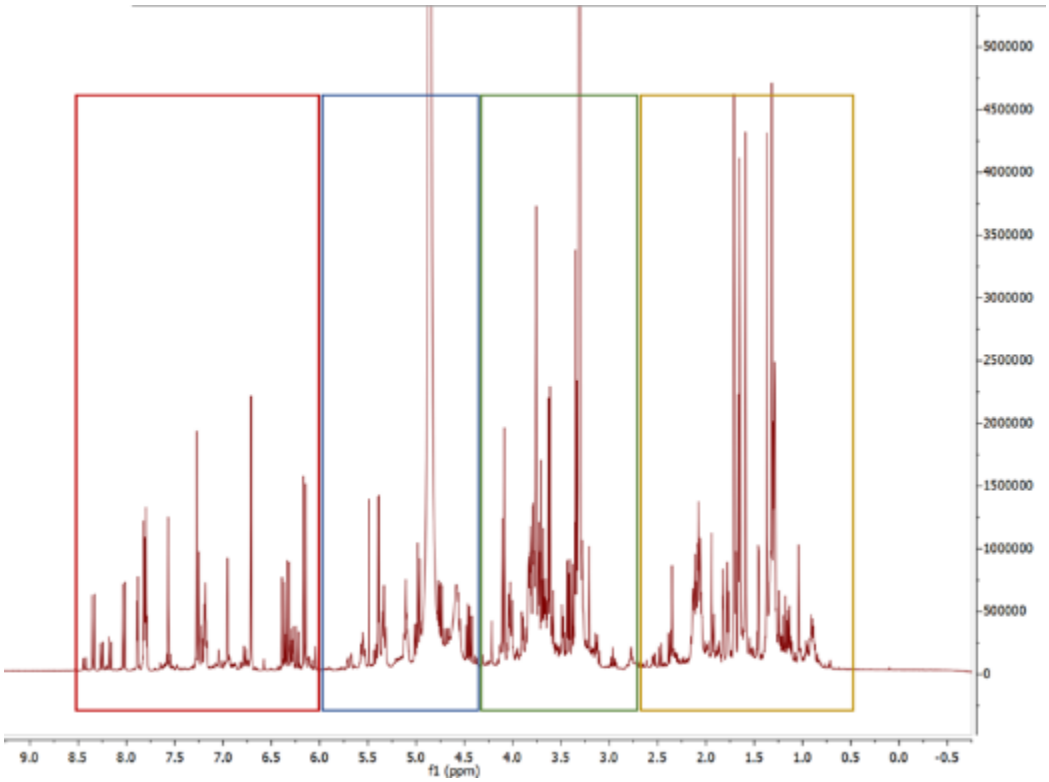


Figure 10 ^{1}H-NMR profile of *P. ostruthium* rhizome extract dissolved in methanol-d4 (MeOD).

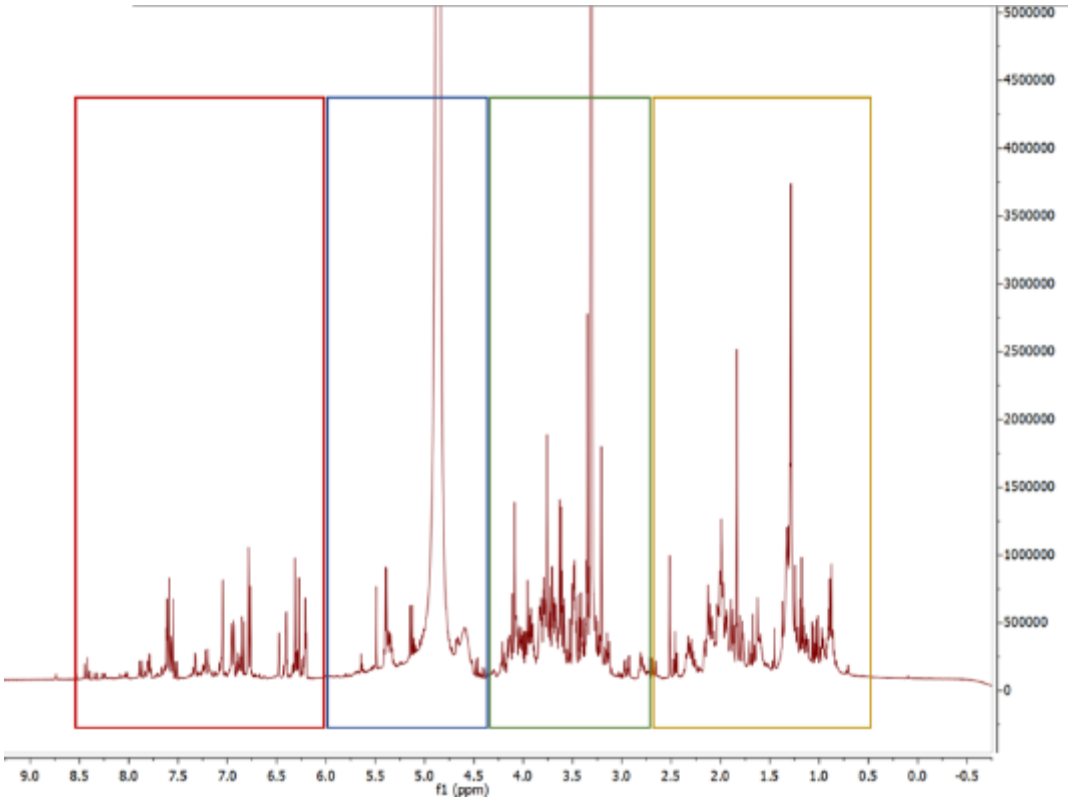


Figure 11 ^{1}H-NMR profile of *P. ostruthium* leaf extract dissolved in methanol-d4 (MeOD).

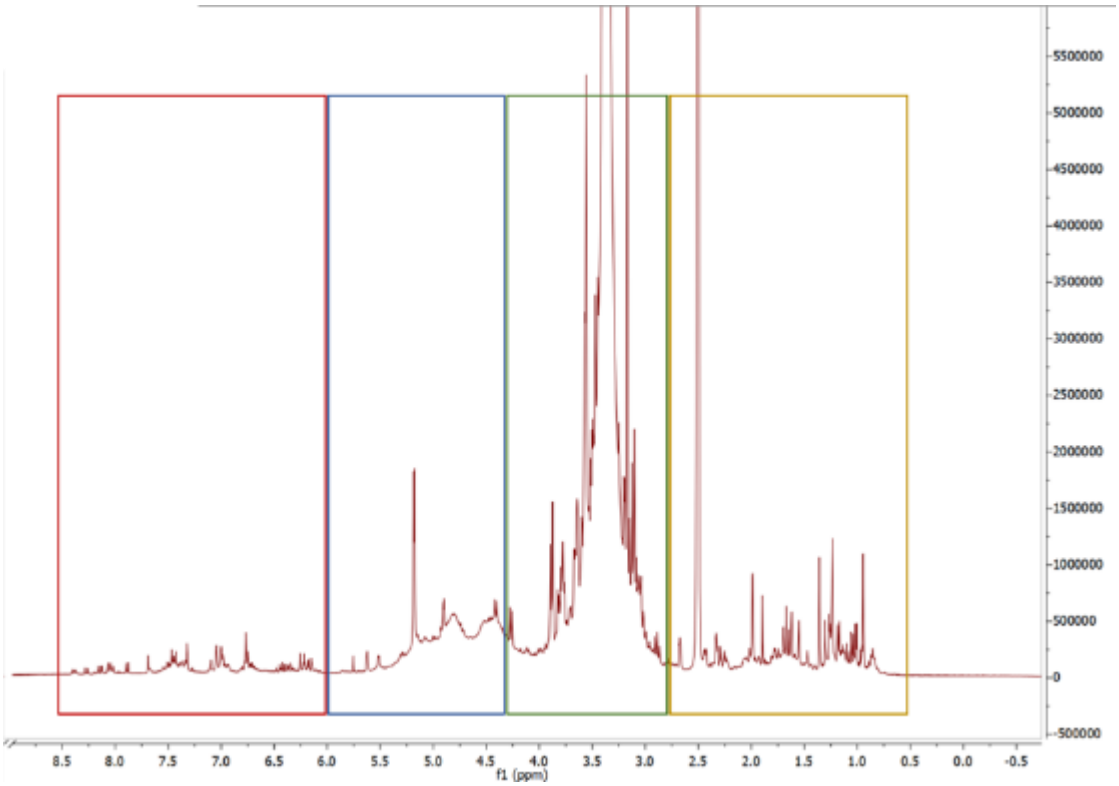


Figure 12 ^{1}H-NMR profile of *P. ostruthium* rhizome extract dissolved in Dimethyl sulfoxide (DMSO)

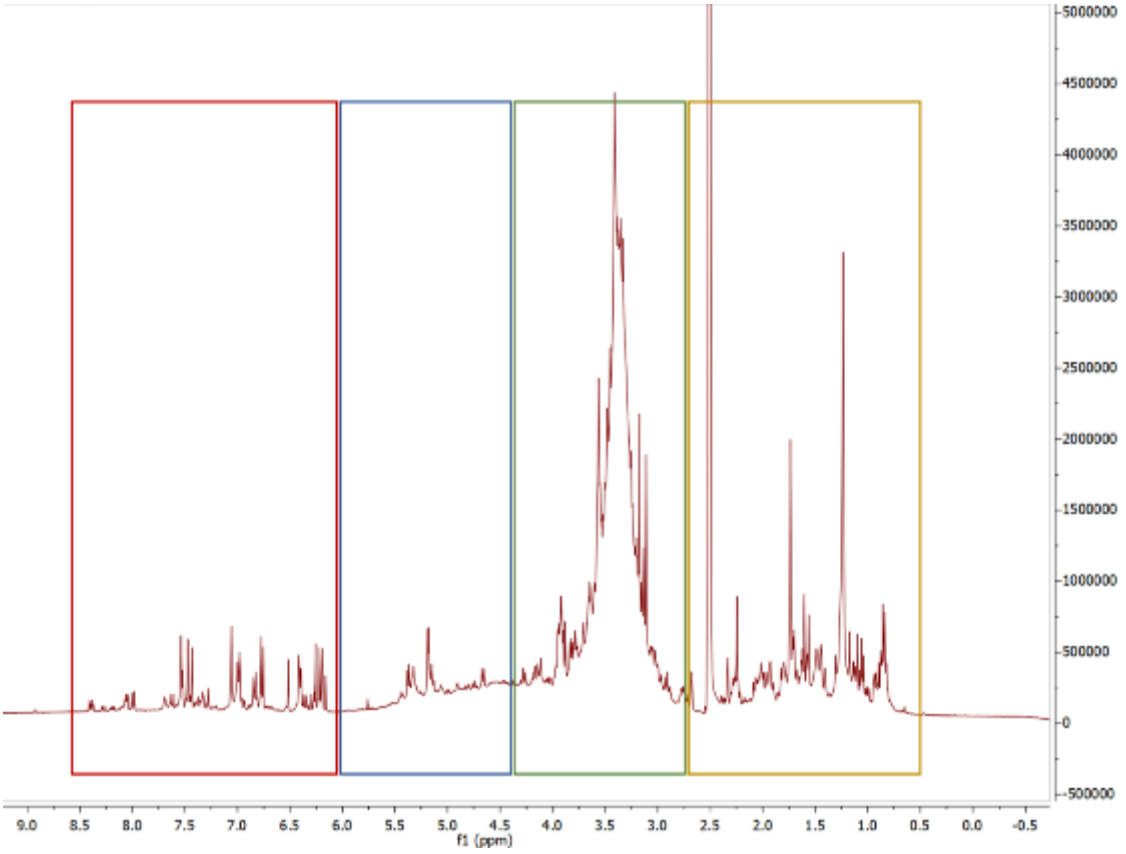


Figure 13 ^{1}H-NMR profile of *P. ostruthium* leaf extract dissolved in Dimethyl sulfoxide (DMSO)

LC-DAD-ESI-MS analysis

The leaves and rhizomes extracts were analysed through LC-DAD-ESI-MS; Data are presented in *Table 2*.

Table 2 Major components in *P. ostruthium* LE and RE identified and quantified by LC-DAD-ESI-MS analysis [136].

	Compound	$[M-H]^-$ (*m/z*)	$[M-H]^+$ (*m/z*)	λ_{max} (nm)	LE	RE
					mg/100 g DE	
1	3-*O*-Caffeoylquinic acid	353	355	296, 326	4.05 ± 0.14 *	195.55 ± 1.67
2	5-*O*-Caffeoylquinic acid	353	353	296, 326	5.14 ± 0.05 [a,*]	114.36 [a] ± 1.44
3	4-*O*-Caffeoylquinic acid	353	355	296, 326	135.0 ± 0.57 [a,*]	16.28 [a] ± 0.08
4	5-*O*-p-Coumaroylquinic acid	337	339	296, 324	3.70 ± 0.02 [b]	-
5	5-*O*-Feruloylquinic acid	367	369	296, 324	5.05 ± 0.08 [c,*]	59.80 [c] ± 0.05
6	4-*O*-Feruloylquinic acid	367	369	296, 324	-	1.93 [c] ± 0.01
7	p-Coumaroyl glucose	325	327	226, 315	0.13 ± 0.00 [b]	-
8	Quercetin-3-*O*-rutinoside	609	611	257, 354	95.28 ± 0.84	-
9	3,4-di-*O*-Caffeoylquinic acid	515	517	296, 324	11.08 ± 0.05 [a,*]	2.69 [a] ± 0.02
10	Hesperidin	609	611	284, 332	-	9.57 ± 0.06
11	Quercetin-3-*O*-(6''acetyl-glucoside)	505	507	256, 356	138.52 ± 1.88 [d]	-
12	3,7-Dimethylquercetin	329	331	257, 358	2.28 ± 0.03 [e]	-
13	Oxypeucedanin-hexoside	465	467	313	46.98 ± 0.42 [f,*]	0.43 [f] ± 0.01
14	Kaempferol 3-*O*-acetyl-glucoside	489	491	265, 328	501.24 ± 0.66 [g]	-
15	Osthenol-7-*O*-glucoside	-	393	270, 320	-	0.73 [h] ± 0.01
16	Oxypeucedanin-malonyl-hexoside	-	553	270, 315	-	0.14 [f] ± 0.00
17	Oxypeucedanin hydrate	-	305	311	1.14 ± 0.01 [f,*]	5.67 [f] ± 0.04
18	Oxypeaucedanin 2'-acetate-3'glucoside	-	509	311	3.25 ± 0.02 [f,*]	2.13 [f] ± 0.01
19	Oxypeucedanin	-	287	309	5.81 ± 0.02 [f,*]	3.05 [f] ± 0.03
20	Oxypeucedanin ethanolate	-	333	311	8.62 ± 0.04 [f]	-
21	Ostruthol	-	387	309	-	1.45 ± 0.02
22	Isoimperatorin	-	271	300	-	29.55 ± 0.08
23	Imperatorin	-	271	310	-	7.31 ± 0.05
24	Ostruthin	-	299	330	-	281.88 ± 2.24
	Percentage distribution (%) of phytochemical classes					
	Phenolic acids				16.97	53.32
	Flavonoids				76.23	1.31
	Coumarins				6.80	45.37

Data are the mean ± standard deviation (S.D.) of three independent experiments in triplicate (n = 3), expressed as mg/100 g dry extract (DE). Quantification was carried out by building external calibration curves of reference standards, whereas the superscript letters (a–h) indicate that the quantification was carried out based on the calibration curves of the following structural analogues: 3-*O*-Caffeoylquinic acid, Coumaric acid, Ferulic acid, isoquercetin, quercetin, oxypeucedanin, Kaempferol 3-*O*-glucoside, and Osthenol, respectively; * = $p < 0.005$ vs. RE.

As shown in *Table 2*, among the major components identified, LE showed the greatest content of flavonoids (76.23% vs. 1.31%), followed by phenolic acids and coumarins. Conversely, RE showed a higher content of phenolic acids and coumarins than LE, 53.32% vs. 16.97%, and 45.37% vs. 6.80%, respectively, and a very low content of flavonoids (1.31%). Kaempferol 3-O-(6''acetyl-glucoside) (501.24 ± 0.66 mg/100 g DE) was the most abundant component identified in LE, followed by quercetin-3-O-(6''acetyl-glucoside) (138.52 ± 1.88 mg/100 g DE), 4-O-caffeoylquinic acid (135.01 ± 0.57 mg/100 g DE), quercetin-3-O-rutinoside (95.28 ± 0.84 mg/100 g DE) and oxypeucedanin-hexoside (46.98 ± 0.42 mg/100 g DE) (*Figure 14*). RE showed instead a completely different phytochemical profile with a preponderance of phenolic acids and coumarins. Ostruthin (281.88 ± 2.24 mg/100 g DE) was the most abundant compound, followed by 3-O-caffeoylquinic acid (195.55 ± 1.67 mg/100 g DE), 5-O-caffeoylquinic acid (114.36 ± 1.44), 5-O-Feruloylquinic acid (59.80 ± 0.05) and isoimperatorin (29.55 ± 0.08) (*Figure 15*).

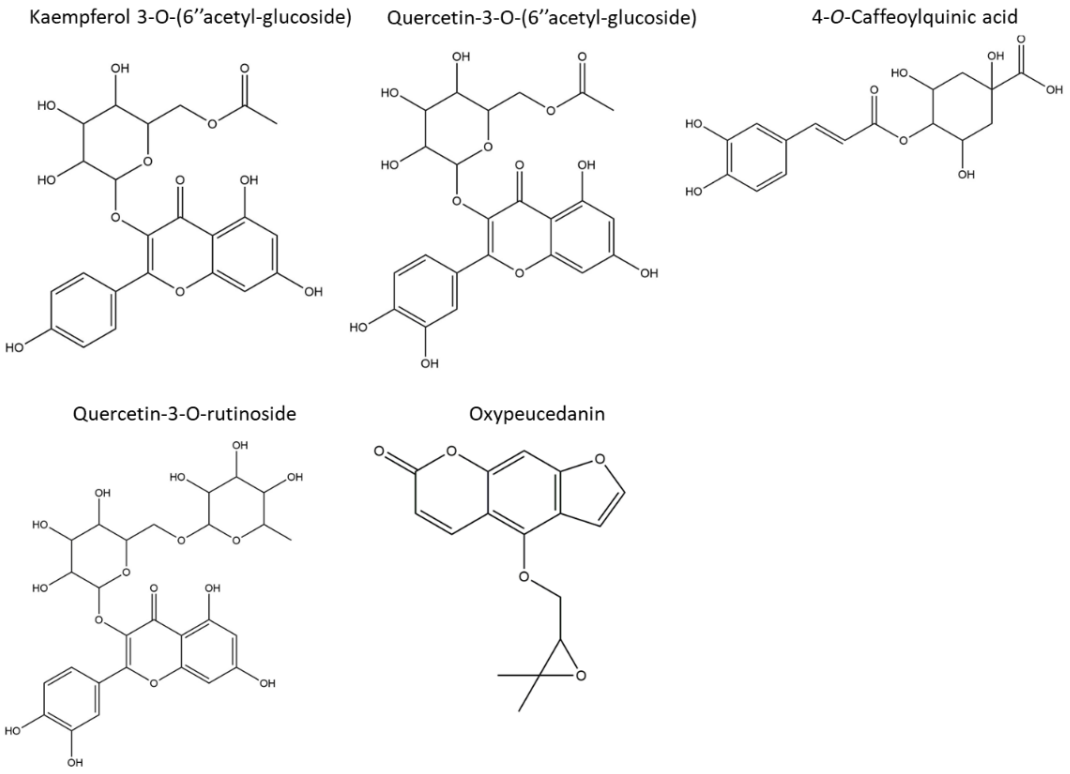


Figure 14 Chemical formulas of the major constituents found in *P. ostruthium*' leaf extract (ChemDraw Professional 15.0).

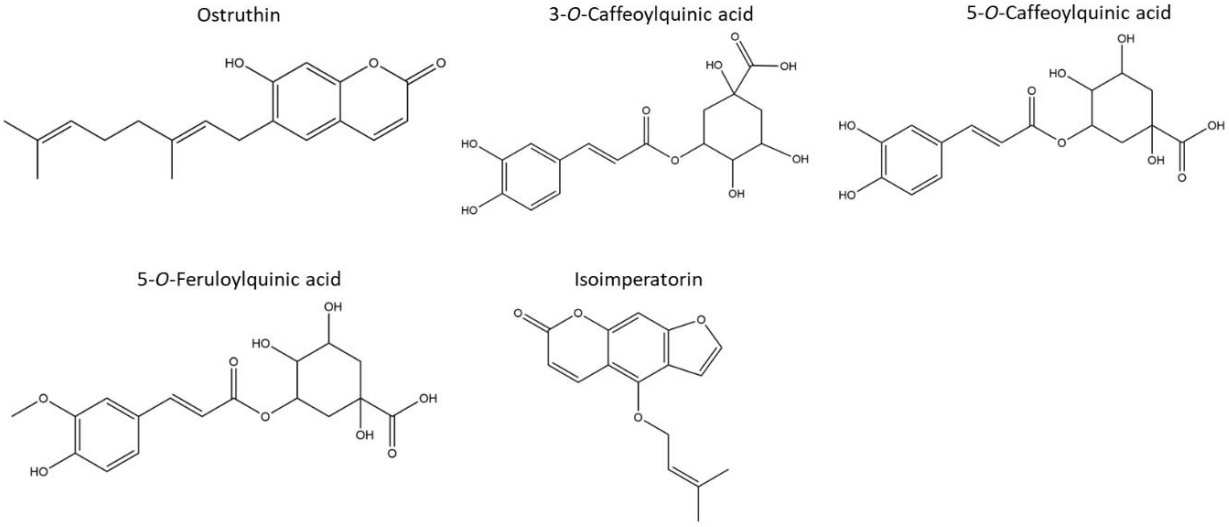


Figure 15 Chemical formulas of the major constituents found in *P. ostruthium*' rhizome extract (ChemDraw Professional 15.0).

Antioxidant, Anti-Inflammatory, Enzyme-inhibition Activities

The antioxidant and anti-inflammatory activities of LE and RE were screened using in vitro cell-free tests based on different environments and reaction mechanisms (*Table 3*). LE showed the best biological activity in all assays according to its higher content in flavonoids, which are known to be involved in free-radical scavenging processes and chelating activity as enzyme-cofactors. The same trend was observed also in the two anti-inflammatory tests, which evaluate the ability of the extracts to counteract heat-induced bovine serum albumin (BSA) denaturation and to inhibit protease activity, playing a pivotal role in many inflammatory diseases.

Table 3 Determination of antioxidant and anti-inflammatory activities of LE and RE by several in vitro colorimetric assays [136].

Assay	LE	RE	Reference Standard [b]
Antioxidant activities			
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	24.11 (20.14–28.87) *	152.73 (61.54–379.06)	8.57 (4.88–10.22) §
Trolox equivalent antioxidant capacity (TEAC)	12.14 (10.34–14.24) *	51.07 (35.56–73.35)	4.89 (2.24–6.95) §
Ferric reducing antioxidant power (FRAP)	19.37 (15.59–24.07) *	47.18 (39.75–56.01)	5.38 (3.86–8.01) §
Oxygen radical absorbance capacity (ORAC)	1.03 (0.76–1.40)	1.35 (1.09–1.69)	0.72 (0.38–0.92) §
Anti-inflammatory activities			
BSA [a] denaturation assay	15.16 (12.97–17.72) *	57.06 (47.72–69.70)	17.58 (15.05–19.68)°
Protease inhibitory activity	24.78 (19.75–31.09)	30.04 (23.42–38.51)	6.88 (3.26–9.44) §

Data are half-maximal inhibitory concentrations (IC_{50}, µg/mL) with confidence limits (C.L.) derived from three independent experiments in triplicate. [a] BSA, Bovine serum albumin; [b] Reference standards: Trolox for DPPH, TEAC, FRAP and ORAC assays; Diclofenac sodium for BSA and protease inhibitory activity assay. * = $p < 0.005$ vs. RE; § = $p < 0.005$ vs. LE and RE; ° = $p < 0.005$ vs. RE.

The in vitro inhibitory effects of LE and RE on pro-inflammatory enzymes, cyclooxygenase (COX-2) and lipoxygenase (LOX), were evaluated. Data are presented in *Table 4*.

Table 4 In vitro inhibition of LE and RE on cyclooxygenase and lipoxygenase enzymatic activities [136].

Enzyme	LE		RE		Standard
	150 µg/mL	300 µg/mL	150 µg/mL	300 µg/mL	
COX-2	67.3 ± 11.5	43.8 ± 4.4	n.d.	n.d.	87.9 ± 0.1 *
LOX	52.0 ± 27.3 ‡	78.7 ± 8.8 #	11.3 ± 11.3	65.4 ± 13.5 #	96 ± 3.5 *

Data are mean percent inhibition ± S.D. ($n = 3$). Reference standard: 3 µg/mL nimesulide for COX-2, and 100 µM nordihydroguaiaretic acid for LOX. ‡ = $p < 0.05$ in a t-test comparison between different concentrations of the same extract; # = $p < 0.05$ in a *t*-test comparison between different extracts at the same dose; * = $p < 0.05$ with respect to all other groups, n.d. = not detectable.

The in vitro inhibitory effects of LE and RE were evaluated on collagenase and tyrosinase enzymes; Data are presented in *Table 5*.

Table 5 In vitro inhibition of LE and RE on collagenase and tyrosinase enzymatic activities.

Enzyme	LE		RE		Standard
	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL	
Collagenase	46.6 ± 3.6	57.5 ± 3.0	30.9 ± 1.5	38.1 ± 1.3	49.8 ± 1.3
Enzyme	**LE**		**RE**		**Standard**
	100 µg/mL	**300 µg/mL**	**100 µg/mL**	**300 µg/mL**	
Tyrosinase	32.2 ± 5.0	37.4 ± 0.7	25.1 ± 5.7	30.6 ± 4.5	35.1 ± 3.7 54.6 ± 4.5

Data are mean percent inhibition ± S.D. ($n = 3$). Reference standard: 3.75 µg/mL Phosphoramidon disodium salt for collagenase, and 2 µg/mL kojic acid and 5 µg/mL *Glycyrrhiza glabra* root methanolic extract for tyrosinase.

Additionally, for LE also the IC_{50} value of inhibition of the Collagenase enzyme was investigated as shown in *Figure 16*.

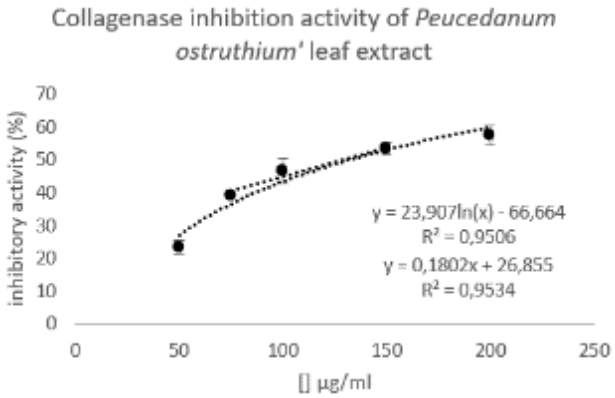


Figure 16 Collagenase inhibition activity of *P. ostruthium'* LE. IC_{50} value was estimated 131.6 µg/mL.

Cell Viability and Wound-Healing Activity

The effects of RE and LE on cell viability have been evaluated by the MTT assay using HaCaT human keratinocytes and L929 murine fibroblasts as skin cell experimental models at the endpoints of 24 and 48 h, showing a dose-dependent decrease of cell viability for increasing extract concentrations, with a steeper slope for exposure to RE in both cell types (*Figure 17*). Values of median inhibitory concentrations (IC_{50}) and threshold effective concentrations (IC_{05}) at 48 h are presented in *Table 6*.

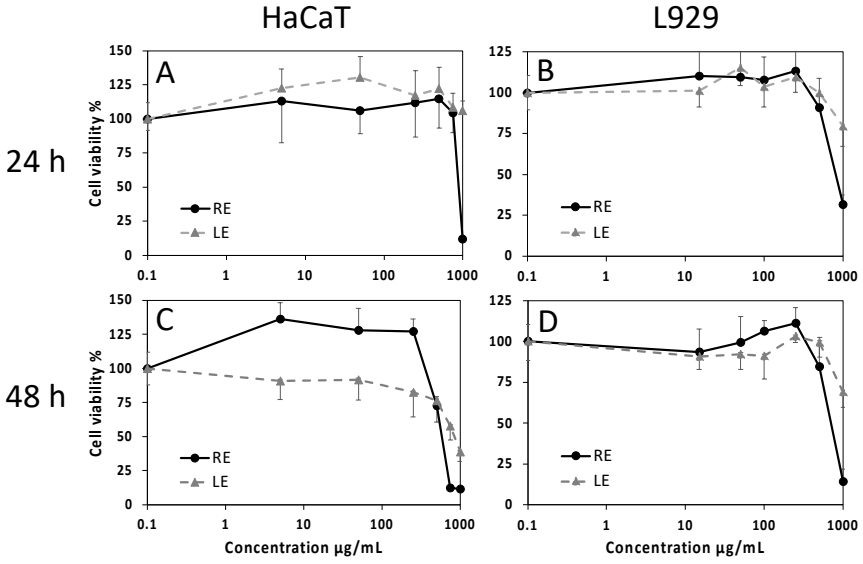


Figure 17 Cell viability evaluated by the MTT assay on HaCaT (**A**,**C**) and L929 (**B**,**D**) exposed for 24 h (**A**,**B**) or 48 h (**C**,**D**) to increasing concentrations of LE or RE. Data are mean absorbances ± S.D. of 570 nm readings, obtained from 6 replicate wells for each condition in two independent experiments [136].

Table 6 Median (IC_{50}) and threshold (IC_{05}) concentrations of LE and RE on HaCaT and L929 evaluated by the MTT assay at 48 h endpoints [136].

	Leaf Extract		Rhizome Extract	
	IC_{50}	IC_{05}	IC_{50}	IC_{05}
HaCaT	897 (760–1059)	252 (113–559)	439 (416–463)	364 (293–451)
L929	1094 (607–1970)	801 (189–3394)	681 (603–768)	385 (320–462)

Data are expressed as extract concentrations in µg/mL. Values of 95% confidence intervals are shown in parentheses.

The effects of extracts on skin cell migration were evaluated by in vitro scratch wound assays on HaCaT and L929 monolayers. On HaCat monolayers wound closure was measured at 24h, while on L929 monolayer the cell migration within the wound space was measured at 48 h. Data are presented in *Figure 18*.

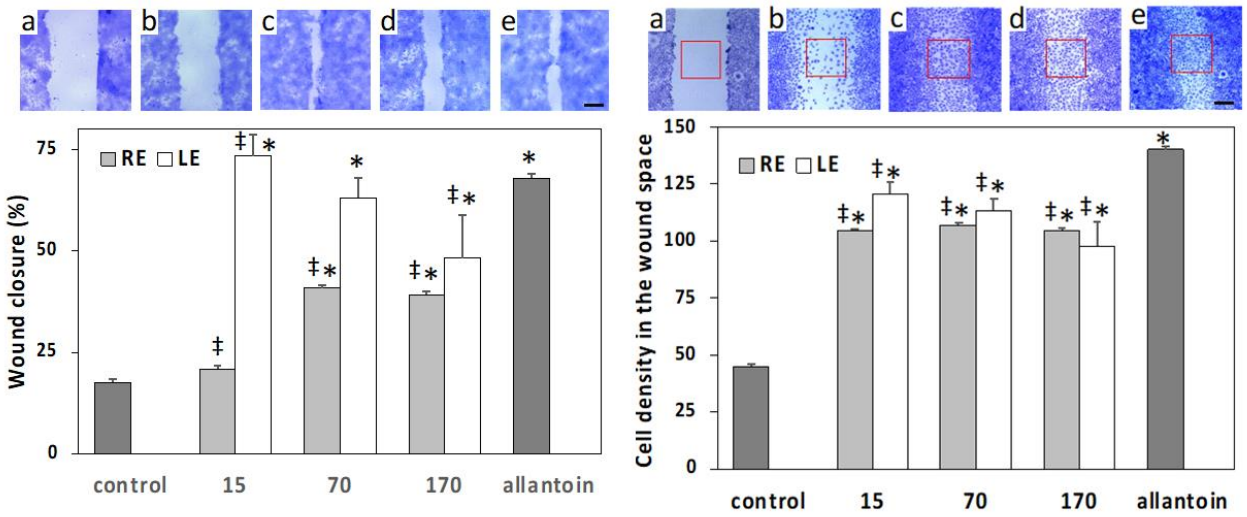


Figure 18 Scratch wound assays conducted on HaCaT human keratinocytes (first panel) and L929 mouse fibroblasts (second panel) exposed to different concentrations of LE or RE (µg/mL). Micrographs show representative wounded cell monolayers at different times after wounding and under different conditions (bar = 250 µm). Data of HaCaT are expressed as means ± S.D. (n = 49–67) of percent wound closure at 24 h. Data of L929 are means ± S.D. (n = 8) of the normalized cell densities within the wound space (number of cells per unit area; red squares in micrographs) at 24 h post wounding. a = wound space just after scratch wounding; b = control at 24 h; c = 15 µg/mL LE at 24 h; d = 70 µg/mL RE at 24 h; e = positive control allantoin at 24 h; * = significantly different from control; ‡ = significantly different from allantoin ($p < 0.01$) [136].

Discussion

Traditional medicine reports the use of *P. ostruthium* for the preparation of medicaments such as infusions, decoctions, or liqueurs. Considering this, to optimize the extraction of bioactive compounds from leaves and rhizomes, a hydroalcoholic mixture was used.

Previous studies [161–163] using LC-ESI-DAD-MS analysis identified 23 main components in both *P. ostruthium* LE and RE. These components belong to various polyphenol classes like coumarins, phenolic acids, and flavonoids. However, important differences in terms of secondary metabolites were found. These differences could be ascribed to the climatic and environmental features of the sampling sites [164] but also to the extraction method adopted [161–163]. Regarding LE, which has been poorly investigated to date, these differences stand out mainly in terms of the relative abundance of flavonoids, especially kaempferol and quercetin derivatives, with respect to phenolic acids such as caffeoyl and feruloylquinic derivatives [161].

Considering RE, according to our data, two previous studies identified caffeoyl derivatives as the preponderant class, followed by coumarins [162,163]. Moreover, even within the same class of compounds, significant differences were recorded in terms of relative abundance. In the present study, a preponderance of ostruthin was highlighted, while previous studies reported a preponderance of oxypeucedanin and derivatives [161].

Considering COX and LOX enzymes, a previous study reported their inhibition by *P. ostruthium* rhizome extracts [141]. However, our data indicate stronger inhibitory activity by LE with respect to RE. Moreover, a stronger effect of LE than RE was also found in the wound healing assay on HaCaT cells, and this finding agrees with a study on a skin ulcer model in rats treated with an ointment containing a *Peucedanum* leaf extract [165].

What emerges for the first time from this study is that leaves and rhizomes are sharply different from a phytochemical point of view. The RE contains about seven times and three times more coumarins and phenolic acids than LE, and a very low content of flavonoids (1.31% in RE vs. 76.23% in LE), represented exclusively by hesperidin, as previously identified [162,163]. These substantial differences translate into different biological activities, with LE proving to be the most promising for antioxidant, anti-inflammatory, and wound healing activities. This is in contrast with previous ideas that the health effects of these extracts are mainly attributable to phenolic acids, i.e., caffeoyl and feruloylquinic derivatives. Flavonoids are probably the most discriminating factor in terms of biological activity, although a synergistic effect of the two classes of compounds cannot be excluded.

In RE, the two major constituents are the coumarins ostruthin and isoimperatorin, which are known for cytotoxic and antiproliferative activities [166,167] and could play a role in the slightly stronger cytotoxic effect of RE with respect to LE on both HaCaT and L929 cells. In addition, a role in the RE inhibition of proinflammatory enzymes could be ascribed to caffeoyl and feruloylquinic derivatives [168]. In the LE, the much higher flavonoid content compared to the RE might be why it shows stronger inhibition of proinflammatory enzymes and better wound healing activity. Quercetin, abundant in LE in a glycosylated form, has been shown to promote wound healing in different studies, including in vitro on HaCaT cells through epithelial–mesenchymal transition [169]. Quercetin has also been reported to inhibit both COX and LOX activities [170] and could be responsible for the stronger inhibition of these enzymes observed in LE with respect to RE.

Lastly, it's important to highlight that the phytochemical profile of LE is safer from a toxicological standpoint due to its lower coumarin content compared to RE. This makes LE a very promising extract for use in nutraceuticals and cosmeceuticals. The scalability of industrial production for the plant has already been achieved, and the rhizome extract is on the market. The introduction of the leaf extract as an herbal healthcare product would result in a considerably improved exploitation of this plant with positive economic and environmental outcomes.

Conclusion

Our study provides new information on the phytochemical composition and medicinal properties of *P. ostruthium*, an alpine species mainly known since ancient times for the traditional use of its rhizome. However, during rhizome harvesting for medicinal purposes, leaves are generally discharged. Considering that in some areas of the Alps, the leaf is used for the treatment of skin problems, we investigated and compared the hydroalcoholic extracts of the two plant parts. The leaf extract (LE) proved to be the most promising due to its strong antioxidant and anti-inflammatory properties. Both extracts had minimal impact on human keratinocytes and murine fibroblasts, yet they displayed notable wound healing properties. This supports the traditional use of this species in treating skin diseases and inflammation. In addition, the low presence of furanocoumarins in the LE makes it an ideal candidate to be included in dermatological and cosmeceutical products. Thanks to the data collected, it is, therefore, possible to suggest the use of the leaves of the plant for phytotherapeutic applications, thus encouraging the recovery of plant material, which is still mostly discarded with a view to a circular economy, even on a small scale.

Scientific contribution

Preliminary data have been presented in abstract and presentation in Scientific Congresses:

- **Danna C.**, Bazzicalupo M., Smeriglio A., Denaro M., Trombetta D., Burlando B., Cornara L. Antioxidant and anti-inflammatory properties of *Imperatoria ostruthium* L. leaf extract. 116° Congresso della Società Botanica Italiana (SBI), IX International Plant Science Conference (IPSC), 8-10 September 2021 (Online)

- **Danna C.**, Bazzicalupo M., Smeriglio A., Denaro M., Trombetta D., Burlando B., Cornara L. Scientific validation of the medicinal properties of *Imperatoria ostruthium* L.: insights of the "Divinum remedium". XVIII Congresso Nazionale di Fitoterapia (S.I.Fit), XI SYRP Conference, 21–23 May 2021 (Online)

- Smeriglio A., **Danna C.**, Bazzicalupo M. et al. The phytochemical profile of leaf and rhizome of *Peucedanum ostruthium* (L.) W. D. J. Koch influences the antioxidant, anti-inflammatory, and wound healing properties of this medicinal plant. 117° Congresso della Società Botanica Italiana (SBI), X International Plant Science Conference (IPSC), 7-10 September 2022, Bologna (Online)

Further studies are in progress to characterize the phytochemical profile and some biological activities of the EOs extracted from the *Peucedanum ostruthium*' leaves and rhizomes.

Detailed results have been published and extensively discussed in the following article published in Open Access by the Editor MDPI under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/):

molecules

MDPI

Article

Anti-Inflammatory and Wound Healing Properties of Leaf and Rhizome Extracts from the Medicinal Plant *Peucedanum ostruthium* (L.) W. D. J. Koch

Cristina Danna [1], Miriam Bazzicalupo [2], Mariarosaria Ingegneri [3], Antonella Smeriglio [3], Domenico Trombetta [3], Bruno Burlando [4,*] and Laura Cornara [1]

1 Department of Earth, Environment and Life Sciences (DISTAV), University of Genova, Corso Europa 26, 16132 Genova, Italy; cristina.danna@edu.unige.it (C.D.); cornaral@gmail.com (L.C.)
2 CREA—Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Corso Inglesi 508, 18038 Sanremo, Italy; miriam.bazzicalupo@gmail.com
3 Department of Chemical, Biological, Pharmaceutical and Enviromental Sciences (ChiBioFarAm), University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy; mariarosaria.ingegneri@unime.it (M.I.); antonella.smeriglio@unime.it (A.S.); domenico.trombetta@unime.it (D.T.)
4 Department of Pharmacy—DIFAR, University of Genova, Viale Benedetto XV 3, 16132 Genova, Italy
* Correspondence: bruno.pietro.burlando@unige.it

Abstract: *Peucedanum ostruthium* (L.) W. D. J. Koch (Apiaceae) is a worldwide perennial herb native to the mountains of central Southern Europe. The rhizome has a long tradition in popular medicine, while ethnobotanical surveys have revealed local uses of leaves for superficial injuries. To experimentally validate these uses, plant material was collected in the Gran Paradiso National Park, Aosta Valley, Italy, and the rhizome and leaves were micromorphologically and phytochemically characterized. Polyphenol-enriched hydroalcoholic rhizome and leaf extracts, used in cell-free assays, showed strong and concentration-dependent antioxidant and anti-inflammatory activities. In vitro tests revealed cyclooxygenase and lipoxygenase inhibition by the leaf extract, while the rhizome extract induced only lipoxygenase inhibition. MTT assays on HaCaT keratinocytes and L929 fibroblasts showed low cytotoxicity of extracts. In vitro scratch wound test on HaCaT resulted in a strong induction of wound closure with the leaf extract, while the effect of the rhizome extract was lower. The same test on L929 cells showed similar wound closure induction with both extracts. The results confirmed the traditional medicinal uses of the rhizome as an anti-inflammatory and wound healing remedy for superficial injuries but also highlighted that the leaves can be exploited for these purposes with equal or superior effectiveness.

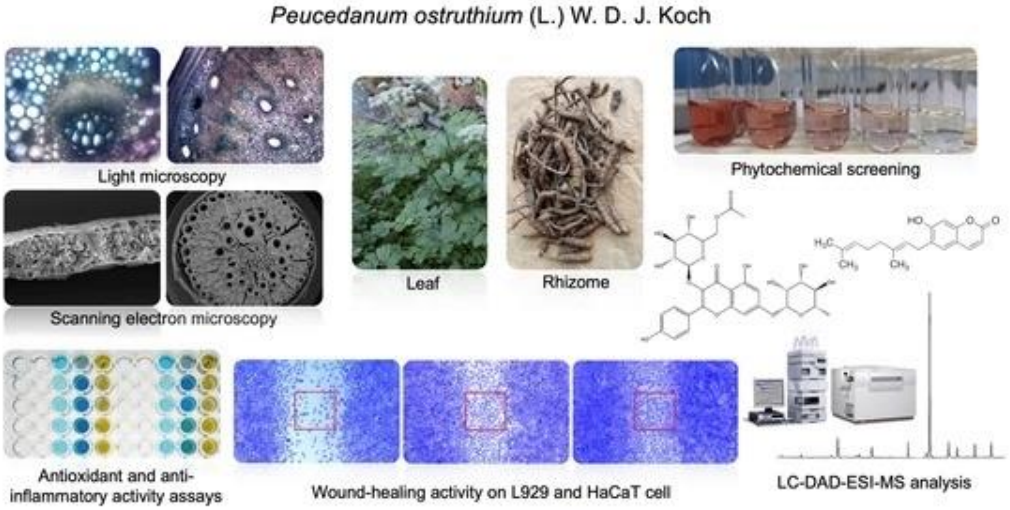
Keywords: plants traditional use; leaf extract; rhizome extract; micromorphology; phytochemical characterization; antioxidant activity; anti-inflammatory activity; wound-healing activity

check for updates

Citation: Danna, C.; Bazzicalupo, M.; Ingegneri, M.; Smeriglio, A.; Trombetta, D.; Burlando, B.; Cornara, L. Anti-Inflammatory and Wound Healing Properties of Leaf and Rhizome Extracts from the Medicinal Plant *Peucedanum ostruthium* (L.) W. D. J. Koch. *Molecules* **2022**, *27*, 4271. https://doi.org/10.3390/molecules27134271

Academic Editor: George Albert Karikas

Received: 13 May 2022
Accepted: 29 June 2022
Published: 2 July 2022



PART II

Eucalyptus sp. pl.: exploitation of the by-products in a circular economy perspective

PART IIA

Eucalyptus EOs as bio-herbicides and bio-controllers

Introduction

Native to Australia, the genus *Eucalyptus* (Myrtaceae) contains about 800–900 species and is largely cultivated around the world in commercial plantations for several purposes, such as cellulose, pulp, gum, EOs, and honey production, as well as for its use in construction and as ornamental plants [171,172].

In Italy, several *Eucalyptus* species were introduced at the beginning of the 1800s by plant collectors to be used as ornamental trees in private gardens [173]. During the early 1900s, these trees became largely cultivated, especially in South Italy, for reclaiming swampy lands and eliminating the outbreak of malaria [174]. Today, the *Eucalyptus* genus is still cultivated in many Italian regions, growing up to 350 m above sea level. Long-established eucalyptus-cut foliage plantations occur in Imperia and Western Liguria (Italy), in the Alpes Maritime (France), and Cornwall (England), while less extensive plantations are also present in Ireland [175]. Nowadays, Italy is one of the main producers and exporters of eucalyptus-cut foliage, which is mainly sold on the Northern European market for floral compositions [176]. In Liguria, among the species cultivated for this purpose, there are *E. cinerea* F. Muell. ex. Benth., *E. gunnii* Hook.f., *E. pulverulenta* Sims cv "Baby blue," *E. nicholii* Maiden & Blakely, and *E. parvula* L.A.S. Johnson & K.D.Hill.

Proper pruning is useful for producing high-quality juvenile stems of *Eucalyptus* species [177], and during harvesting, stems are carefully selected for branch quality (color and wax density, the shape of the foliage, etc.). As a result, a large amount of waste biomass is produced. It is estimated that, in the Province of Savona, Western Liguria, where these plantations have an extension of 100 ha, a total of about 1000 tons per year of cut foliage is produced, with waste of more than 4% (4 tons), which is currently buried or burned (Coldiretti, Savona; personal communication). However, it must be noted that this waste plant material is still rich in bioactive compounds with interesting biological properties, so it can be used for different applications in the food, medicinal, or agricultural sectors.

Recent studies have shown the potential of remnants from *Eucalyptus* pruning for transformation into added-value products [178,179]. The genus *Eucalyptus* is, in fact, characterized by the presence of many secondary compounds, such as phenolics, essential oils, waxes, and resins, which can be useful as pharmaceutical products, natural pesticides, insecticides, and herbicides [172,180–182].

It is well known that the use of synthetic compounds for weed control and pest control has harmed the environment and human health. Glyphosate-based formulations, extensively used as herbicides worldwide, have been recently referred to as "probably carcinogenic" [183]. For this reason, in addition to the development of resistance in weeds and pathogens, many countries have recently restricted or banned the use of such formulations [64,184]. Essential oils (EOs) that show bioherbicidal potential and are not persistent in the environment represent a valid alternative to synthetic chemicals in integrated weed management. Therefore, *Eucalyptus* EOs—rich in 1,8-cineole—can play an important role in modern agriculture to reduce the use of harmful herbicides and pesticides thanks to their allelopathic effect on weeds and their interaction with noxious insects [55,185,186]. Phytotoxic effects of different *Eucalyptus* EOs have been reported on many common weed species (e.g., *Amaranthus blitoides*, *A. hybridus, Chenopodium album, Cynodon dactylon*, and *Portulaca oleracea*) [187–190]. However, to prevent these same EOs from causing damage to some crops, it is essential to evaluate their ability to act in a selective manner [191,192]. The phytotoxicity of weeds is probably

due to the alteration of several biochemical and physiological processes, affecting seed germination and/or hypocotyl and radical elongation [193–196]. In several cases, essential oils have demonstrated antibacterial and antifungal activity against pathogens affecting food or crops [197,198]. From this perspective, *Eucalyptus* species could represent a great source of EOs to be applied in the agricultural sector for the protection of crops and foodstuffs from pathogens.

The bioactivities depend on the quantitative and qualitative composition of the EO of each *Eucalyptus* species. Therefore, the identification of the species source of EOs is important, especially when pruning material comes from mixed plantations where different species are found side by side. Micro-morphological analyses are an essential starting point for quality control, especially for plant remnants and by-products [199]. These analyses involve the use of light microscopy (LM) and scanning electron microscopy (SEM), allowing us to characterize the plant material, highlight contaminations [200], and establish the correct botanical identity.

The micro-morphological characterization of pruning remnants from different *Eucalyptus* species, namely *E. gunnii*, *E. pulverulenta* "Baby blue," *E. cinerea*, and *E. nicholii*, cultivated for cut foliage in the hinterland of Western Liguria (Italy), was carried out. In addition, the phytochemical profile of the EOs obtained from the biomass of each of these species was analyzed. These EOs were then tested for selective phytotoxicity on weeds and crop seeds and against bacteria and fungi pathogens. Finally, their eco-compatibility was also evaluated for safe applications in agriculture as bioherbicides.

Materials and Methods

Standards and Reagents

Standards and reagents were purchased from Sigma Aldrich (Milan, Italy), where not otherwise specified.

Plant Material

E. gunnii (EG) and *E. pulverulenta* 'Baby blue' (EP) were collected in January 2021 in commercial plantations in the hinterland of Western Liguria (Finale Ligure, Savona, Italy) in Tovo San Giacomo (coordinates = 44.19683185◦ N, 8.26007775◦ E) and in Magliolo (coordinates = 44.1972954◦ N, 8.2558821◦ E), respectively. A voucher specimen of each species (GE5187 and GE5188, respectively) was deposited in the herbarium of the Department of Earth, Environment and Life Sciences, University of Genoa, Italy.

E. cinerea (EC) and *E. nicholii* (EN) were collected in February 2022 in commercial plantations in the hinterland of Western Liguria (Finale Ligure, Savona, Italy) at Tovo San Giacomo (coordinates = 44.19683_ N, 8.26005_ E) and at Magliolo (coordinates = 44.191671_ N, 8.252987_ E), respectively. A voucher specimen of each species (GDOR n. 60106 and GDOR 60105, respectively) was deposited in the herbarium of the Department of Earth, Environment and Life Sciences, University of Genoa, Italy.

The plants were approximately 10 months old and about 3 m high at the time of collection. About 1 kg of branches bearing juvenile foliage of each species was collected for both micromorphological analyses and EOs' extraction.

Extraction of Essential Oils

The foliage of the juvenile branches bearing were shattered and then subjected to hydrodistillation for 3 h using a Clevenger apparatus, according to the standard procedure described in the European Pharmacopoeia [201]. The EOs were dried over Na_2SO_4 and kept under N_2 at 4 ◦C in the dark, until analysis.

Micromorphological Analyses

Juvenile leaves were analysed through light microscopy (LM) and scanning electron microscopy (SEM). A preliminary morphological study of the leaves of each species was carried out with a LEICA M205 C stereomicroscope (Leica Microsystems, Wetzlar, Germany) to evaluate the shape, colour, venation pattern, and presence of waxes and oil glands.

Leaf epidermal peelings were obtained from both the abaxial and adaxial surfaces using the nail polish technique [202]. Six images for surface of each selected leaf were captured at 10× magnification, and the oil gland density was estimated according to the overlying cells on the epidermal surfaces on an area corresponding to 0.962 mm^2 using a Leica D.M. 2000 microscope equipped with a digital camera (DFC 320, Leica Microsystems, Wetzlar, Germany) [203]. All the captured images were analysed using image processing software ImageJ [204], which enables measurements and counting, allowing to obtain quantitative data. The statistical analysis was based on one-way analysis of variance (ANOVA) Student t with Bonferroni post-hoc tests for multiple pairwise mean comparisons, using the Data Analysis Tool Pack in Excel.

Transversal sections of fresh, healthy leaves were obtained by free-hand sectioning using a double-edged razor blade. Samples were mounted on glass slides in water and observed in LM. Different staining were used for anatomical and histochemical analyses: Toluidine Blue pH 4.0, Phloroglucinol-HCl, Sudan III, Fluorol-yellow [205,206].

Selected leaves of EG and EP were fixed, dehydrated, and paraffin embedded. Eight-micron thick cross sections were obtained using an automatic advance rotative microtome (Leica RM 2255, Leica Biosystems, Heidelberg, Germany) and stained with Toluidine Blue pH 4.0 and Masson's trichrome stain [207].

For SEM analyses, small portions of each leaves' species were fixed in 70% ethanol-FineFix working solution (Milestone s.r.l., Bergamo, Italy) for 24 h at 4 ◦C, dehydrated through ethanol series [148], and then critical point dried in CO_2 (CPD, K850 2M Strumenti s.r.l., Rome, Italy). Finally, the samples were mounted on the aluminium stubs using glued carbon tabs, sputter-coated with 10 nm gold [208], and observed with a Vega3 Tescan LMU SEM (Tescan USA Inc., Cranberry Twp, PA, USA) at an accelerating voltage of 20 kV.

GC-FID and GC-MS Analysis

Analytical gas chromatography (GC) was carried out on a Perkin-Elmer Sigma-115 gas chromatograph (Perkin Elmer, Waltham, MA, USA) equipped with a flame ionization detector (FID) and a data handling processor. The separation was achieved using a HP-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent, Roma, Italy). The analysis was also run using a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm i.d., 0.25 µm film thickness, Agilent, Roma, Italy). GC/MS analysis was performed on an Agilent 6850 Ser. II apparatus (Agilent, Roma, Italy), fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d., 0.33 µm film thickness, Agilent, Roma, Italy), coupled to an Agilent Mass Selective Detector MSD 5973. Detailed information regarding the elution program and the experimental parameters are extensively reported by Danna et al. [209].

Most constituents were identified by GC by comparison of their Kovats retention indices (Ri) (determined relative to the retention times (tR) of n-alkanes (C10–C35)), with either those of the literature [210–213] and mass spectra on both columns or those of authentic compounds available in our laboratories by means of NIST 02 and Wiley 275 libraries [214]. The component relative concentrations were obtained by peak area normalization. No response factors were calculated.

Phytotoxic Activity and α-Amylase Inhibitory Assay

The phytotoxic activity was evaluated on germination and radical elongation of several plant species: weeds (*Lolium multiflorum* Lam., *Portulaca oleracea* L.*, Sinapis alba* L.), horticultural crops (*Cucumis sativus* L., *Lactuca sativa* L., *Lepidium sativum* L., *Pisum sativum* L., *Raphanus sativus* L., *Solanum lycopersicum* L.), and the aromatic plant *Ruta graveolens* L. These seeds are often used for their easy and well-known germinability. *C. sativus, L. sativa*, *L. sativum*, *P. sativum*, *R. graveolens*, *R. sativus*, and *S. lycopersicum*, and were purchased from Blumen group SRL (Emilia-Romagna, Bologna, Italy). *L. multiflorum* seeds were purchased from Fratelli Ingegnoli Spa (Milano, Italy), *P. oleracea* seeds from W. Legutko SRL (Jutrosin, Poland), and the seeds of S. alba were collected from a wild population near the university campus in Fisciano (Salerno, Italy). A seed was considered germinated when the protrusion of the root became evident [215]. The seed germination and radical elongation experiments were performed as described by Danna et al. and Malaspina et. al. [209,216].

Screening of plant material for α-amylase inhibition was carried out for EG and EP EOs according to Xiao and coworkers [217] and Sudha and coworkers [218], based on the starch-iodine test slightly modified. The methodology applied is also reported by Danna et al. [209].

Ecotoxicity: Brine Shrimp Lethality Assay

The Ecotoxicity of the EG and EP EOs, was evaluated through the brine shrimp (*Artemia salina*) lethality assay carried out according to Caputo and coworkers [219]. The methodology applied is also reported by Danna et al. [209].

Plant Pathogens: Antimicrobial and Antifungal activities

The EOs of EC and EN were tested against three Gram-negative bacterial pathogens: *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot disease affecting especially Brassicaceae, and *Enterobacter cloacae* and *Citrobacter freundii*, clinical pathogens and emergent plant pathogens. The antimicrobial activity was evaluated through dilution broth susceptibility assays [220], measuring the bacterial concentrations via a Model 680 Microplate Reader at 600 nm (Bio-rad laboratories, Segrate, Italy) [221].

The EOs of EC and EN were also tested against two fungal phytopathogens: *Fusarium oxysporum* f. sp. *lycopersici* (4287), soilborne vascular fungus, and *Botrytis cinerea*, polyphagous foliar and soft rot pathogen. The growth inhibitory activity was carried out according to Vitale et al. [222]. Optical density at 600 nm (O.D. 600) was measured at 10 min intervals using a Tecan Infinite 200 Pro microplate reader (Tecan, Männedorf, Switzerland).

The methodologies applied are extensively described by Malaspina et al. [216].

Statistical Analysis

Results were analysed by one-way analysis of variance (ANOVA) using GraphPad Prism 6.0 (Software Inc., San Diego, CA, USA), expressed as means ± standard deviation (S.D.). Results were considered significant at $p < 0.05$.

Results

Macro and Micromorphological studies

Morphological analyses were performed on juvenile leaves of *E. gunnii* (EG) and *E. pulverulenta* 'Baby blue' (EP) collected during January 2021, and of *E. cinerea* (EC) and *E. nicholii* (EN) collected during February 2022 in the hinterland of Western Liguria (Italy) (*Figure 1*A–D).

Figure 1 Plantations of *E. gunnii (A), E. pulverulenta 'Baby blue' (B), E. cinerea (C)* and *E. nicholii (D)* located in the hinterland of Western Liguria (Italy) [209,216].

The leaves of all the *Eucalyptus* species analysed are glabrous and leathery, showing different shapes and colours depending on the abundance and arrangement of waxes. EG shows alternate dark green leaves, varying from sessile and rounded to pedunculate and more elongated. EP is characterized by blue grey opposite leaves, sessile with a rounded shape. EC leaves are opposite, sessile, oval/rounded, blue-grey, and densely reticulated. EN leaves are alternate, pedunculated, elongated, and dark green (*Figure 2*).

Figure 2 Juvenile branches of *E. gunni, E, pulverulenta 'Baby-blue', E. cinerea* and *E. nicholii.*

The leaves of each species were analysed through LM and SEM. All the species presented amphistomatal leaves with anomocytic stomata, oval or rounded, appearing at the same level as the neighbouring epidermal cells and as the overlying cells surrounding the oil cavities; trichomes are absent. Epidermal cells in all the species are polygonal isodiametric or slightly elongated in shape. Numerous papillae were observed in EG, EC and EN, while they were not visible in EP. The venation is pinnate-reticulate in all the species, appearing more or less prominent also depending on the wax deposition on the cuticle surfaces (larger in EP and EC, finer in EG and EN). Different arrangements of the waxes were found e.g., tubular waxes and crystalloid waxes (rosettes). In leaf transversal sections, the oil glands appear distributed throughout the mesophyll and are more protruding in the upper surface in all the species. The oil cavities cut close to the gland midpoint appeared spherical/ellipsoidal or pear-shaped. Each gland had a single internal epithelial layer, and the amorphous content was visible within the cavity. Oxalate calcium crystals were observed in all the species, within the mesophyll and in the surfaces. The midrib, in the transversal section, is slightly convex on both sides and consists of one or two large bicollateral vascular bundles in an open arc, with dorsal trace types more or less evident. The anatomical characteristics for all the species are reported in the above figures: *E. gunnii* (*Figure 3* and *Figure 4*), *E. pulverulenta* 'Baby blue' (*Figure 5* and *Figure 6*), *E. cinerea* (*Figure 7* and *Figure 8*) and *E. nicholii* (*Figure 9* and *Figure 10*).

Leaf epidermal peelings analysed via LM, and processed with the software Image J, showed the oil gland mean density of the abaxial and adaxial surfaces of all the species (*Table 1*).

Table 1 *E. gunnii, E. pulverulenta, E. cinerea* and *E. nicholii* oil glands mean density on the leaf surfaces [209,216].

Species	Oil Gland Mean Density (No. glands per mm^2 ± SD)	
	Abaxial	Adaxial
E. gunnii	2.1 ± 0.7	4.2 ± 1.2
E. pulverulenta	7.5 ± 1.0	13.2 ± 1.8
E. cinerea	9.6 ± 3.2	12.2 ± 2.4
E. nicholii	22.1 ± 4.9	18.3 ± 4.2

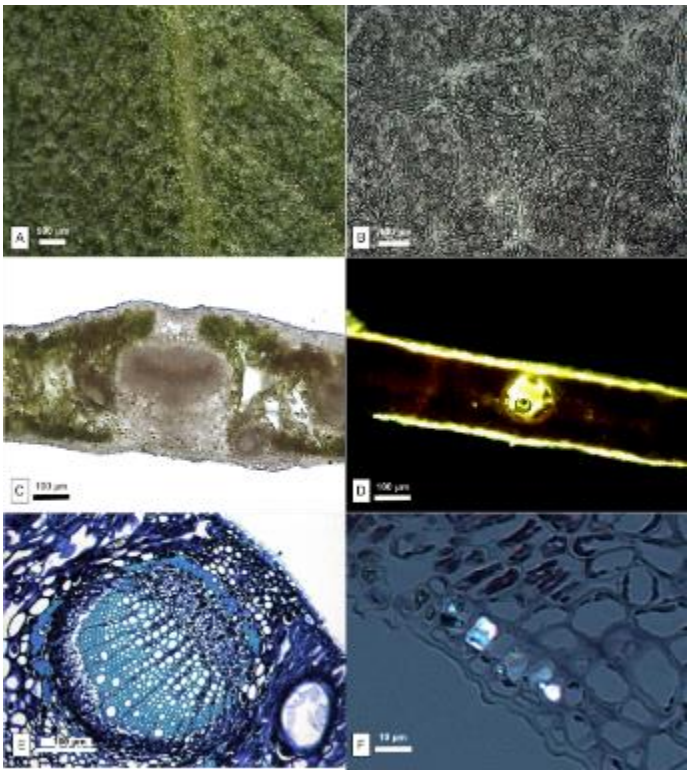


Figure 3 *E. gunnii* in LM. A: Abaxial surface (stereomicroscopy); B: abaxial surface peeling obtained via nail-polish technique; C: Transverse section through the midrib showing vascular bundles and oil cavities; D: oil gland in transversal leaf section (Fluorol-yellow stain); E: Paraffin-transverse sections through leaf midrib (TBO stain); F: Calcium oxalate crystals in the mesophyll (polarized light).

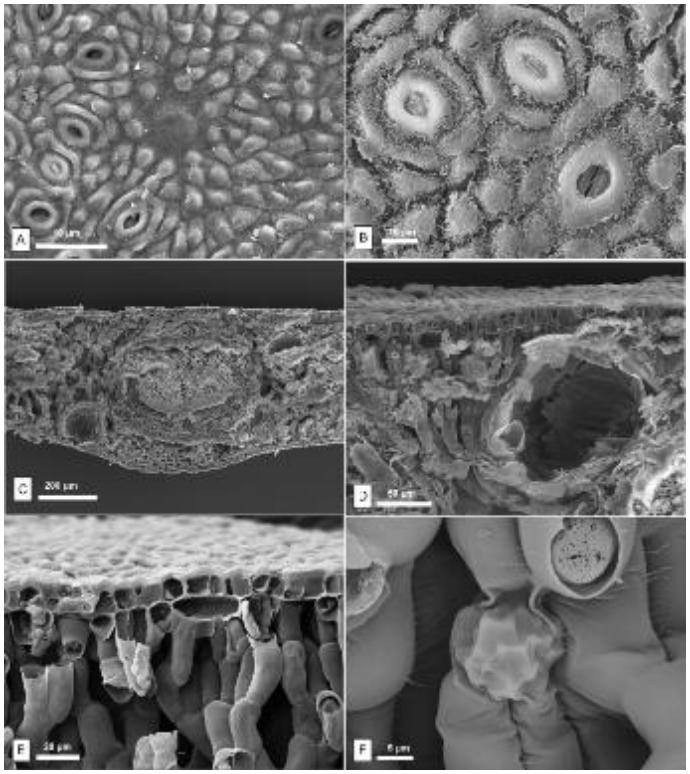


Figure 4 *E. gunnii* in SEM. A: Overlying cells surrounding oil cavities in the abaxial surface; B: abaxial surface, stomata, papillae and waxes focus; C: Leaf transversal section of the midrib; D: oil cavity within the mesophyll; E: papillae in transversal section; F: calcium oxalate crystal.

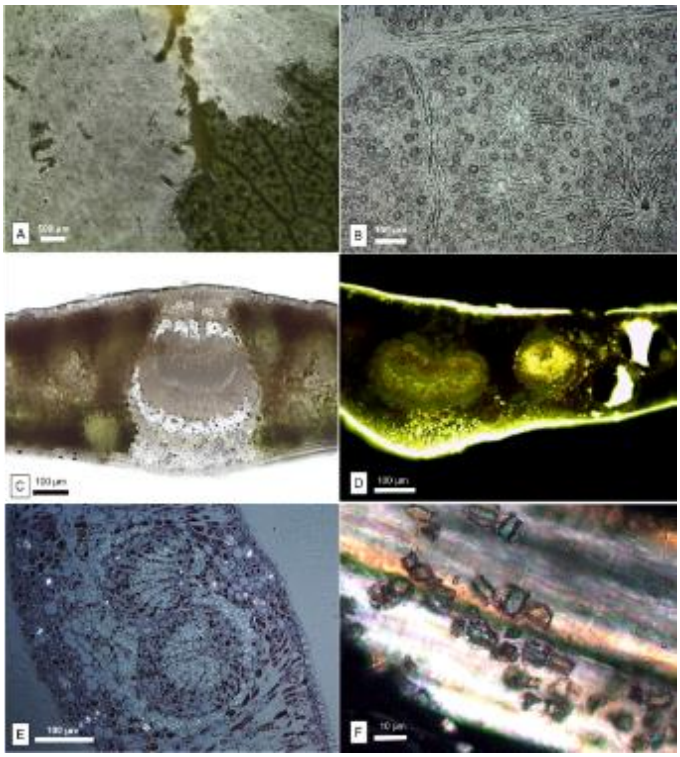


Figure 5 *E. pulverulenta* in LM. A: Abaxial surface (stereomicroscopy); B: abaxial surface peeling obtained via nail-polish technique; C: Transverse section through the midrib showing vascular bundle and oil cavities; D: Transverse section through the midrib and oil glands (Fluorol-yellow stain); E: Paraffin-transverse sections through leaf midrib in polarized light showing calcium oxalate Crystals (Masson's trichrome stain); F: Calcium oxalate crystals in the surface (polarized light).

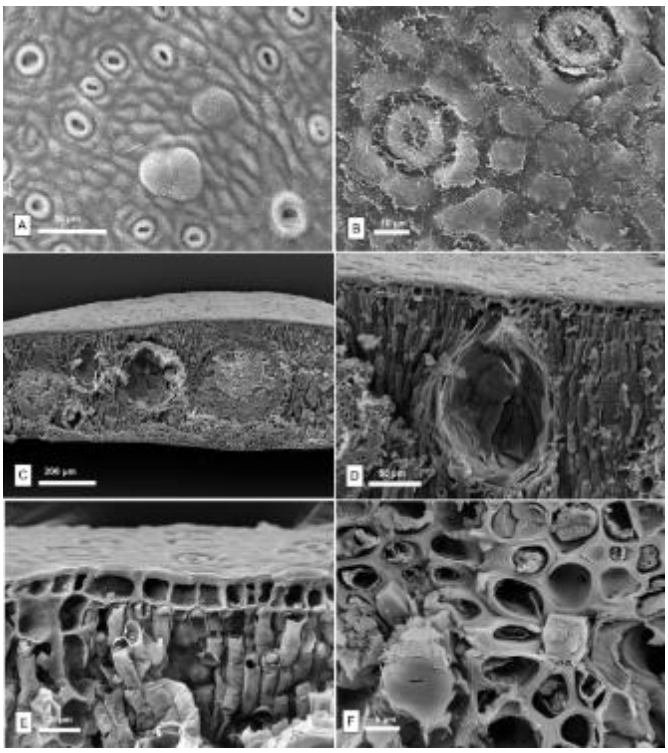


Figure 6 *E. pulverulenta* in SEM. A: Overlying cells surrounding oil cavities in the abaxial surface; B: abaxial surface, stomata, papillae and waxes focus; C: Leaf transversal section of the midrib; D: oil cavity within the mesophyll; E: papillae in transversal section; F: calcium oxalate crystal.

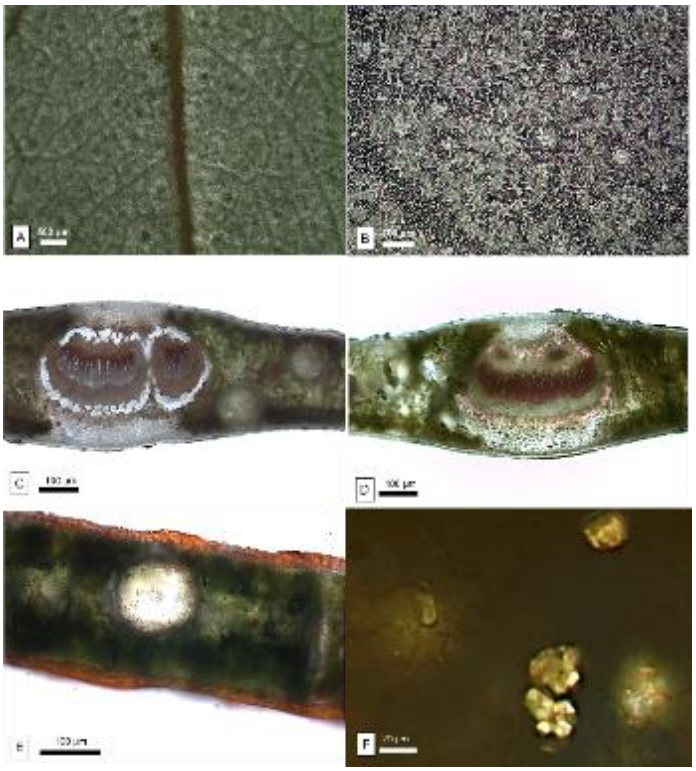


Figure 7 *E. cinerea* in LM. A: Abaxial surface (stereomicroscopy); B: abaxial surface peeling obtained via nail-polish technique; C: Transverse section through the midrib showing vascular bundles and oil cavities; D: Transverse section through the midrib and oil glands (Phloroglucinol-Hcl stain); E: transverse section with oil gland (Sudan stain); F: Calcium oxalate crystals in the mesophyll (polarized light).

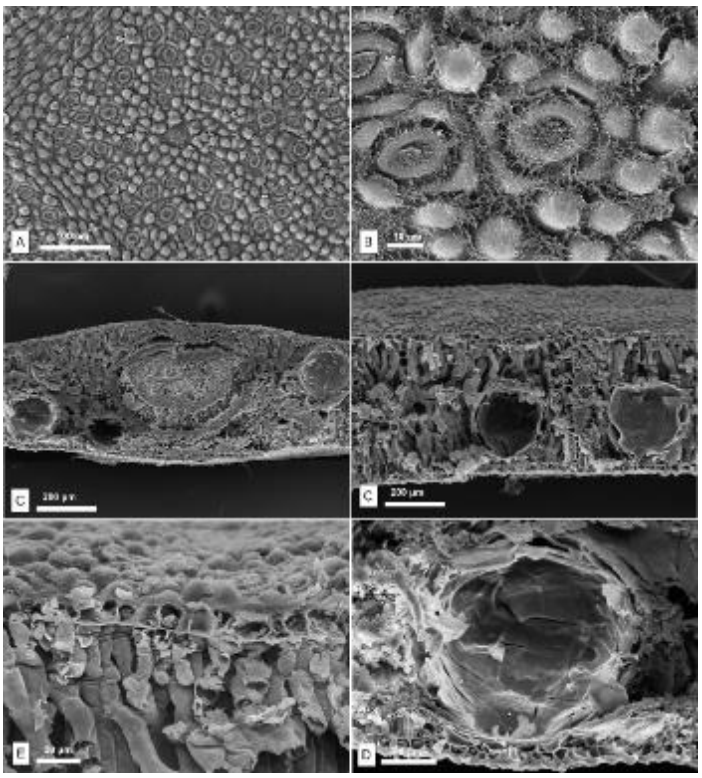


Figure 8 *E. cinerea* in SEM. A: abaxial surface, stomata and overlying cells surrounding oil cavities; B: abaxial surface, stomata, papillae and waxes focus; C: Leaf transversal section of the midrib; D: oil cavities within the mesophyll; E: papillae in transversal section; F: oil gland focus.

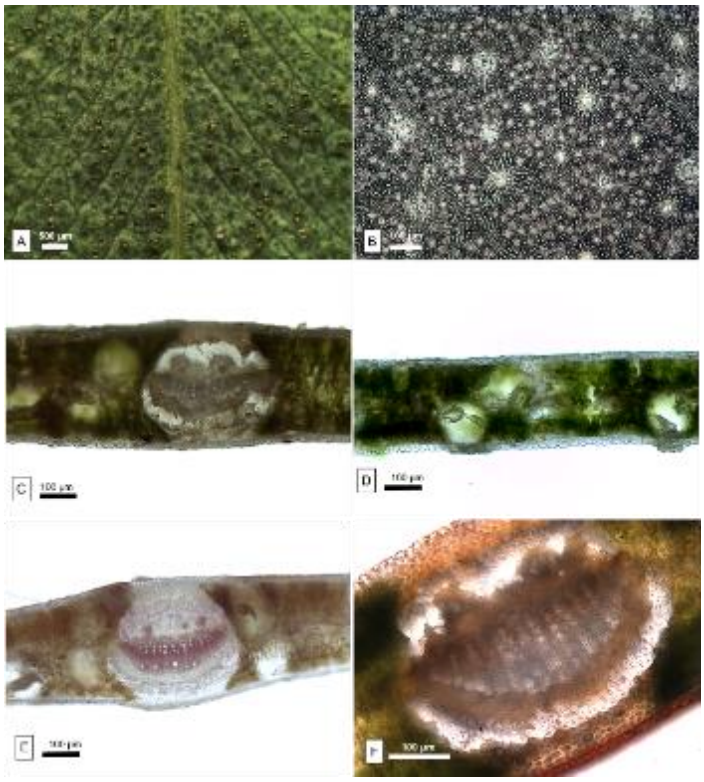


Figure 9 *E. nicholii* in LM. A: Abaxial surface (stereomicroscopy); B: abaxial surface peeling obtained via nail-polish technique; C: Transverse section through the midrib showing vascular bundles and oil cavities; D: Transverse section showing oil glands; E: Transverse section through the midrib with oil glands (Phloroglucinol-Hcl stain); F: Transverse section midrib focus (Sudan stain).

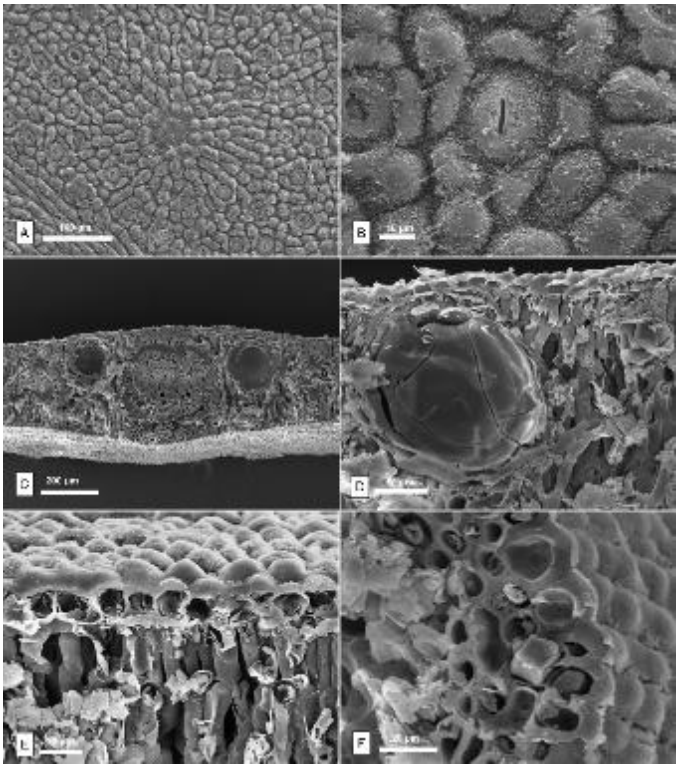


Figure 10 *E. nicholii* in SEM. A: abaxial surface, stomata and overlying cells surrounding oil cavities; B: abaxial surface, stomata, papillae and waxes focus; C: Leaf transversal section of the midrib; D: oil cavity within the mesophyll; E: papillae in transversal section; F: calcium oxalate crystal.

Chemical Composition of Essential Oils

The hydrodistillation of the aerial parts of the *Eucalyptus* species analysed furnished pale yellow EOs, with a yield of 1.1% (EG) and 1.6% (EP) on a dry mass basis, and of 2.6% (EC) and 3.7% (EN), on a fresh weight basis. The EOs composition with retention indices and area percentages for each compound are reported in *Table 2* (EG), *Table 3* (EP), *Table 4* (EC); *Table 5* (EN); the compounds are listed according to their elution order on a HP-5MS column.

Table 2 Chemical composition of the essential oil extracted from the leaves of *E. gunnii* (EG) determined via GC-FID and GC-MS spectra analysis [209].

Compound	EG %	KI [a]	KI [b]	Identification [c]
Santolina triene	t	848	1043	1,2
α-Pinene	13.1	872	1028	1,2,3
Camphene	0.1	874	1075	1,2,3
β-Pinene	0.3	901	1120	1,2,3
dehydro-1,8 Cineole	t	912	1095	1,2
Myrcene	0.3	925	1173	1,2,3
δ-2-Carene	0.1	934	1146	1,2,3
p-Cymene	t	945	1279	1,2,3
1,8-Cineole	74.7	959	1220	1,2,3
(Z)-β-Ocimene	t	976	1240	1,2,3
(E)-β- Ocimene	1.9	983	1260	1,2,3
γ-Terpinene	0.3	1016	1254	1,2,3
dehydro-Linalool	0.5	1043		1,2
1,3,8-*p*-Menthatriene	0.4	1048		1,2
allo-Ocimene	0.1	1055	1382	1,2
neo-Isopulegol	t	1064		1,2
Borneol	0.2	1074	1715	1,2,3
endo-Fenchol	0.2	1077		1,2
Terpineol	4.2	1099	1710	1,2,3
cis-Verbenol	0.2	1104	1663	1,2
Verbenone	0.2	1131		1,2
Thymol	0.1	1205	2172	1,2,3
δ-Elemene	0.1	1216	1460	1,2,3
γ-Elemene	0.1	1226	1651	1,2,3
Myltayl-4(12)-ene	0.1	1233		1,2
dihydro-Eugenol	0.5	1242		1,2
α-Copaene	t	1262	1477	1,2,3
Sibirene	t	1294		1,2
Caryophyllene	0.2	1296	1607	1,2,3
α-Guaiene	0.2	1372	1583	1,2
Total	**98.1**			
Monoterpene hydrocarbons	17.1			
Oxygenated monoterpenes	80.3			
Sesquiterpenes hydrocarbons	0.7			
Oxygenated sesquiterpenes				

a, b The Kovats retention indices determined relative to a series of n-alkanes (C10–C35) on the apolar HP-5 MS and the polar HP Innowax capillary columns, respectively; c identification method: 1 = comparison of the Kovats retention indices with published data, 2 = comparison of mass spectra with those listed in the NIST 02 and Wiley 275 libraries and with published data, and 3 = coinjection with authentic compounds; t = trace (<0.1%).

Table 3 Chemical composition of the essential oil extracted from the leaves of *E. pulverulenta* var Baby-blue (EP) determined via GC-FID and GC-MS analysis [209].

Compound	EP %	KI [a]	KI [b]	Identification [c]
1-Methyl-1,3-cyclohexadiene	t	759	1183	1,2
3-Methyl-2-buten-1-ol	t	761	1328	1,2
n-Octane	t	773		1,2
(2Z)-Hexenol	t	787		1,2
α-Pinene	4.8	872	1028	1,2,3
Camphene	0.1	874	1075	1,2,3
β-Pinene	0.5	901	1120	1,2,3
Myrcene	0.4	925	1173	1,2,3
δ-2-Carene	0.2	934	1146	1,2,3
β-Phellandrene	t	944	1206	1,2,3
1,8-Cineole	75.5	959	1220	1,2,3
(E)-β- Ocimene	1.0	983	1260	1,2,3
γ-Terpinene	0.9	1016	1254	1,2,3
dehydro-Linalool	0.5	1043		1,2
endo-Fenchol	1.0	1077		1,2
Terpineol	1.5	1099	1710	1,2,3
cis-Verbenol	0.1	1104	1663	1,2
Verbenone	0.2	1131		1,2
Thymol	0.3	1205	2172	1,2,3
dihydro-Eugenol	7.5	1242		1,2
α-Copaene	0.1	1262	1477	1,2,3
Sibirene	0.7	1294		1,2
α-Guaiene	0.3	1372	1583	1,2
Cycloisolongifol-5-ol <trans>	0.2	1395		1,2
Globulol	1.9	1457	2095	1,2
Total	**97.7**			
Monoterpene hydrocarbons	15.4			
Oxygenated monoterpenes	79.1			
Sesquiterpenes hydrocarbons	1.1			
Oxygenated sesquiterpenes	2.1			

a, b The Kovats retention indices determined relative to a series of n-alkanes (C10–C35) on the apolar HP-5 MS and the polar HP Innowax capillary columns, respectively; c identification method: 1 = comparison of the Kovats retention indices with published data, 2 = comparison of mass spectra with those listed in the NIST 02 and Wiley 275 libraries and with published data, and 3 = coinjection with authentic compounds; t = trace (<0.1%).

Table 4 Chemical composition of the essential oil extracted from the leaves of *E. cinerea* (EC) determined via GC-FID and GC-MS analysis [216].

Compound	EC %	Ki[a]	Ki[b]	Identification[c]
α-Pinene	7.3	858	1028	1,2,3
Camphene	0.2	869	1075	1,2,3
β-Pinene	0.3	893	1105	1,2,3
3-*p*-Menthene	0.1	903		1,2
β-Myrcene	0.2	913	1174	1,2,3
α-Phellandrene	0.8	921	1176	1,2,3
α-Terpinene	3.3	934	1188	1,2,3
Eucalyptol (1,8-cineole)	67.7	949	1213	1,2,3
γ-Terpinene	1.2	971	1254	1,2,3
p-Mentha-3,8-diene	0.2	982	1259	1,2
Terpinolene	3.5	998	1265	1,2,3
Linalool	0.2	1018	1553	1,2,3
allo-Ocimene	0.1	1039	1409	1,2
neo-allo-Ocimene	0.3	1042		1,2
Borneol	0.1	1067	1715	1,2,3
neoiso-Pulegol	0.2	1070		1,2
Terpinen-4-ol	0.6	1079	1705	1,2,3
α-Terpineol	3.9	1093	1720	1,2,3
Linalyl acetate	0.1	1219	1565	1,2
α-Terpinyl acetate	5.2	1234	1687	1,2
α-Copaene	0.3	1270	1498	1,2,3
α-Gurjunene	0.1	1287	1529	1,2
(E)-Caryophyllene	0.5	1295	1575	1,2,3
Aromadendrene	0.3	1308	1628	1,2
α-Humulene	0.1	1323	1651	1,2
allo-Aromadendrene	0.1	1330	1661	1,2
β-Selinene	0.2	1347	1697	1,2
Viridiflorene	0.5	1366	1713	1,2
Total	**97.6**			
Monoterpene hydrocarbons	17.5			
Oxigenated monoterpenes	78.0			
Sesquiterpene hydrocarbons	2.1			
Oxigenated sesquiterpenes	0			

a, b The Kovats retention indices determined relative to a series of n-alkanes (C10–C35) on the apolar HP-5 MS and the polar HP Innowax capillary columns, respectively; c identification method: 1 = comparison of the Kovats retention indices with published data, 2 = comparison of mass spectra with those listed in the NIST 02 and Wiley 275 libraries and with published data, and 3 = coinjection with authentic compounds; t = trace (<0.1%).

Table 5 Chemical composition of the essential oil extracted from the leaves of *E. nicholii* (EN) determined via GC-FID and GC-MS analysis [216].

Compound	EN %	Ki[a]	Ki[b]	Identification[c]
α-Pinene	3.7	858	1028	1,2,3
Camphene	0.2	869	1075	1,2,3
β-Pinene	0.2	893	1105	1,2,3
3-*p*-Menthene	0.2	903		1,2
β-Myrcene	0.1	913	1174	1,2,3
α-Phellandrene	0.8	921	1176	1,2,3
α-Terpinene	3.3	934	1188	1,2,3
Eucalyptol (1,8-cineole)	79.5	949	1213	1,2,3
γ-Terpinene	0.9	971	1254	1,2,3
p-Mentha-3,8-diene	0.1	982	1259	1,2
Terpinolene	2.5	998	1265	1,2,3
Linalool	0.3	1018	1553	1,2,3
trans-Pinocarveol	0.3	1041	1641	1,2
Pinocarvone	0.1	1063	1587	1,2
Borneol	0.1	1067	1715	1,2,3
neoiso-Pulegol	0.1	1070		1,2
Terpinen-4-ol	0.7	1079	1705	1,2,3
α-Terpineol	3.9	1093	1720	1,2,3
δ-Elemene	0.1	1220	1480	1,2,3
(E)-Caryophyllene	0.3	1295	1575	1,2,3
allo-Aromadendrene	0.2	1330	1661	1,2
Viridiflorene	0.1	1366	1713	1,2
Total	**97.7**			
Monoterpene hydrocarbons	12.0			
Oxigenated monoterpenes	85.0			
Sesquiterpene hydrocarbons	0.7			
Oxigenated sesquiterpenes	0			

a, b The Kovats retention indices determined relative to a series of n-alkanes (C10–C35) on the apolar HP-5 MS and the polar HP Innowax capillary columns, respectively; c identification method: 1 = comparison of the Kovats retention indices with published data, 2 = comparison of mass spectra with those listed in the NIST 02 and Wiley 275 libraries and with published data, and 3 = coinjection with authentic compounds; t = trace (<0.1%).

Several compounds were identified: 30 for EG, accounting for 98.1% of the total EO, 25 for EP, accounting for 97.7% of the total EO, 28 for EC, accounting for 97.6% of the total EO, and 22 for EN, accounting for 97.7% of the total EO. The oxygenated monoterpenes are the main constituents in all the EOs analysed, with a percentage of 80.3% for EG, 79.1% for EP, 78.0% for EC and 85.0% for EN. 1,8-Cineole was the main constituent, with a percentage of 67.7% in EC, 74.7% in EG, 75.5% in EP, and 79.5% in EN.

Other components comprising a percentage greater than 1% differed in the several species analysed: in EG were abundant α-pinene (13.1%), terpineol (4.2%), (E)-β-ocimene (1.9%); in EP dihydro-eugenol (7.5%) and α-pinene (4.8%), globulol (1.9%), terpineol (1.5%); in EC α-pinene (7.3%), and terpinene derivatives: α-terpinene (3.3%), γ-terpinene (1.2%), terpinolene (3.5%), α-terpineol (3.9%) and α-terpinyl acetate (5.2%); in EN α-pinene (3.7%), α-terpinene (3.3%), terpinolene (2.5%), and α-terpineol (3.9%).

Phytotoxic and Anti-α-Amylase Activity

Data concerning the phytotoxic activity, evaluated by seeds germination and radicle elongation, exerted by the EOs tested on different plant species are reported in *Table 6* for *E. gunnii*, *Table 7* for *E. pulverulenta*, *Table 8* for *E. cinerea*, *Table 9* for *E. nicholii*.

Table 6 Phytotoxic activity of the *E. gunnii* essential oil, applied at different concentrations (μg/mL) to seeds of diverse plant species [209].

Seeds		N° Germinated seeds				Radicle lenght			
		1000	500	250	100	1000	500	250	100
L. sativa	Mean ± SD	7.7 ± 0.6	8.3 ± 0.6	8.0 ± 0.0	9.5 ± 0.7	1.7 ± 0.6	2.5 ± 0.5	2.3 ± 0.6	2.6 ± 0.7
	%	9.4	2.3	0	0	37.0	7.4	17	3
P. oleracea	Mean ± SD	0.3 ± 0.6 *	2.3 ± 2.3	2.7 ± 2.1	3.3 ± 2.1	2.3 ± 0.4	2.1 ± 0.2	2.2 ± 0.3	2.0 ± 0.1
	%	94.6	58.9	51.8	41.1	0	0	0	0
R. sativus	Mean ± SD	2.7 ± 0.6 **	3.7 ± 0.6 *	5.0 ± 2.6	3.7 ± 0.6 *	1.0 ± 0.2	1.2 ± 0.6	1.5 ± 0.3	1.7 ± 0.4
	%	61.5	47.2	28.6	47.8	0	0	0	0
S. lycopersicum	Mean ± SD	2.6 ± 1.5 **	4.5 ± 0.7 *	5.7 ± 0.6	5.6 ± 0.6	1.3 ± 0.5 **	2.2 ± 1.9 *	2.3 ± 1.7 *	2.5 ± 1.8 *
	%	67.5	43.8	27.8	30	82.6	70.7	69.3	66.7
P. sativum	Mean ± SD	9.0 ± 1.7	9.0 ± 1.7	7.3 ± 2.1	9.3 ± 1.2	4.1 ± 1.0	4.0 ± 1.0	4.5 ± 1.1	3.9 ± 0.9
	%	7.2	7.2	24.7	4.1	0	0	0	0
C. sativus	Mean ± SD	6.0 ± 0.0	5.3 ± 0.6	5.7 ± 0.6	4.3 ± 0.6	6.0 ± 1.6	7.0 ± 1.0	6.9 ± 1.7	6.4 ± 0.8
	%	25	33.7	28.7	46.2	15.5	1.4	2.8	9.9
L. sativum	Mean ± SD	9.0 ± 1.0	8.3 ± 0.6	7.5 ± 0.7	9.1 ± 0.3	2.0 ± 1.4	3.0 ± 1.2	3.2 ± 1.3	2.5 ± 1.8
	%	5.5	2.3	11.8	0	63.0	44.4	40.7	53.7
L. multiflorum	Mean ± SD	7.0 ± 2.0	7.5 ± 0.7	7.7 ± 0.6	7.0 ± 2.6	2.6 ± 0.6 *	3.5 ± 1.1	4.0 ± 1.0	3.5 ± 1.0
	%	12.5	6.2	3.7	12.5	53.5	37.5	28.6	37.5

Results are reported as the mean ± SD of three experiments. * $p < 0.05$. ** $p < 0.01$. vs control (inhibition = 0), according to two-way ANOVA followed by Tuckey's multiple comparison test, at the significance level of $p < 0.05$.

Table 7 Phytotoxic activity of the *E. pulverulenta* essential oil, applied at different concentrations (μg/mL) to seeds of diverse plant species [209].

Seeds		N° Germinated seeds				Radicle lenght			
		1000	500	250	100	1000	500	250	100
L. sativa	Mean ± SD	9.0 ± 0.0	8.0 ± 0.0	8.5 ± 0.7	8.5 ± 0.7	2.2 ± 0.5	2.1 ± 0.3	2.2 ± 0.5	2.1 ± 0.5
	%	0	5.9	0	0	4.5	9.5	4.5	9.5
P. oleracea	Mean ± SD	1.7 ± 1.5 **	2.0 ± 1.7 **	4.3 ± 1.5	2.7 ± 1.2 *	1.9 ± 0.4	2.1 ± 0.3	2.3 ± 0.2	2.2 ± 0.2
	%	74.7	70.2	35.9	59.8	0	0	0	0
R. sativus	Mean ± SD	4.0 ± 0.0	3.7 ± 1.2 *	3.7 ± 1.5 *	6.0 ± 1.7	1.8 ± 0.5	1.5 ± 0.8	1.7 ± 0.3	2.4 ± 0.6
	%	36.6	41.3	41.3	38.4	0	0	0	0
S. lycopersicum	Mean ± SD	8.3 ± 0.6	7.3 ± 0.6	7.3 ± 0.6	8.2 ± 0.6	3.2 ± 1	4.5 ± 1.7	4.6 ± 1.7	5.8 ± 1.8
	%	0	8.7	8.7	0	57.3	40	38.7	22.7
P. sativum	Mean ± SD	9.3 ± 0.6	8.7 ± 0.6	10 ± 0.0	9.7 ± 0.6	3.9 ± 0.6	3.7 ± 0.8	4.5 ± 1.1	4.8 ± 0.9
	%	4.1	10.3	3	0	0	0	0	0
C. sativus	Mean ± SD	5.3 ± 0.6	5.7 ± 0.6	6.0 ± 0.0	6.3 ± 0.6	5.4 ± 0.7	7.5 ± 0.9	6.7 ± 1.1	7.2 ± 1.9
	%	11.6	5	0	0	23.9	0	5.6	0
L. sativum	Mean ± SD	7.0 ± 0.0	6.7 ± 1.5	6.7 ± 2.1	8.0 ± 1.7	1.1 ± 0.2 **	1.9 ± 0.4 *	2.2 ± 0.8 *	2.5 ± 0.7 *
	%	12.5	16.2	16.2	0	76.0	58.7	52.2	45.6
L. multiflorum	Mean ± SD	7.3 ± 0.6	6.0 ± 1.0	7.0 ± 0.0	8.3 ± 0.6	1.6 ± 0.7 **	1.7 ± 0.4 **	2.3 ± 0.7 **	3.4 ± 0.7
	%	0	14.2	0	0	70.4	68.5	57.4	37.0

Results are reported as the mean ± SD of three experiments. * $p < 0.05$. ** $p < 0.01$. vs control (inhibition = 0), according to two-way ANOVA followed by Tuckey's multiple comparison test, at the significance level of $p < 0.05$.

Table 8 Phytotoxic activity of the *E. cinerea* essential oil, applied at different concentrations (μg/mL) to seeds of diverse plant species [216].

	N° Germinated Seeds						
	L. multiflorum	*S. alba*	*C. sativus*	*L. sativa*	*P. sativum*	*R. sativum*	*R. graveolens*
Control	6.3 ± 1.5	8.3 ± 1.5	9.3 ± 1.2	7.3 ± 1.5	9.7 ± 0.6	6.3 ± 1.2	6.3 ± 2.5
Treatment (μg/mL)							
100	3.3 ± 2.1	6.0 ± 1.7	8.7 ± 0.6	8.0 ± 2.0	8.7 ± 2.3	2.0 ± 1.0 **	1.0 ± 1.0 **
250	8.0 ± 1.0	1.0 ± 1.0 ****	9.7 ± 0.6	9.0 ± 1.0	8.7 ± 1.5	2.0 ± 2.0 **	0.3 ± 0.6 ***
500	6.0 ± 3.6	0.7 ± 1.2 ****	10.0 ± 0.0	7.3 ± 1.5	8.7 ± 1.5	0.0 ± 0.0 ***	2.0 ± 1.0 **
1000	6.7 ± 1.5	0.0 ± 0.0 ****	8.7 ± 1.2	8.0 ± 0.0	8.0 ± 1.0	0.3 ± 0.6 ***	0.3 ± 0.6 ***
	Radicle length						
	L. multiflorum	*S. alba*	*C. sativus*	*L. sativa*	*P. sativum*	*R. sativum*	*R. graveolens*
Control	1.2 ± 0.6	0.4 ± 0.2	6.3 ± 0.9	3.2 ± 1.2	6.0 ± 1.8	3.1 ± 1.0	1.7 ± 0.8
Treatment (μg/mL)							
100	0.4 ± 0.0	0.2 ± 0.1	3.4 ± 0.7 ***	1.4 ± 0.5	3.5 ± 1.3	2.2 ± 0.0**	0.0 ± 0.0 **
250	0.9 ± 0.4	0.0 ± 0.0 **	2.3 ± 0.5 ****	2.5 ± 0.7	2.4 ± 0.8 *	0.0 ± 0.0 **	0.0 ± 0.0 **
500	1.1 ± 0.5	0.0 ± 0.0 **	2.3 ± 0.4 ****	1.8 ± 0.7	2.9 ± 0.9 *	0.0 ± 0.0 **	0.9 ± 0.0
1000	0.8 ± 0.4	0.0 ± 0.0 **	2.3 ± 0.8 ****	2.3 ± 0.8	2.8 ± 1.0 *	0.0 ± 0.0 **	0.0 ± 0.0 **

Results are reported as the mean ± SD of three experiments. Significant differences among the dose concentrations of the EO treatments: *P < 0.05; ** P < 0.01, *** P < 0.001, **** P < 0.00001.

Table 9 Phytotoxic activity of the *E. nicholii* essential oil, applied at different concentrations (μg/mL) to seeds of diverse plant species [216].

	N° Germinated Seeds						
	L. multiflorum	*S. alba*	*C. sativus*	*L. sativa*	*P. sativum*	*R. sativum*	*R. graveolens*
Control	5.3 ± 2.1	9.7 ± 0.6	9.0 ± 1.0	8.7 ± 0.6	10.0 ± 0.0	6.0 ± 1.0	8.0 ± 2.0
Treatment (μg/mL)							
100	5.7 ± 1.5	6.0 ± 1.0 ***	10.0 ± 0.0	8.0 ± 0.0	9.3 ± 0.6	2.0 ± 1.0 **	4.7 ± 3.5
250	4.3 ± 1.2	4.0 ± 1.0 ****	10.0 ± 0.0	8.7 ± 0.6	9.0 ± 0.0	2.0 ± 1.0 **	1.0 ± 1.0 **
500	5.3 ± 3.2	0.0 ± 0.0 ****	9.0 ± 1.0	9.0 ± 1.7	9.7 ± 0.6	0.7 ± 0.6 ***	1.0 ± 1.0 **
1000	5.7 ± 2.3	0.0 ± 0.0 ****	10.0 ± 0.0	8.0 ± 1.0	9.0 ± 1.0	0.7 ± 1.2 ***	0.0 ± 0.0 **
	Radicle length						
	L. multiflorum	*S. alba*	*C. sativus*	*L. sativa*	*P. sativum*	*R. sativum*	*R. graveolens*
Control	3.4 ± 0.8	0.6 ± 0.2	6.7 ± 0.9	2.7 ± 1.0	6.3 ± 1.9	2.8 ± 1.1	1.2 ± 0.5
Treatment (μg/mL)							
100	0.9 ± 0.3 ***	0.4 ± 0,2	2.6 ± 0.7 ***	2.6 ± 0.8	3.4 ± 0.9 *	1.3 ± 0.0 **	0.8 ± 0.0
250	0.6 ± 0.2 ****	0.2 ± 0.1 *	2.7 ± 0.5 ***	1.5 ± 0.5	2.9 ± 0.7 *	1.9 ± 0.0	0.0 ± 0.0 ***
500	0.6 ± 0.2 ****	0.0 ± 0.0 ***	1.9 ± 0.6 ****	2.6 ± 0.9	3.7 ± 1.2	0.0 ± 0.0 ****	0.0 ± 0.0 ***
1000	0.9 ± 0.4 ***	0.0 ± 0.0 ***	2.0 ± 0.9 ****	2.4 ± 0.8	2.9 ± 1.2 *	0.0 ± 0.0 ****	0.0 ± 0.0 ***

Results are reported as the mean ± SD of three experiments. Significant differences among the dose concentrations of the EO treatments: *P < 0.05; ** P < 0.01, *** P < 0.001, **** P < 0.00001.

The EOs showed variable phytotoxicity on the seeds depending on the plant species tested and the concentration used. Regarding EG and EP EOs, EG EO inhibited the germination of *P. oleracea*, *R. sativus*, and *S. lycopersicum* seeds, while affecting only radical elongation in *S. lycopersicum* seeds. Instead, EP EO inhibited the germination of *P. oleracea* and *R. sativus* and affected the radical elongation of *L. sativum* and *L. multiflorum.* Moreover, EG and EP EOs did not show any effects against the germination and/or radical elongation of *L. sativa*, *P. sativum*, and *C. sativus*. Regarding EC and EN, both EOs, at the highest concentrations tested, completely inhibited the germination and the radicle elongation of the weed species *S. alba* L. and *L. multiflorum* Lam. On the contrary, germination was stimulated in the case of *L. sativa* L. for EC and *C. sativus* L. for EN. Among the crop plants, both EC and EN EOs showed no significant inhibition of germination in all tested seeds, except for *R. sativus* L. The aromatic plant *R. graveolens* L. was the most sensitive species, being significantly affected by the EC and EN EOs both in germination and radicle elongation.

α-Amylase is a key enzyme for seed growth regulation, being involved in the hydrolysation of the starch during the seed germination process [223]. For this reason, after the determination of the phytotoxic activity of the EOs, their activity on α-amylase regulation was studied for the EG and EP EOs (*Table 1*0).

Table 10 *α*-Amylase inhibitory activity of *E.gunnii* and *E. pulverulenta* 'Baby blue' EOs [209].

Essential Oil	IC_{50} (μg/mL)
E. pulverulenta	35.9 ± 3.6
E. gunnii	524.1 ± 10.3
Acarbose (positive control)	130.2 ± 12.4

Data are expressed as mean ± S.D. (*n* = 3).

Ecotoxicity

The ecotoxicity of EG and EP EOs was evaluated using the toxicological test on *Artemia salina*, a marine zooplanktonic organism, which thanks to its easy cultivation, availability, low cost, and adaptation to adverse conditions represents a golden standard in toxicological assays [224]. Both EOs were tested in 0.01–100 mg/mL dose range. Until 10 mg/mL concentration, the tested EOs did not shown on the nauplii any toxicity or swimming alteration behaviour at the endpoints of 24 and 48 h. On the contrary, at the highest dose (100 mg/mL) both EOs showed some toxicity at 24 h (16.66 vs. 13.33% for EG and EP EOs, respectively), and at 48 h (85.0 vs. 50% for EG and EP EOs, respectively).

Antimicrobial Activity

The antimicrobial activity of the EOs of EC and EN was tested on emergent bacterial plant pathogens of agricultural interest *Xanthomonas campestris pv. campestris*, *Enterobacter cloacae*, and *Citrobacter freundii*. the Minimum Inhibitory concentration (MIC) was evaluated for the EOs, and for some pure components at different endpoints (2, 6, 12, and 24 h) (*Table 11* and *Figure 11*).

Table 11 Minimum Inhibitory Concentration (MIC) of the *E. cinerea* and *E. nicholii* EOs [216]..

Treatment	MIC (% v/v)		
	Xanthomonas campestris pv. *campestris*	*Enterobacter cloacae*	*Citrobacter freundii*
***E. cinerea* EO**	0,01%	0,01%	0,01%
***E. nicholii* EO**	0,01%	0,01%	0,1%

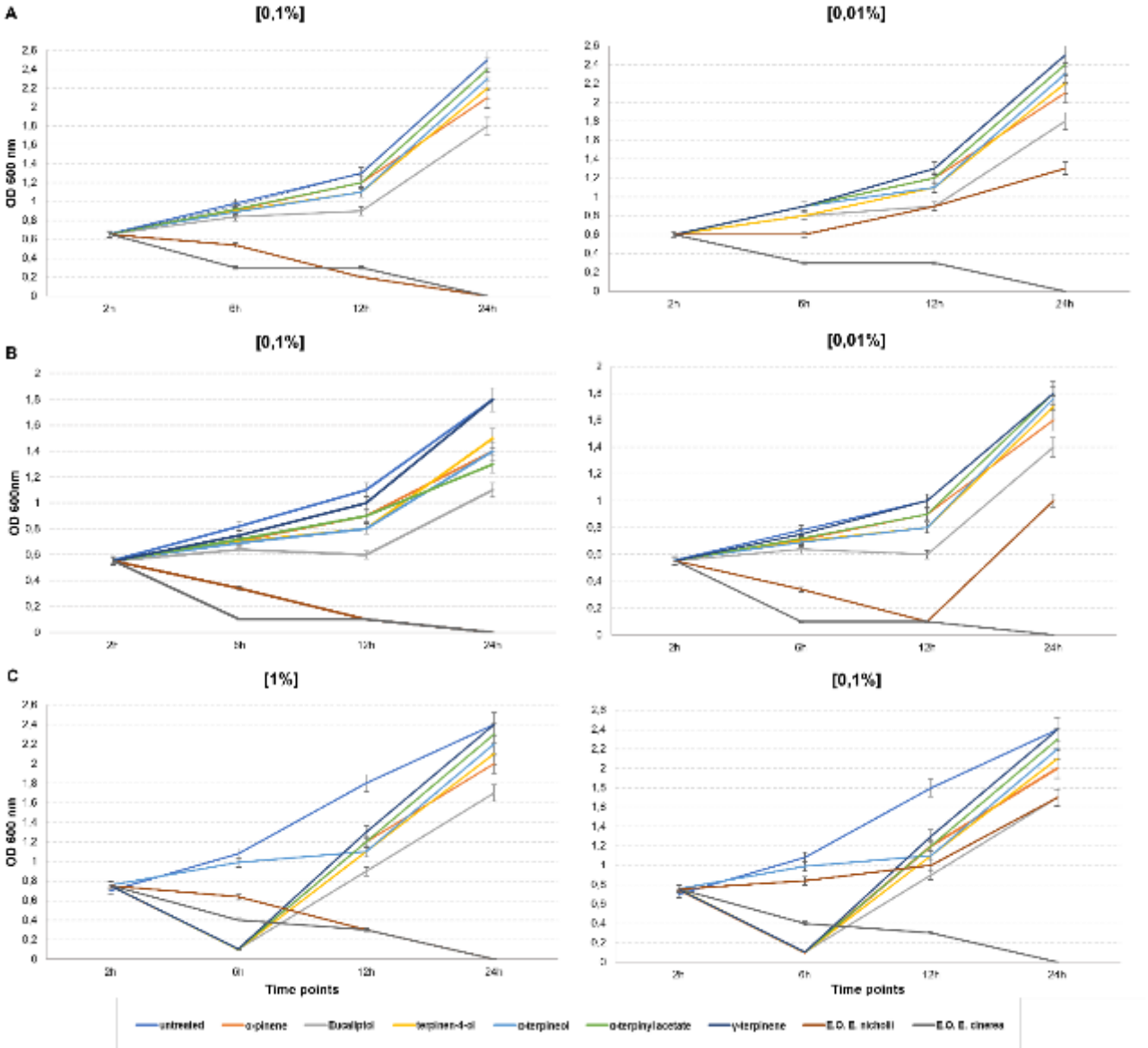


Figure 11 Antibacterial activity of different concentrations (0, 0.01, 0.1 and 1% v/v) *E. cinerea* and *E. nicholii* EOs, and six pure EO compounds on: (A) *Xanthomonas campestris* pv. *Campestris*, (B) *Enterobacter cloacae*, (C) *Citrobacter freundii* 24 h after treatment [216].

Antifungal Activity

The biological activity of the EOs of EC and EN and of some the pure compounds was evaluated on the plant fungal pathogens *Fusarium oxysporum* f.sp. *lycopersici* and *Botrytis cinerea*. The results (*Figure 12*) indicated that, at the lowest concentration of 0.1% v/v, the EN EOs was more efficient in controlling the fungi respect to the EC EO. This greater inhibition could be attributed to the higher concentration of eucalyptol in the EO of EN compared to that of EC (79.5% and 67.7%, respectively). In general, the pure α-terpineol and α-pinene compounds demonstrated little antifungal activity while γ-terpinene seems to be a promising compound in the fungal growth control.

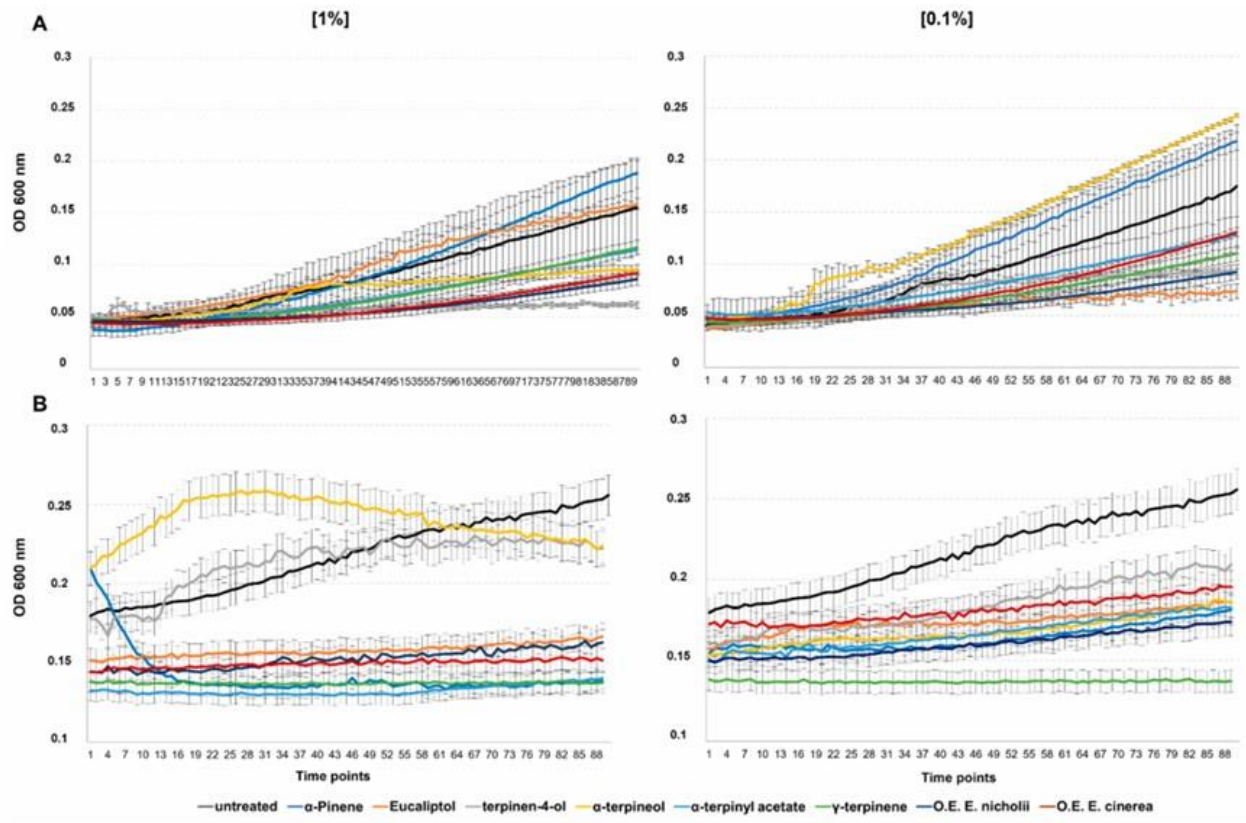


Figure 12 Antifungal activity of *E. cinerea* and *E. nicholii* EOs and six pure compounds applied at different concentrations to the fungal pathogens: (A) *F. oxysporum* and (B) *B. cinerea*. After 80 min of incubation, measurements were conducted at 10 min[216].

Discussion

The genus *Eucalyptus* has a high aesthetic and economic value for the floriculture sector and its fronds are increasingly used in the markets of Northern Europe for flower arrangements. Based on the data collected by the General Census of Agriculture, it was estimated that in 2005, eucalyptus production already accounted for 22% of the frond crops harvested in Western Liguria (provinces of Imperia and Savona) that went to the ornamental floral sector, with an extension of cultivation covering over 350 ha. The growing relevance of these species has been reported by the flower market of Sanremo, whereby 50% of the green fronds marketed in the 2014–2015 period consisted of eucalyptus [225]. Thus, with the augmented eucalyptus production in recent years to meet the demand, there has also been a corresponding increase in the quantity of frond by-products derived from this activity. This fact raises the problem of the elimination of vegetative wastes, but, at the same time, it provides novel opportunities for the recovery and valorization of this plant material as a source of natural value-added products. In this research, we focused on four *Eucalyptus* species cultivated in Liguria and sold in the European market for ornamental purposes: *E. gunnii*, *E. pulverulenta* "Baby blue," *E. cinerea*, and *E. nicholii*.

Micromorphological characterization is fundamental for the evaluation of the raw plant material, whether crushed or powdered, and necessary for the standardization or quality control of the plant by-product inputs in the recycling process. In these fragmented tissues, it is still possible to analyze the anatomical vegetative features, corroborate the conformity of the starting material, and note any adulterations or contamination from foreign organisms. In the genus *Eucalyptus*, the macromorphological characteristics of taxonomic value are the shape, color, and vein pattern of the leaves, while the important micromorphological features include the type of stomata, morphology of epicuticular waxes, the presence of epidermal papillae that are more or less prominent, and the shape of the midrib section shape [226]. The features evidenced agree with data previously reported for other *Eucalyptus* species [203,227,228].

The phytochemical profile of the EOs obtained from the different species allows the identification and quantification of the phytocompounds. In our study, the major compounds found in all the analyzed EOs were 1.8-cineole. Its presence was recorded at 79.5% in EN, 75.5% in EP, 74.7% in EG, and 67.7% in EC. Similar compositions were observed regarding α-pinene and terpinene derivatives (α-terpinene, γ-terpinene, terpinolene, α-terpineol, and α-terpinyl acetate), as well as other main constituents.

A comparison with essential oils of the same species grown in different regions allowed us to highlight similarities and differences in the phytochemical profiles. The chemical composition of EG EO is very similar to a Serbian sample presenting a high percentage of 1.8-cineole (67.8%), and α-pinene as the second major compound with a percentage of 14.1% [229]. EG EO differs from the Argentina sample in which 1.8-cineole is detected as the major compound (17.9%), and in which high percentages of p-cymene (12.3%), spathulenol (12.3%), and α-phellandrene (7.0%) are found [230]. EG EO also differs from the Sardinian sample in which the 1.8-cineole is found as the major compound (33%), with high amounts of trans-sabinene hydrate acetate (15%), globulol (10.3%), and longicyclene (9.1%) [219]. EG EO is rich in oxygenated monoterpenes, compared to an EG Tunisian sample, with a higher percentage of oxygenated sesquiterpenes (spathulenol, globulol, and viridiflorol) [231]. The chemical composition of EP EO in our sample agrees with data reported in previous studies carried out on samples from Morocco [232], Australia [233], and Tuscany [234], in which 1.8-cineole was the main constituent, ranging from 75.1 to 85.1%, and α-pinene was present in comparable amounts, ranging from 2.1 to 4.0%. Some differences were noted, e.g., α-terpinyl acetate was present in EOs of samples from Tuscany (Italy) and Australia, but it is absent in our sample. On the contrary, dihydroeugenol, present in our sample, was absent in the EP EO collected in Morocco, Australia, and Tuscany. The chemical composition of EC EO was similar to the EO of samples from Northwest Tunisia, presenting 1.8-cineole as a major compound

(61%), and high percentages of camphene (15.13%) and globulol (4.06%) [235]. Greater similarity in the EOs, with a range of 58.7%-90.1% and 1.8-cineole as a major compound, was found in species coming from Egypt [236], Tunisia [237,238], Iran [239], India [240], Argentina [241,242], and Brazil [243,244]. Interestingly, environmental conditions, and thus geographic locations, and season of harvesting have a decisive influence on the composition of the EOs as reported in the study conducted on EC grown in Egypt [245]. Although data in the literature on the chemical composition of EO EN are scarce [246], the confirmation of 1,8-cineole as the main compound (with values of 82.2%) was provided by Lee and collaborators [247].

In general, the phytochemical profile of the EOs obtained from different plant species is important to compare their biological activities and to discriminate specific compounds with potential effects as antibacterial, antifungal, and herbicidal agents.

The phytotoxicity of the EOs was confirmed in the literature, and the bioactivity is primarily attributed to the high percentage of eucalyptol and terpinene derivatives [248–251]. Different mechanisms of action could be involved [56,252] and these compounds were proposed both for direct use as a bioherbicide and as a starting point for the synthesis of herbicide [253,254]. Potential applications of *Eucalyptus* EOs for their phytotoxic effects on weeds have been reported, e.g., against monocotyledons such as *Cynodon dactylon* L., *Digitaria sanguinalis* (L.) Scop., and *Echinochloa crus-galli* (L.) P. Beauv [184,187,189], and dicotyledons such as *Acroptilon repens* (L.) DC., *Amaranthus blitoides* S.Watson, *A. retroflexus* L., *A. viridis* L., *Chenopodium album* L., and *Parthenium hystorophorus* L. *Portulaca oleracea* L. [184,187–190,255–257]. However, the phytotoxic effects of EOs on crops were also referred to in the literature [191,192,258,259], making it essential to assess their selectivity.

In general, the EOs of the *Eucalyptus* species investigated here showed phytotoxicity toward the tested weeds (*Lolium multiflorum, Portulaca oleracea, and Silene alba)*, varying in relation to the concentration used and the species examined with variable effects both on seed germination and radicle elongation. On the contrary, all the EOs showed no significant inhibition of seed germination of crop plants (*Cucumis sativus*, *Lactuca sativa*, *Lepidium sativum*, and *Pisum sativum*). In some cases, germination was even stimulated, except for *Raphanus sativus* and *Solanum lycopersicum,* and the aromatic plant *Ruta graveolens*. Radicle elongation of *Solanum lycopersicum*, *Lepidium sativum,* and *Ruta graveolens* was also decreased in some cases. Considering the results obtained in the α-amylase inhibition test, it is possible to hypothesize that the variability in EO phytotoxic activities may be also related to the difference in the starch reserves of the seeds of the considered species.

The *Eucalyptus* EOs are known to be responsible for antibacterial and antifungal activities [231,260,261]. Regarding the results obtained testing EC and EN EOs on bacteria pathogens, EC showed the highest antimicrobial activity. In particular, the presence in EC of δ-elemene, α-terpinyl acetate, α-copaene, α-gurjunene, aromadendrene, α-humulene, and β-selinene, compounds that are absent in the EO of EN, could contribute to increasing the antimicrobial activity against the Gram-negative bacteria tested. Moreover, EC EO, in addition to being known for its antibacterial activity against many Gram-positive and Gram-negative bacteria and human pathogens [243,261], is effective against plant pathogenic bacteria [262].

Several studies have highlighted the antimicrobial properties of the single components analysed in our study; in particular, the antimicrobial activity of α-pinene [263], terpinen-4-ol [264], and α-terpineol [265] was determined and highlighted by the different MIC values, whereas γ-terpinene did not show antimicrobial activity [266]. Interestingly, the present study showed that all pure components tested had an antimicrobial activity lower than that of each whole EO, suggesting a strong synergistic effect of their various constituents [267]. Regarding the results obtained testing EC and EN EOs on fungi pathogens, EN showed the highest antifungal activity. The antifungal activity of terpenes can be directly related to the incorporation of these compounds into cellular membranes, which compromises their structural integrity. On the contrary, differences in the lipid

composition in the membrane of diverse fungi may be responsible for the effect of these molecules [268]. Sterols are one class of lipids present in the membrane of fungi that may be targeted by these EOs [269]. In conclusion, it can be hypothesized that the major antifungal activity of EN with respect to EC could be ascribed to the higher percentage of eucalyptol (79.5%).

Finally, from the preliminary data on toxicity tests carried out, the *Eucalyptus* EOs analysed appear safe in a wide dose range (0.01–10 mg/mL) for aquatic organisms; therefore, they could be proposed for use in agriculture as eco-sustainable bio-herbicides.

Conclusion

Using a multidisciplinary approach, we investigated the micromorphology and phytochemistry of *E. gunnii* (EG), *E. pulverulenta* "Baby blue" (EP), *E. nicholii* (EN), and *E. cinerea* (EC), as well as the phytotoxic and antimicrobial activities of their EOs.

The essential oils from the *Eucalyptus* species tested, containing a high percentage of eucalyptol and terpinene derivatives, showed selective phytotoxicity, as well as antibacterial and antifungal activities against bacteria and fungi that cause plant diseases. These data indicate that *Eucalyptus* by-products can be a rich source of bioactive metabolites, which can be useful as agrochemicals, thus transforming waste into a value-added resource. However, it is also well known that essential oils can exert biological effects on various plant species and soil microorganisms, with potential risks to biodiversity. Therefore, it is desirable to implement careful management of these biomasses, avoiding their indiscriminate reintroduction into the environment. An in-depth study of the biological effects of their active metabolites is thus necessary to direct these compounds to the most suitable uses.

The chosen approach meets the principles of the circular economy and current directives aimed at developing a suitable method to discard agricultural by-products while reducing the negative impacts of chemical pesticides both on the agrosystems and on public health.

Scientific contribution

Preliminary data have been presented in a presentation in Scientific Congress:

- Malaspina P, **Danna C.**, Cornara L, Aicardi P, Woo S.L., Papaianni M., Polito F., De Feo V. Characterization of *Eucalyptus cinerea* and *E. nicholii* by-products for agricultural applications. 117° Congresso della Società Botanica Italiana (SBI), X International Plant Science Conference (IPSC), 7-10 September 2022, Bologna (Online)

Detailed results have been published and extensively discussed in the following articles published in Open Access by the Editor MDPI under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/):

molecules

MDPI

Article

Eucalyptus gunnii and *Eucalyptus pulverulenta* 'Baby Blue' Essential Oils as Potential Natural Herbicides

Cristina Danna [1], Laura Cornara [1], Antonella Smeriglio [2], Domenico Trombetta [2,*], Giuseppe Amato [3], Pierluca Aicardi [4], Laura De Martino [3,*], Vincenzo De Feo [3] and Lucia Caputo [3]

[1] Department of Earth, Environment and Life Sciences, University of Genova, 16132 Genova, Italy; cristina.danna@edu.unige.it (C.D.); laura.cornara@unige.it (L.C.)
[2] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98166 Messina, Italy; asmeriglio@unime.it
[3] Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy; giuseppe.amato1996@gmail.com (G.A.); defeo@unisa.it (V.D.F.); kaputo@unisa.it (L.C.)
[4] Pierluca Aicardi, Coldiretti Savona, 17100 Savona, Italy; pierluca.aicardi@coldiretti.it
* Correspondence: dtrombetta@unime.it (D.T.); ldemartino@unisa.it (L.D.M.); Tel.: +39-09-0676-6458 (D.T.); +39-08-996-8292 (L.D.M.)

Abstract: The phytotoxicity and eco-compatibility of essential oils (EOs) from *Eucalyptus gunnii* (EG) and *E. pulverulenta* 'Baby Blue' (EP), cultivated in Italy for their cut foliage, were investigated. Leaf micromorphology, EOs phytochemical characterization, and phytotoxicity were analysed. EP revealed a significantly higher oil gland density and a higher EO yield with respect to EG. In both EOs, 1,8-cineole was the major compound (~75%), followed by α-pinene in EG (13.1%) and eugenol in EP (7.5%). EO phytotoxicity was tested on both weeds (*Lolium multiflorum*, *Portulaca oleracea*) and crops (*Raphanus sativus*, *Lactuca sativa*, *Lepidium sativum*, *Solanum lycopersicum*, *Pisum sativum*, *Cucumis sativus*). EG EO inhibited germination of *P. oleracea*, *R. sativus*, and *S. lycopersicum* seeds (ranging from 61.5 to 94.6% for the higher dose used), while affecting only radical elongation in *S. lycopersicum* (ranging from 66.7 to 82.6%). EP EO inhibited germination of *P. oleracea* and *R. sativus* (ranging from 41.3 to 74.7%) and affected radical elongation of *L. sativum* and *L. multiflorum* (ranging from 37.4 to 76.0%). None of the EOs affected the germination and radical growing of *L. sativa*, *P. sativum*, and *C. sativus*. Moreover, EP EO was more active than EG EO in inhibiting α-amylase, a key enzyme for seed growth regulation. Brine shrimp lethality assay showed that both EOs are safe for aquatic organisms, suggesting their high eco-compatibility. The data collected provide useful information for future applications of these EOs in agriculture as safe and selective bioherbicides.

Keywords: *Eucalyptus*; oil cavities; micromorphology; essential oils; natural herbicides; phytotoxicity; α-amylase; eco-compatibility

Citation: Danna, C.; Cornara, L.; Smeriglio, A.; Trombetta, D.; Amato, G.; Aicardi, P.; De Martino, L.; De Feo, V.; Caputo, L. *Eucalyptus gunnii* and *Eucalyptus pulverulenta* 'Baby Blue' Essential Oils as Potential Natural Herbicides. *Molecules* **2021**, *26*, 6749. https://doi.org/10.3390/molecules26216749

Academic Editor: Francesca Mancianti

Received: 4 October 2021
Accepted: 4 November 2021
Published: 8 November 2021

plants

MDPI

Article

Eucalyptus cinerea and *E. nicholii* by-Products as Source of Bioactive Compounds for Agricultural Applications

Paola Malaspina [1], Marina Papaianni [2,*], Marta Ranesi [2], Flavio Polito [3], Cristina Danna [1], Pierluca Aicardi [4], Laura Cornara [1], Sheridan L. Woo [5] and Vincenzo De Feo [3]

[1] Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy
[2] Department of Agricultural Sciences, University of Naples Federico II, Via Università 133, 80055 Portici, Italy
[3] Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy
[4] Coldiretti Savona, Piazza Giulio II 4-7, 17100 Savona, Italy
[5] Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
* Correspondence: marina.papaianni@unina.it

Abstract: The cultivation of different species of *Eucalyptus* has recently expanded in Liguria (Italy) due to the growing demand of the North European floricultural market. Eucalyptus tree branches are cut and selected for their quality, resulting in large amounts of waste biomass to be disposed of. The aim of our study was to evaluate the phytotoxic and antimicrobial activities of essential oils (EOs) from pruning wastes of *E. cinerea* (EC) and *E. nicholii* (EN), for potential applications in agriculture. Phytochemical analyses showed eucalyptol (1,8-cineole) as the major component in both EOs, but the EO yield of EN was higher than that of EC, in agreement with a significantly higher oil gland density on EN leaves. EOs from both species showed phytotoxicity on both weeds tested, but no significant inhibition on horticultural crop seed germination, except for *Raphanus sativus*. The EO from EC showed the strongest antibacterial activity, while the EO from EN showed the strongest antifungal activity. Concluding, EOs from *Eucalyptus* pruning may be used as possible alternatives to synthetic herbicides and pesticides, acting as antimicrobial and antifungal agents, thus representing a safe strategy for crop management programs.

Keywords: waste reuse; plant metabolites; essential oils; micromorphology; alternative pesticides; phytotoxicity

Citation: Malaspina, P.; Papaianni, M.; Ranesi, M.; Polito, F.; Danna, C.; Aicardi, P.; Cornara, L.; Woo, S.L.; De Feo, V. *Eucalyptus cinerea* and *E. nicholii* by-Products as Source of Bioactive Compounds for Agricultural Applications. *Plants* **2022**, *11*, 2777. https://doi.org/10.3390/plants11202777

PART IIB

Eucalyptus EOs as repellents, insecticides and acaricides

Introduction

The genus *Eucalyptus* L'Hér., native to Australia, belongs to the Myrtaceae family and includes about 900 species [270]. The genus was first named *Aromadendron* Andrews ex Steud. by Dr William Anderson in 1776, and then described and renamed *Eucalyptus* by the French botanist Charles Louis L'Héritier de Brutelle in 1788. Nowadays, the common term eucalypts is not equivalent to the systematic concept of *Eucalyptus* [271–273]. A division of the genus into several subgenera was proposed in 1971 by Pryor and Johnson, and later in 1995 Hill and Johnson formally established the diversification and segregation of the genera *Angophora* Cav., *Corymbia* K.D.Hill & L.A.S. Johnson, and *Eucalyptus*, altogether traditionally known as Eucalyptus. *Eucalyptus* itself includes two large subgenera, *Monocalyptus* and *Symphyomyrtus*, and several smaller ones (e.g., *Eudesmia*) [274–276]

Eucalyptus today represents the most planted broad-leaved forests in the world, also finding widespread use in reforestation programs [277] (e.g., *Figure 1*). According to a survey carried out in 65 countries with extensive plantations, the total area of *Eucalyptus* plantations worldwide is estimated to have exceeded 22.57 million hectares [278].

Figure 1 Plantations (*Arboretum*) of *Eucalyptus wandoo* (A) and *E. robusta* (B) in Tunisia [279].

Eucalyptus plants have been known since ancient times for the multiple uses of their components (wood, fibers, cellulose, dyes, pulp, gum, resin, and EOs), which can find applications in different sectors such as construction, and medicinal and phytosanitary preparations [280]. In traditional medicines of different countries, many *Eucalyptus* species are indicated as important plants for the control of ticks and mosquitoes, which are vectors of parasites and pathogens, such as Lyme disease, malaria, and filariasis, and viruses, such as dengue, West Nile, and Zika virus, compromising public health and requiring control strategies [281,282].

Nowadays, several applications have been exploiting the allelopathic and repellent properties of the *Eucalyptus* bioactive compounds and the activities of their essential oils (EOs). Synthetic chemical compounds used as weeds and pest controllers have heavily harmed the environment, and resistance phenomena on the

target organisms are now recognized for several synthetic controllers. Therefore, it is deemed necessary to research alternative substances to cope with these problems [61,283–287].

In this regard, plants giving large biomasses rich in essential oils, such as the *Eucalyptus* species, can represent a great source of natural compounds with interesting and useful bioactive properties [179]. The high EO yield and the rich and varied phytochemical profiles allow their use in sectors other than human public health and medicine. Moreover, EOs are easily isolable and eco-friendly, being biodegradable and not persisting in the environment, and presenting low or no toxicity against vertebrates [288–290]. However, the use of biorational insecticides could have potentially unintended effects on the environment, such as negative effects on pollinators; but its effects on human health have not yet been fully clarified. Unfortunately, especially for EOs, only little attention has been paid so far to this aspect and further investigations are needed for their future use in pest management programs [60].

Exhaustive reviews have previously analysed the properties of the *Eucalyptus* EOs as antioxidant, anti-inflammatory, antimicrobial, anthelmintic, antiviral, insecticidal, herbicidal, etc. [55,172,182,280,291,292]; however, an exhaustive general review focusing on the effect of these EOs on insects and mites is lacking. Therefore, the purpose of the present review was to provide the readers with the most recent information concerning the repellent, insecticidal, and acaricidal activities of the EOs of several *Eucalyptus* species. For this purpose, data on the phytochemical profile of the EOs tested in relation to these biological activities were also reported. This review was mainly focused on the research on this topic carried out in the last 10 years, but it also provided data from older articles. The goal was to summarize and describe the spectrum of insects and mites on which the *Eucalyptus* EOs seem to act and to promote the EO's application in the areas of food storage, plant protection, and human health.

Methods

Literature review

Search for studies, starting on Jan 2021 using ISI Web of Science, Google Scholar databases, Science Direct, PubMed, Academia.edu (years 1990–2022), was carried out using the following search terms: *Eucalyptus* essential oil, insecticide, acaricide, repellent, deterrent. The references section of relevant literature was also scanned to look for studies that might have been missed. All the studies were filtered for inclusion in this literature review excluding the ones referred for *Eucalyptus* species that are no more taxonomically considered into the *Eucalypus* genus (e.g. *Corymbia*).

Data collection and evaluation

Two Excel databases (one for insects and one for mites) were set up, in which each row is related to the information of a single animal tested species, including the following information as a column: reference; *Eucalyptus* species/hybrids studied; tested properties and method applied; animal species tested and their developmental stage; effects of EOs; results of tested active compounds; sectors of interest (human health, animal health, food storage, crop protection). Nomenclature follows the GBIF Backbone Taxonomy ("GBIF-Global Biodiversity Information Facility,").

Additionally, a database regarding phytochemistry was generated, in which each row is related to the information of a single species of *Eucalyptus*, including the following information as a column: reference; country of collection; *Eucalyptus* species/hybrids, plant section used; extraction method; analytical method; number of identified compounds; main constituents (%); EOs yield (%). The *Eucalyptus* species investigated were unified as nomenclature using Plants of the World ("Plants of the World Online|Kew Science,"), and some synonimous referred in the analysed literature were also provided.

Results

Eucalyptus species

The total number of *Eucalyptus* taxa reported in the literature analysed is 71 species and 5 hybrids (*Table 1*); However, in some articles, data are simply related to the generic term *Eucalyptus* sp. The most studied *Eucalyptus* species are *E. globulus* Labill., *E. camaldulensis* Dehnh. and *E. cinerea* F.Muell. ex Benth., which are also the most cultivated [271,293].

Table 1 *Eucalyptus* species and hybrids analysed in the literature [294].

***Eucalyptus* species**
Eucalyptus amygdalina Labill.
Eucalyptus andrewsii Maiden
Eucalyptus approximans Maiden
Eucalyptus astringens (Maiden) Maiden
Eucalyptus badjensis Beuzev. & M.B.Welch
Eucalyptus benthamii Maiden & Cambage
Eucalyptus bicolor A.Cunn. ex Hook. (syn *E. largiflorens* F.Muell.)
Eucalyptus bicostata Maiden, Blakely & Simmonds
Eucalyptus blakelyi Maiden
Eucalyptus botryoides Sm.
Eucalyptus caesia Benth.
Eucalyptus camaldulensis Dehnh.
Eucalyptus cinerea F.Muell. ex Benth.
Eucalyptus cloeziana F.Muell.
Eucalyptus codonocarpa Blakely & McKie
Eucalyptus crebra F.Muell.
Eucalyptus curtisii Blakely & C.T.White
Eucalyptus dalrympleana Maiden
Eucalyptus dives S.Schauer
Eucalyptus dorrigoensis (Blakely) L.A.S.Johnson & K.D.Hill
Eucalyptus dundasii Maiden
Eucalyptus dunnii Maiden
Eucalyptus elata Dehnh.
Eucalyptus fastigata H.Deane & Maiden
Eucalyptus fraxinoides H.Deane & Maiden
Eucalyptus globulus Labill.
Eucalyptus globulus subsp. *globulus* Labill.
Eucalyptus globulus subsp. *maidenii* (F.Muell.) J.B.Kirkp.
Eucalyptus grandis W.Hill ex Maiden
Eucalyptus gunnii Hook.f.
Eucalyptus intertexta R.T.Baker
Eucalyptus lehmannii (Schauer) Benth.
Eucalyptus leucoxylon F.Muell.
Eucalyptus macrorhyncha F.Muell. ex Benth.
Eucalyptus mannifera Mudie
Eucalyptus michaeliana Blakely
Eucalyptus microtheca F.Muell.
Eucalyptus moorei Maiden & Cambage
Eucalyptus nicholii Maiden & Blakely
Eucalyptus nitens (H.Deane & Maiden) Maiden
Eucalyptus nobilis L.A.S.Johnson & K.D.Hill
Eucalyptus nortonii (Blakely) L.A.S.Johnson
Eucalyptus obliqua L'Hér. (syn *E. procera* Dehnh.)
Eucalyptus ovata Labill.
Eucalyptus pauciflora subsp. *niphophila* (Maiden & Blakely) L.A.S.Johnson & Blaxell
Eucalyptus pellita F.Muell.
Eucalyptus phoenicea F.Muell.
Eucalyptus polyanthemos S.Schauer
Eucalyptus polybractea R.T.Baker
Eucalyptus punctata DC.
Eucalyptus pyrocarpa L.A.S.Johnson & Blaxell
Eucalyptus radiata Sieber ex DC.
Eucalyptus radiata subsp. *robertsonii* (Blakely) L.A.S.Johnson & Blaxell
Eucalyptus resinifera J.White
Eucalyptus robusta Sm.
Eucalyptus rossii R.T.Baker & H.G.Sm.
Eucalyptus rubida H.Deane & Maiden
Eucalyptus rudis Endl.
Eucalyptus saligna Sm.
Eucalyptus sargentii Maiden
Eucalyptus siderophloia Benth.
Eucalyptus sideroxylon A.Cunn. ex Woolls
Eucalyptus smithii R.T.Baker
Eucalyptus spathulata Hook.
Eucalyptus sphaerocarpa L.A.S.Johnson & Blaxell
Eucalyptus staigeriana F.Muell. ex F.M.Bailey
Eucalyptus stellulata Sieber ex DC.
Eucalyptus tereticornis Sm.
Eucalyptus tindaliae Blakely (syn *E. phaeotricha* Blakely & McKie)
Eucalyptus torquata Luehm.
Eucalyptus umbellata Dum.Cours.
Eucalyptus umbra R.T.Baker
Eucalyptus urophylla S.T.Blake
Eucalyptus viminalis Labill.
Eucalyptus sp
***Eucalyptus* hybrids**
Eucalyptus alba Reinw. ex Blume x *E. tereticornis* Sm.
Eucalyptus badjensis Beuzev. & M.B.Welch x *E. nitens* (H.Deane & Maiden) Maiden
Eucalyptus grandis W.Hill ex Maiden x *E. camaldulensis* Dehnh.
Eucalyptus grandis W.Hill ex Maiden x *E. tereticornis* Sm.
Eucalyptus urophylla S.T.Blake x *E. grandis* W.Hill ex Maiden

Eucalyptus essential oils: chemical composition

The chemical composition of the EOs depends on different intrinsic and extrinsic factors including the species, the age of the plant, and the chemical-physical conditions of the growth and harvesting environment (season, location, climate, soil, and developmental stage). In addition, it also depends on the processes used for the isolation and on the methods of EO characterization. All these variables determine the final spectra of the detected chemicals [295,296].

The oil yield reported in the analyzed literature for *Eucalyptus* plants is quite heterogeneous, ranging from 0.07 to 6.73 %. Different analytical methods such as HPLC, GC-MS, GC-EIMS, GC-FID, UV-VIS, and H-NMR were employed to highlight the chemical profile of the *Eucalyptus* EOs. The major constituents of the EOs of the species analyzed in this study are reported in the review of Danna et al. in Supplementary Materials [294].

Eucalyptus EOs can be divided into several categories according to their chemotypes [296]. The major constituent of most *Eucalyptus* EOs is 1.8-cineole (also known as eucalyptol) (*Figure 2* and *Figure 3*). Other important major constituents, monoterpenes and sesquiterpenes, are α-pinene, p-cymene, α-terpineol, limonene, ɣ-terpinene, α-phellandrene, β-pinene, globulol, aromadendrene, and β-phellandrene (*Figure 2* and *Figure 3*). Moreover, other constituents are also reported such as viridiflorol, spathulenol, α-eudesmol, o-cymene, 4-terpineol, piperitone, and pinocarveol. It is worth mentioning that, despite being the most common major component in many species, 1.8-cineole does not appear as dominant in some *Eucalyptus* species such as *E. staigeriana* and *E. elata* (*Figure 3*). Furthermore, in the case of *E. globulus*, the concentration of the different compounds can vary among different populations e.g. from India and Tunisia (*Figure 3*) depending on several parameters as previously mentioned.

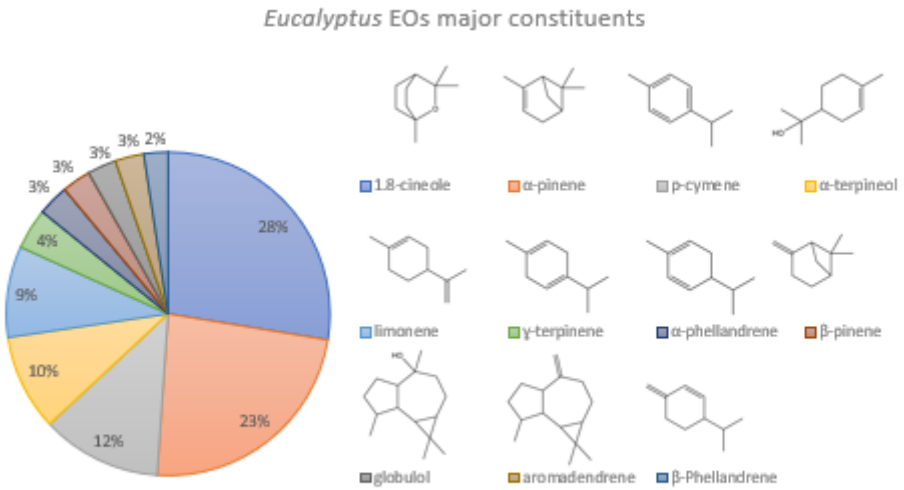


Figure 2 *Major constituents of the* *Eucalyptus* EOs in the analysed literature [294].

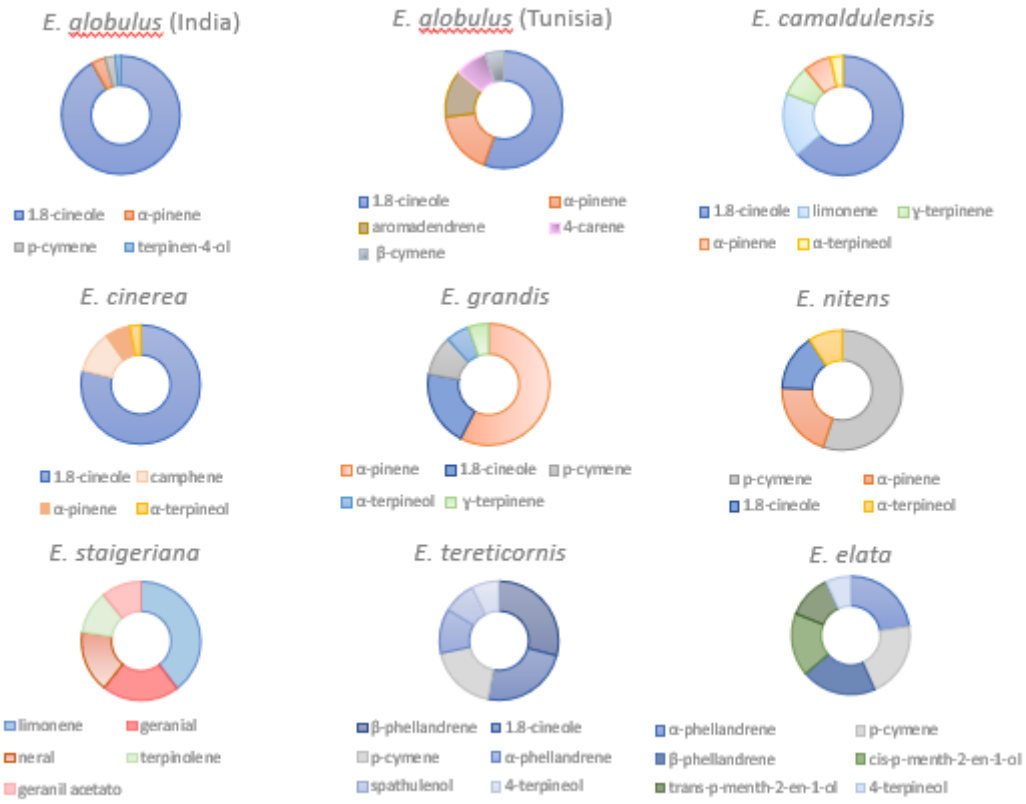


Figure 3 Examples of EOs phytochemical profile reported from different *Eucalyptus* populations [294]: *E. globulus* (India) [297], *E. globulus* (Tunisia) [298], *E. camaldulensis* [299], *E. cinerea* [238], *E. grandis* [241,300,301], *E. nitens* [302], *E. staigeriana* [303,304] , *E. tereticornis* [230,241] and *E. elata* [305].

Eucalyptus EOs potentialities in pest control

The biological properties of *Eucalyptus* EOs are linked to the concentration of their constituents, and minor compounds can play a synergistic role either by enhancing the biological activity of the main components or exerting antagonistic/additive effects [306]. The bioactivities of the EOs can be exploited and applied by humans, thus exploiting the entire spectrum of their action [55,172,182,280].

Insects and mites are severe pests in the strict sense of human concern. Indeed, several species can cause important damage to the crops (e.g., *Tetranychus* spp.), to the food stocks (e.g., *Tribolium* spp., *Sitophylus* spp.), to the animals (e.g., *Varroa* spp.), and to human health (e.g., *Aedes* spp., *Musca* spp.), as they are involved in the transmission of diseases or the cause of home infestations.

Natural plant-derived insecticides can be applied as a strategy in integrated pest management (IPM) programs, and laboratory investigations represent an important step in understanding insect-insecticide or insect-plant-insecticide interactions [307]. Generally, insecticides of botanical origin represent excellent substitutes for synthetic pesticides as they are considered biodegradable and non-persistent in the environment [284,287,308]. However, laboratory and field investigations of non-target effects and unintended effects on insect's biological and reproductive traits need to be improved, particularly in cases of sublethal exposures [60,309,310].

Demand for *Eucalyptus* EOs is estimated in terms of kilotons and USD millions in a report that provides actual data for the years 2017, 2018, 2019, 2020, and 2021 and forecasts up to 2028. The volume of the global essential oils market in 2021 was estimated at around 150 kilotons of which 10% was represented by the *Eucalyptus* oil market (estimated around 15 kilotons). Previous estimations in 2017 and 2018 were around 12 and 13 kilotons, thus highlighting a growing market for the *Eucalyptus* EOs with an increase of revenues (USD Million) of 232.56 and 409.61 respectively [311].

Eucalyptus globulus, E. camaldulensis, and *E. cinerea* are the most cultivated *Eucalyptus* species worldwide; they have rich scientific literature and could be the most promising species for the extraction of EOs on an industrial scale. The potentiality of the *Eucalyptus* EOs is related to the abundant biomasses, the high oil yield obtained during the extraction, and their chemical content. Since the compounds are present in different amounts depending on the species and populations, the choice of the species from which to extract EOs and their possible combination must also be investigated to optimize their application in pest management programs on a local scale.

Repellent, insecticidal and acaricidal activity tests

Insects and mites have biological cycles composed of different developmental stages, each of which with peculiar characteristics not only from a morphological but also from a physiological and biological point of view. For this reason, the *Eucalyptus* EOs are generally tested on different stages of development: eggs, larvae, pupae/nymphae, and adults. Several parameters are monitored using different methodologies to evaluate the repellent power and the insecticidal and acaricidal activities of *Eucalyptus* EOs.

Therefore, contact and topical toxicity, fumigant toxicity, fecundity tests, ovicidal activity tests, repellent activity, antifeedant activity, acetylcholinesterase inhibition, and antennal response have been considered in the analyzed literature. Results, evaluated at different endpoints, are expressed as lethal concentration/dose, lethal time, percentage, and indexes of deterrence [72]. In addition to laboratory tests, field tests have been carried out to ensure the development of strategies applicable in the territory, ensuring the management of the plant pests in plantations [312].

Several solvents have been used to dissolve the EOs such as acetone, dimethyl sulfoxide (DMSO), ethanol, methanol, paraffin (mineral) oil, ultrapure water with 0.1% polysorbate 20, etc. The applied solvent could significantly alter the results obtained, and, therefore, a careful selection of the medium dilution for the tested species must be made [61,313].

In addition, in the last years, new approaches based on polymeric encapsulates of essential oils and nano-emulsions have been explored to maximize the effectiveness of the action of the EOs. The significant results in improving the performance obtained thanks to the increased bioavailability and durability of the EOs can open new perspectives for the preparation of new formulations for pest control [314–318]. However, it should be noted that although nanoformulations (nano-encapsulations, nano-emulsions, and nano-suspensions) can improve the efficiency of pesticides by enhancing permeability, solubility, and stability, it is not yet clear whether they will only produce benefits or represent a new class of hazards [319].

Insects and acari pests tested

All the substances or mixtures that allow to prevent, destroy, repel, or mitigate insects and mites can be defined respectively repellents, insecticides or acaricides. Several studies were carried out demonstrating the insecticidal (*Table 2*) and acaricidal (*Table 3*) activities on different target species, using mainly *in vitro* tests and additionally in field tests.

A total of 117 articles reporting repellent, insecticide or acaricide properties of *Eucalyptus* EOs have been analysed: among them, 95 deal with insects and 22 with mites. The data collected shows that *Eucalyptus* EOs have been tested on 50 insect species and 13 acari species. The sectors of interest reported (impact) refer to human health (HH), animal health (AH), plant protection (PP), food storage (FS).

Table 2 Literature of Insects species tested with *Eucalyptus* EOs for repellent and insecticidal/ovicidal activities [294].

Insects tested species	Impact	*Eucalyptus* species
ANOPLURA		
Pediculidae		
Pediculus humanus capitis De Geer, 1778	HH	*E. camaldulensis* [301]; *E. dunnii* [301]; *E. globulus* [320–323]; *E. globulus* subsp. *globulus* [324]; *E. globulus* subsp. *maidenii* [324]; *E. grandis* [301]; *E. gunnii* [324]; *E. sideroxylon* [324]; *E. tereticornis* [301]; *E. grandis* x *E. camaldulensis* [301]; *E. grandis* x *E. tereticornis* [301]; *Eucalyptus* sp [316]
BLATTOIDEA		
Blatellidae		
Supella longipalpa (Fabricius, 1798)	HH	*Eucalyptus* sp [325]
Blattella germanica (Linnaeus, 1767)	HH	*E. camaldulensis* [241,326]; *E. cinerea* [241]; *E. dunnii* [241]; *E. globulus* subsp. *globulus* [241]; *E. globulus* subsp. *maidenii* [241]; *E. gunnii* [241]; *E. grandis* [241]; *E. saligna* [241]; *E. sideroxylon* [241]; *E. tereticornis* [241]; *E. viminalis* [241]; *E. grandis* x *E. tereticornis* [241]; *E. grandis* x *E. camaldulensis* [241]
COLEOPTERA		
Bostrichidae		
Rhyzopertha dominica (Fabricius, 1792)	FS	*E. astringens* [327]; *E. blakelyi* [247]; *E. cinerea* [302]; *E. codonocarpa* [247]; *E. dundasii* [328]; *E. lehmani* [327]; *E. microtheca* [329]; *E. nicholii* [247]; *E. nigra* [302]; *E. nitens* [302]; *E. obliqua* [329]; *E. pellita* [302]; *E. phoenicea* [302]; *E. resinifera* [302]; *E. spatulata* [329]; *E. torquata* [329]; *E. umbra* [302]; *E. alba* x *E. tereticornis* [302]
Bruchidae		
Acanthoscelides obtectus Say, 1831	FS	*E. globulus* [330]; *E. saligna* [331]
Chrysomelidae		
Callosobruchus maculatus (Fabricius, 1775)	FS	*E. astringens* [327]; *E. bicolor* [332]; *E. camaldulensis* [333–335]; *E. globulus* [336,337]; *E. intertexta* [333]; *E. lehmani* [327]; *E. sargentii* [333]; *E. staigeriana* [304]
Caryedon serratus (Olivier, 1790)	FS, PP	*Eucalyptus* sp [338]
Curculionidae		
Sitophilus granarius (Linnaeus, 1758)	FS	*E. dives* [339]; *E. globulus* [339]
Sitophilus oryzae (Linnaeus, 1763)	FS	*E. approximans* [247]; *E. bicostata* [247]; *E. blakelyi* [247]; *E. caesia* [247]; *E. camaldulensis* [333]; *E. cinerea* [237,247]; *E. codonocarpa* [247]; *E. curtisii* [247]; *E. dives* [247,340]; *E. elata* [247]; *E. globulus* subsp. *maidenii* [237,247]; *E. intertexta* [333]; *E. leucoxylon* [247]; *E. macrorhyncha* [247]; *E. mannifera* [247]; *E. michaeliana* [247]; *E. moorei* [247]; *E. nicholii* [247]; *E. nortonii* [247]; *E. obliqua* [341]; *E. ovata* [247]; *E. pauciflora* subsp. *niphophila* [247]; *E. polyanthemos* [247]; *E. rossii* [247]; *E. sargentii* [333]; *E. sideroxylon* [247]; *E. stellulata* [247]; *Eucalyptus* sp [314,342];
Sitophilus zeamais Motschulsky, 1855	FS	*E. benthamii* [343]; *E. camaldulensis* [344]; *E. dives* [340]; *E. dunnii* [343]; *E. globulus* [343344]; *E. saligna* [331,343–345]; *E. viminalis* [343]
Nitidulidae		
Carpophilus hemipterus (Linnaeus, 1758)	FS	*E. cinerea* [237]; *E. globulus* subsp. *maidenii* [237]
Scolytidae		
Hypothenemus hampei (Ferrari, 1867)	FS	*E. cinerea* [346]; *E. nigra* [346]; *E. punctata* [346]; *E. pyrocarpa* [346]; *E. resinifera* [346]; *E. sphaerocarpa* [346]; *E. alba* × *E. tereticornis* [346]
Silvanidae		
Oryzaephilus surinamensis (Linnaeus, 1758)	FS	*E. dundasii* [328]; *E. globulus* [347]
Tenebrionidae		
Tribolium castaneum (Herbst, 1797)	FS	*E. astringens* [327]; *E. blakelyi* [247]; *E. camaldulensis* [333]; *E. codonocarpa* [247]; *E. globulus* [348–350]; *E. intertexta* [333]; *E.*

		lehmani [327]; *E. nicholii* [247]; *E. saligna* [331]; *E. sargentii* [333]; *Eucalyptus* sp [314,351]
Tribolium confusum Jaqcquelin du Val, 1868	FS	*E. camaldulensis* [352]; *E. globulus* [353–355]; *E. saligna* [345]
DIPTERA		
Culicidae		
Aedes aegypti (Linnaeus, 1762)	HH	*E. badjensis* [356]; *E. benthamii* var. *benthamii* [356];; *E. botryoides* [356]; *E. camaldulensis* [230,357–359]; *E. cinerea* [357,360]; *E. dalrympleana* [356]; *E. dunnii* [230,357]; *E. dorrigoensis*[356]; *E. fastigata* [356]; *E. globulus* [361–363]; *E. globulus* subsp. *globulus* [230,357]; *E. globulus* subsp. *maidenii* [230,357]; *E. grandis* [300,357]; *E. gunnii* [357]; *E. nitens* [364,365]; *E. nobilis* [356]; *E. polybractea* [356]; *E. radiata* ssp *radiata* [356]; *Eucalyptus radiata* subsp. *robertsonii* [356]; *E. resinifera* [356]; *E. robusta* [356]; *E. rubida* [356]; *E. saligna* [230,357,366]; *E. sideroxylon* [357]; *E. smithii* [356]; *E. urophylla* [358]; *E. tereticornis* [230,357]; *E. viminalis* [230,357]; *E. badjensis* × *E. nitens* [356]; *E. grandis* × *E. camaldulensis* [230,357]; *E. grandis* × *E. tereticornis* [230,357]
Aedes albopictus (Skuse, 1894)	HH	*E. camaldulensis* [358]; *E. globulus* [362]; *E. nitens* [364]; *E. radiata* [367]; *E. urophylla* [358]
Anopheles gambiae Giles, 1902	HH	*E. tereticornis* [368]
Anopheles pseudopunctipennis Theobald 1901	HH	*E. nitens* [365]
Anopheles stephensi Liston, 1901	HH	*E. camaldulensis* [369]; *E. globulus* [370]; *E. tereticornis* [371]
Culex pipiens Linnaeus, 1758	HH	*E. globulus* [372]; *E. camaldulensis* [373]
Culex pipiens quinquefasciatus Say, 1823	HH	*E. benthamii* [374]; *E. cloeziana* [374]; *E. globulus* [374,375]; *E. umbellata* [374]; *Eucalyptus* sp [376]
Hippoboscidae		
Melophagus ovinus (Linnaeus, 1758)	AH	*E. globulus* [377]
Muscidae		
Haematobia irritans (Linnaeus, 1758)	AH	*E. badjensis* [305]; *E. badjensis* × *E. nitens* [305]; *E. botryoides* [305]; *E. cinerea* [239]; *E. dalrympleana* [305]; *E. dorrigoensis*[305]; *E. elata* [305]; *E. fraxinoides* [305]; *E. fastigata* [305]; *E. globulus* [315,378]; *E. nobilis* [305]; *E. obliqua* [305]; *E. polybractea* [305]; *E. radiata* subsp. *radiata* [305]; *Eucalyptus radiata* subsp. *robertsonii* [305]; *E. resinifera* [305]; *E. rubida* [305]; *E. smithii* [305]
Musca domestica Linnaeus, 1758	AH, HH, FS	*E. camaldulensis* [379]; *E. cinerea* [242,380]; *E. glubulus* [315,362,370,381]
Psychodidae		
Lutzomyia longipalpis Lutz and Neiva 1912	HH	*E. globulus* [382]; *E. staigeriana* [382]
Tephritidae		
Ceratitis capitata (Wiedemann, 1824)	FS	*E. cinerea* [238]; *E. globulus* subsp. *maidenii* [238]
HEMIPTERA		
Aphididae		
Aphis nerii Boyer de Fonscolombe, 1841	PP	*E. globulus* [383]
Myzus persicae Sulzer, 1776	PP	*E. globulus* [384]
Pseudococcidae		
Phenacoccus solenopsis Tinsley 1898	PP	*E. globulus* [385]
Reduviidae		
Rhodnius neglectus Lent, 1954	HH	*E. urophylla* x *E. grandis* [386]
HYMENOPTERA		
Apidae		

Tetragonisca angustula (Latreille, 1811)		*E. andrewsii* [387]; *E. cinerea* [387]; *E. nigra* [387]; *E. pyrocarpa* [387]; *E. punctata* [387]; *E. resinifera* [387]; *E. siderophloia* [387]; *E. sphaerocarpa* [387]; *E. alba* x *E. tereticornis* [387]
Formicidae		
Atta sexdens var. *rubropilosa* Forel, 1908	PP	*E. camaldulensis* [299]; *E. cloesiana* [299]; *E. grandis* [299]; *E. saligna* [299]; *E. urophylla* [299];
Solenopsis saevissima (Smith, 1855)		*E. andrewsii* [387]; *E. cinerea* [387]; *E. nigra* [387]; *E. pyrocarpa* [387]; *E. punctata* [387]; *E. resinifera* [387]; *E. siderophloia* [387]; *E. sphaerocarpa* [387]; *E. alba* x *E. tereticornis* [387]
ISOPTERA		
Termitidae		
Odontotermes assamensis Holmgren, 1913	PP, BI	*E. globulus* [297]
LEPIDOPTERA		
Crambidae		
Cydalima perspectalis (Walker, 1859)	PP	*Eucalyptus* sp [388]
Gelechiidae		
Pectinophora gossypiella (Saunders, 1844)	PP	*E. globulus* [389]
Sitotroga cerealella (Olivier, 1789)	FS	*E. dives* [340]; *E. saligna* [331]
Geometridae		
Thyrinteina arnobia (Stoll, 1782)	PP	*E. camaldulensis* [390]; *E. grandis* [390]; *E. saligna* [390]; *E. urophylla* [390]
Lymantriidae		
Orgyia trigotephras Boisduval, 1829	PP	*E. globulus* [298]; *E. lehmannii* [298]
Noctuidae		
Helicoverpa armigera (Hübner, 1808)	PP	*E. globulus* [389,391]
Spodoptera frugiperda J.E.Smith, 1797	PP	*E. staigeriana* [303]
Pieridae		
Ascia monuste (Linneaus, 1764)	PP	*E. andrewsii* [387]; *E. cinerea* [387]; *E. nigra* [387]; *E. pyrocarpa* [387]; *E. punctata* [387]; *E. resinifera* [387]; *E. siderophloia* [387]; *E. sphaerocarpa* [387]; *E. alba* x *E. tereticornis* [387]
Plutellidae		
Plutella xylostella (Linnaeus, 1758)	PP	*E. andrewsii* [244]; *E. crebra* [244]; *E. punctata* [244]; *E. pyrocarpa* [244]; *E. siderophloia* [244]; *E. sphaerocarpa* [244]
Pyralidae		
Ectomyelois ceratoniae Zeller, 1839	FS	*E. astringens* [392]; *E. camaldulensis* [392,393]; *E. lehmannii* [392]; *E. leucoxylon* [392,393]; *E. rudis* [392]
Ephestia cautella (Walker, 1863)	FS	*E. astringens* [392]; *E. camaldulensis* [392]; *E. lehmannii* [392]; *E. leucoxylon* [392]; *E. rudis* [392]
Ephestia kuehniella Zeller, 1879	FS	*E. astringens* [392]; *E. camaldulensis* [352,392]; *E. lehmannii* [392]; *E. leucoxylon* [392]; *E. rudis* [392]
Plodia interpunctella (Hübner, 1813)	FS	*E. globulus* [394]
Tortricidae		
Thaumatotibia leucotreta (Meyrick, 1913)	PP	*E. globulus* [389]
THYSANOPTERA		
Ploeothridae		
Gynaikothrips ficorum (Marchal, 1908)	PP	*E. globulus* [383]

Sectors of interest (impact): human health (HH), animal health (AH), plant protection (PP), food storage (FS). building infestation (BI).

Table 3 Literature of Acari species tested with *Eucalyptus* EOs for repellent and acaricidal/ovicidal activities [294].

Acari Tested species	Impact	Eucalyptus species
IXODIDA		
Ixodidae		
Amblyoma variegatum Fabricius, 1794	AH	*E. tereticornis* [395]
Hyalomma anatolicum Koch, 1844	AH	*E. camaldulensis* [396]
Rhipicephalus annulatus (Say, 1821)	AH	*E. globulus* [397,398]; *Eucalyptus* sp [399]
Rhipicephalus bursa Canestrini & Fanzago, 1878	AH	*E. globulus* [400]
Rhipicephalus microplus Canestrini, 1888	AH	*E. globulus* [401–403]; *E. staigeriana* [401]
MESOSTIGMATA		
Dermanissidae		
Dermanyssus gallinae (De Geer, 1778)	AH	*E. globulus* [404–406]; *E. radiata* [404]; *E. staigeriana* [404]
Varroidae		
Varroa destructor Anderson & Trueman, 2000	AH	*E. amygdalina* [407]; *E. camaldulensis* [408]; *E. globulus* [407]; *E. robusta* [407]; *E. sideroxylon* [407]; *Eucalyptus* sp [409]
Varroa jacobsoni Oudemans, 1904	AH	*Eucalyptus* sp [410]
SARCOPTIFORMES		
Acaridae		
Tyrophagus longior Gervais, 1844	FS	*E. globulus* [411]
Tyrophagus putrescentiae (Schrank, 1781)	FS	*E. dives* [340]
Pyroglyphidae		
Dermatophagoides pteronyssinus (Trouessart, 1897)	HH	*Eucalyptus* sp [412]
Sarcoptidae		
Sarcoptes scabiei (DeGeer, 1778)	AH	*E. camaldulensis* [413]
TROMBIDIFORMES		
Tetranychidae		
Tetranychus urticae Koch, 1836	PP	*E. approximans* [414]; *E. bicostata* [414]; *E. blakelyi* [414]; *E. condonocarpa* [414]; *E. dives* [414]; *E. elata* [414]; *E. globulus* [415]; *E. maidenii* [414]; *E. mannifera* [414]; *E. nicholii* [414]; *E. ovata* [414]; *E. sideroxylon* [414]; *Eucalyptus* sp [416]

Sectors of interest (impact): human health (HH), animal health (AH), plant protection (PP), food storage (FS).

Discussion

From time immemorial, *Eucalyptus* plants have been used worldwide as defense from pest species, e.g., by placing their leaves between grains during storage [417,418], using macerates and decoctions as repellent preparations [419], and fumigation practices for repellent purposes [420].

The analyzed literature, including mainly articles relating to laboratory investigations, allowed us to give a more precise picture of the action of *Eucalyptus* EOs on different species of insects and mites. Moreover, the data presented above clearly indicate which species of *Eucalyptus* and insects or mites received the most attention. Key factors include distribution, especially for the species widespread globally, and economic factors, especially for species with a strong impact on human health, crop protection, and food conservation. As already reported, *E. globulus, E. camaldulensis,* and *E. cinerea* present the most extensive literature for insecticidal and acaricidal properties. *Pediculus humanus, Aedes aegypti*, and *Musca domestica* have been of great interest in human health, being parasites or vectors of human diseases [421–423]. *Haematobia irritans* received attention as responsible for animal diseases [423,424]. *Tribolium castaneum*, *Callosobruchus maculatus*, *Sitophilus oryzae, S. granaries* and *Rhizoperta dominica* are the most studied insect target species responsible for food losses [425]. The insect *Ascia monuste* and the mite *Tetranichus urticae* have been studied in relation to crop damage [426,427].

Nowadays, the importance of finding alternative biopesticides is also related to the phenomena of increased resistance, widespread at a geographical level, on the target species, e.g., for biopesticides such as pyrethroids, organophosphates, carbamates, and organochlorines [428,429].

This research does not claim to be exhaustive but aims to help in organizing data for the application of the *Eucalyptus* EOs as biopesticides, schematizing the abundant literature on this topic. In the published article mentioned below, a brief discussion of the results obtained for each species tested was presented.

Detailed data concerning the phytochemistry of the *Eucalyptus* species analysed, including information about the country, the portion of the plant used, the identified compounds, and their relative percentage (covering at least 70% of the chemical spectrum of each sample analysed), as well as the specific EO yields and percentage (%) obtained, are reported in the published article. The correlation between bioactivity and phytochemical profile is of primary importance to understand which of the main bioactive compounds are responsible for toxicity, repellent, and antifeedant actions. The results differ depending on the test applied and the developmental stages analysed. 1.8-cineole, being a major compound of the *Eucalyptus* genus, was found to be responsible for several bioactivities on most of the tested species, with only a few exceptions. In any case, most articles refer to the importance of the phytocomplex, reporting the synergistic action of major and minor compounds.

Detailed information regarding insects and mites, the adopted tests (contact, topical, fumigant, etc.), the developmental stages analysed, and the relative toxicity results are reported in the published article. The correlation between the bioactivity and the adopted tests produced several different results depending on the above-mentioned parameters.

As suggested from the literature analysed, while laboratory investigations are abundant, the literature concerning field investigations is lacking in experiments and few *Eucalyptus*-based pesticides are nowadays available on the market. To achieve long-term commercial success, testing of performance in-field conditions and regulatory aspects is needed as a key step in finding effective solutions in the biopesticides market [430].

Conclusion

This literature survey suggests the development of environmentally friendly commercial products to control insects and mites, decreasing the use of synthetic pesticides. *Eucalyptus* EOs could be commercially exploited to prevent infestations of stored products (used as fumigants or employed for packaging) and to control infestations in the agricultural field (crop protection). In addition, *Eucalyptus* EOs can be applied in the sanification of domestic environments and for human and animal protection. The research of alternative pesticides is closely aligned with environmental sustainability research. Given that *Eucalyptus* trees are extensively cultivated, with a considerable amount of waste biomasses being produced, research in this field also fits with a circular economy approach, as it promotes the valorisation and reuse of waste biomass.

Scientific contribution

Preliminary data have also been presented in a presentation in Scientific Congress:

- **Danna C.**, Malaspina P., Cornara L., Vanin S. *Eucalyptus* EOs: Chemical composition and applications in Pests control – A review in progress. 2nd Conference of young botanists (CYBO), February 9-10, Bozen.

Detailed data, including supplementary materials, have been published and extensively discussed in the following article published in Open Access by the Editor ELSEVIER under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/):

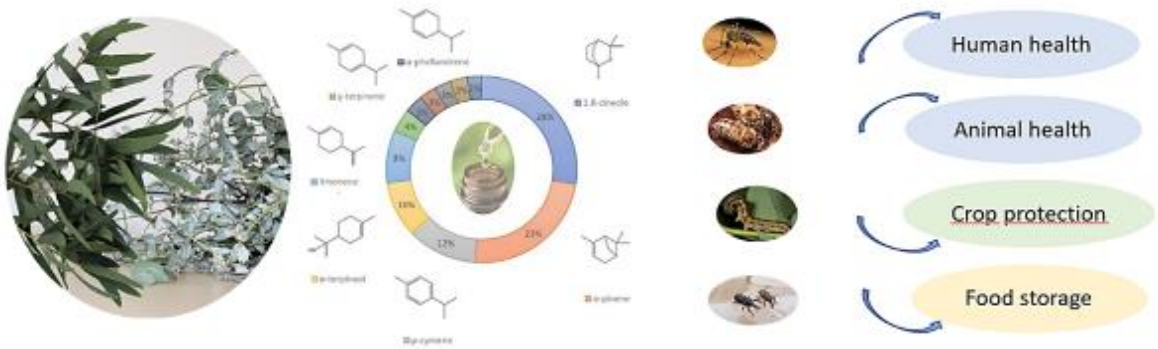
Crop Protection
Available online 28 July 2023, 106319
In Press, Journal Pre-proof What's this?

Eucalyptus essential oils in pest control: A review of chemical composition and applications against insects and mites

Cristina Danna [a], Paola Malaspina [a], Laura Cornara [a], Antonella Smeriglio [b], Domenico Trombetta [b], Vincenzo De Feo [c], Stefano Vanin [a]

Show more

https://doi.org/10.1016/j.cropro.2023.106319 Get rights and content



General Discussion

Since the discussion on the scientific results has already been addressed in the sections corresponding to the various studies conducted in the doctoral project, the topic is engaged here in more general terms.

Man has always developed traditional practices thanks to prolonged observations and direct experiences in the natural environment. Traditions are, therefore, an expression of adaptation to a specific environment, reflecting both the possibilities and the difficulties of life in the same context. Traditions are not fixed elements, but evolve, being the products of specific times and spaces. Disappeared traditions offer glimpses of the past, while traditions that still exist describe the context in which we live. Future traditions will be the fruit of new needs.

The present Ph.D. project tried to integrate Ethnobotany, Circular Economy, and Sustainability with a close look at traditions to investigate possible future applications.

During the ethnobotanical survey, I had the opportunity to get a glimpse into everyday life as it used to take place in the past in the alpine areas. Modern life has completely revolutionized within the succession of a few generations. By interviewing elderly people, and thanks to their memory and stories, I gained some images of life as it could be at the beginning of '900, and the evolutionary process of the mode of life that followed. Surely during my investigation, I was principally focused on documenting the usage of plants; however, it would be limiting to say that it was just that. The documentation of the plants used and still in use was the targeted scientific objective of this research, but the gain I had for my personal growth was much further. The interviews, the dialogues, and the stories allowed me to make a sort of journey through the time, identifying myself with environmental and social difficulties. In an environment such as that of the alpine valleys, where the summer season is short and winter dominates with massive snow, it was essential to manage reserves and create stocks from natural resources and the products of cultivation and livestock. The deep knowledge of the environment and its resources, and the jobs to be carried out marked by seasonal rhythms were then a requirement for survival. Waste was not conceived, and the principles of circularity were constantly applied.

During the investigations aimed at validating the traditional uses of *Imperatoria,* I had the opportunity to look at the methods used in today's pharmacognosy. I entered the world of micromorphology, phytochemistry, and pharmacology using different types of analyses aimed at bringing traditional knowledge into modern phytotherapy. Studying *Peucedanum ostruthium,* I dealt with a species that grows wild or in small cultivations, contributing to the valorisation of a species that in the pharmacopoeia tradition has been considered pivotal in the healing of various ailments (just think of the imperial name and the mentioned epithets given to it). However, today this species is not exploited to its full potential, as only its underground portion continues to be used. Thanks to the present research the potential of the aerial part (currently underused or discarded) of the plant has been highlighted, paving the way for new phytotherapeutic applications.

As a result of academic studies related to the environment and sustainability, and the clear awareness that economic laws guide today's choices, I then addressed the topic of bioherbicides and biopesticides, as important steps in modern agriculture, from a circular economy perspective. During the research conducted on different *Eucalyptus* species and their EOs, I highlighted the need to increase the role of bio-controllers, particularly focusing on products of plant origin in integrated management programs. Studying *Eucalyptus,* I dealt with species that are globally widespread in forests, small cultivations, and large plantations to valorise their by-products, focusing above all on their possible applications in the agricultural sector.

Future perspectives in the relation Man-Plants

As ethnobotany teaches, the relationship between man and nature is still evolving. The theme of sustainability is crucial for humans to make careful choices regarding the relationship they establish with the environment, with nature: "A human culture cannot long survive without the basis of sustainable agriculture and ethical land management." [431] It is essential to study how to harmoniously integrate humans with the environment, developing mutually supportive relationships between them and identifying and proposing models of agriculture geared toward natural conservation and environmental sustainability. Ethnobotany, the science that studies the human–plant relationship and interactions in different cultures, can lay the foundation and become a tool to achieve this goal [98]. Practical examples of farming techniques aimed at environmental sustainability were formulated, reevaluating traditional agricultural practices and knowledge, e.g., biodynamic agriculture [432], organic agriculture [433], natural farming [434], synergistic farming [435], and sustainable agricultural social system [436]. Movements and concepts such as the circular economy [437], permaculture [431], environmental sustainability, and sustainable development [27] are based on similar principles and key cornerstones: the main goal is to create virtuous circles that eliminate or minimize the problem of waste, turning these materials into resources.

The circular economy proposes a self-regenerating economic system in which the terms “source“ and “sink” can be considered two sides of the same coin, and in which the idea of a cycle arises from the depiction of the balanced transformation between the two sides. Entering the perspective of the circular economy requires reconceptualization and a reshaping of language. A term such as "waste" can be replaced by words such as "resource," "source," and "possibility". The idea of the regenerative cycle, to be inspired and aimed at, comes from observing what normally occurs in the natural world. Observation of the natural world also expresses another key concept: that of the network, the world of interactions in terms of energy and matter flows within its mesh made of nodes and connectors. In the globalization period, this fact is evident, and integrated forms of land and resource management are needed to ensure the health and well-being of humans and their societies.

General Conclusion

The research carried out during the Ph.D. has focused on the study of extracts and essential oils mainly obtained from plant byproducts to identify bioactive compounds for health and environmental applications. The research concerned the possibility of obtaining bioactive compounds from different types of plants according to sustainability and circular economy criteria. The field of inquiry took its source from traditional ethnobotanical knowledge, seeking scientific validation and innovative applications.

To begin with, the selection of the plants to be investigated was inspired by species used in traditional medicine. I then focused the study on a species, *Peucedanum ostruthium,* of which the rhizomes/roots are mainly used, discarding a significant aerial biomass. The extracts obtained from this waste biomass were then studied from a pharmacognostic point of view and tested for their bioactivity, identifying possible applications in skin care.

Subsequently, the study turned to the recovery and valorisation of cultivation and waste processing of some species of the *Eucalyptus* genus cultivated in Liguria as ornamental fronds. Essential oils (EOs) obtained from these waste materials were investigated and tested, confirming their possible uses in environmental applications, such as biopesticides, biocontrol agents, and bioherbicides.

Extracts and EOs recovered as by-products from MAPs tested in the present Ph.D. research showed interesting bioactivities, useful for both health and environmental applications. These results suggest their possible use for new phytotherapeutic formulations and plant protection products.

In Macro - In Micro

Nature is as beautiful as it can be

We are allowed to explore it through our senses

Traditions are the result of handed-down explorations

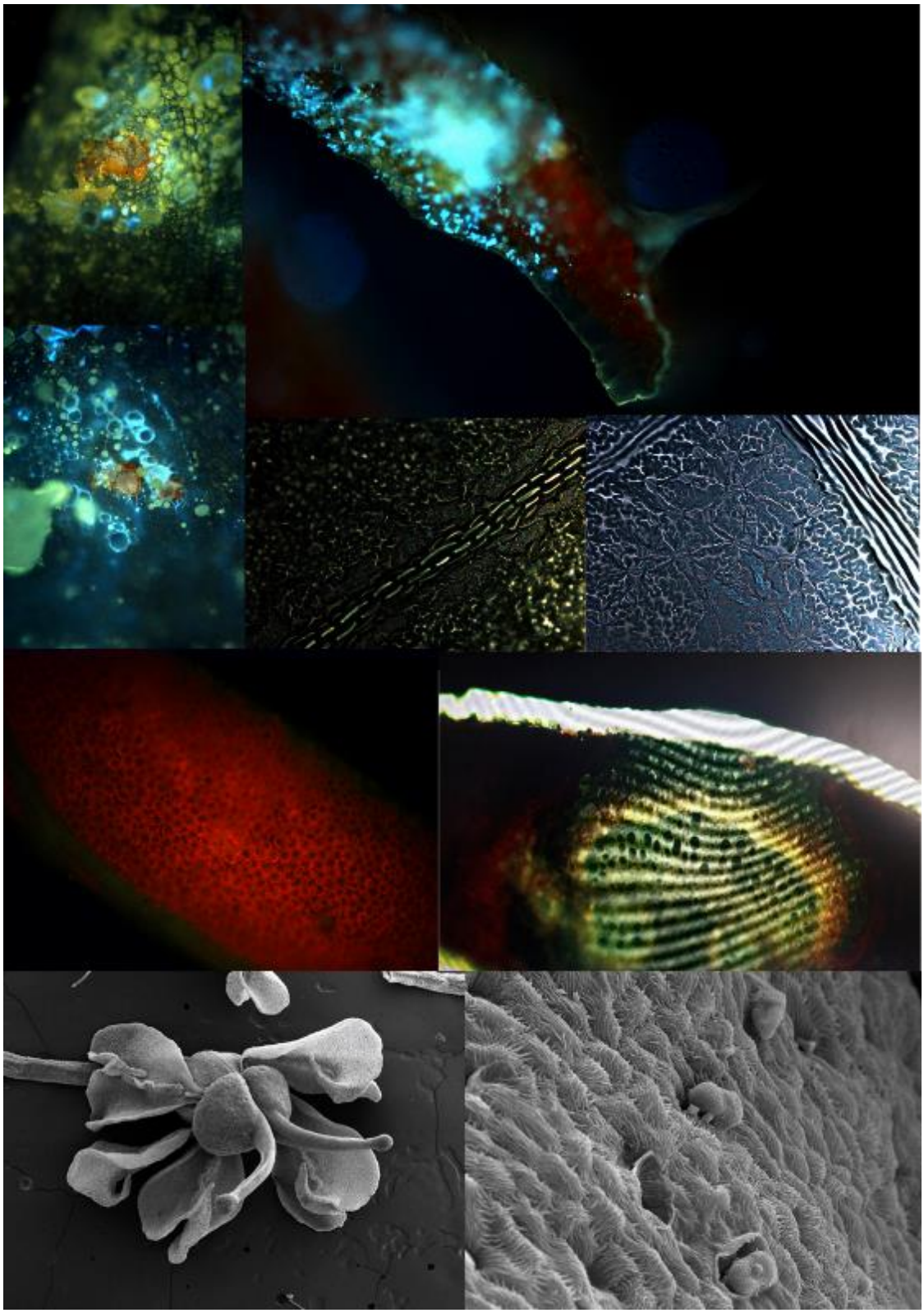
We seek to understand Nature

Let's not stop marveling at it

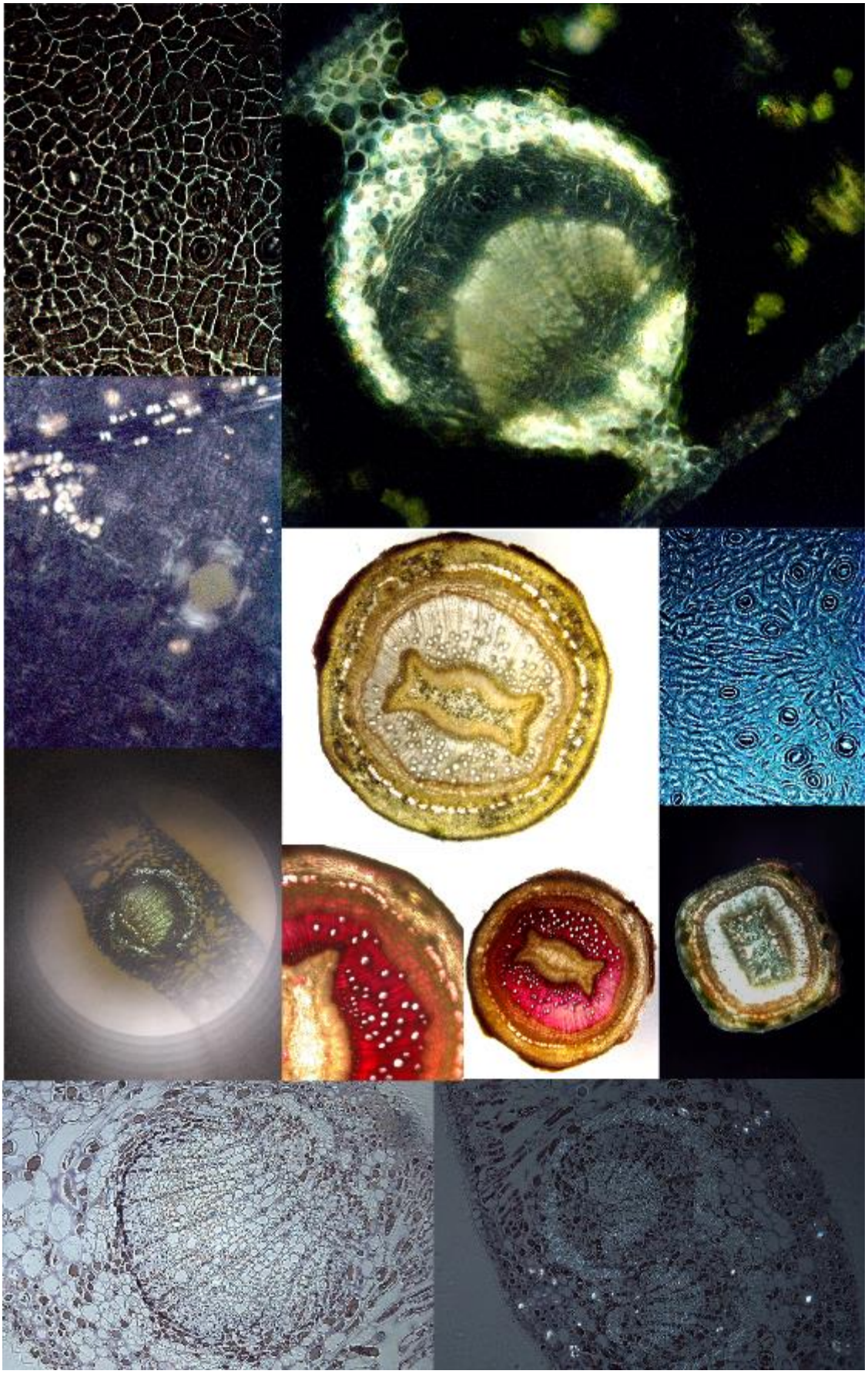
Life is an intriguing gift

Science and Art - microscopic photography

Imperatoria



Eucalyptus



Acknowledgements

First, I wish to thank my advisor, Prof. Laura Cornara, who has accompanied and supported me throughout my Ph.D. studies. Second, I would like to thank Prof. Stefano Vanin, who in turn accompanied me in this period of research. I also wish to appreciate all the collaborators from the University of Messina (Prof. Antonella Smeriglio and Prof. Domenico Trombetta), the University of Salerno (Prof. Vincenzo De Feo), and the University of Genoa (Prof. Bruno Burlando, Dr. Miriam Bazzicalupo, Dr. Paola Malaspina, Dr. Laura Negretti, and Prof. Mauro Mariotti) for being helpful with their skills and tools in the research. Many colleagues and researchers within the University of Genoa have helped me in different situations during my PhD studies, and their advice/teaching has been an integral part of the research. I would like to thank the members of the CYBO organizing committee for taking part in this experience. I owe a debt of gratitude to the coordinator of the Ph.D. school, Prof. Marco Scambelluri, and all the Ph.D. course commission STAT for the commitment to offer formative courses and seminars. I thank Dr. Laura Poggio, representative of the Gran Paradiso National Park's botanical sector, and all the informants who participated in the interviews and shared their memories. I would like to thank Prof. Nektarios Aligiannis for his hospitality at the National and Kapodistrian University of Athens, and the entire research team; Evelina Tsioutsiou it was a pleasure to meet you. My appreciation also extends to my friends who have accompanied me over these years. Finally, my deepest gratitude goes to my family for their invaluable support throughout my path.

The alpine valleys are my country of origin, and it was inspiring to delve into the past that forged the branch of my family on the paternal side. I, therefore, dedicate the work of ethnobotany and further investigations on *Peucedanum ostrutium* (*Imperatoria)* to my home region, Aosta Valley.

Liguria, on the contrary, has been a home for these years of university education. Although I did not investigate native species during my Ph.D. studies, I dedicate the research related to *Eucalyptus* species, nowadays cultivated in the region, to the Liguria region that welcomed me for several years, and which has previously housed part of my family on the maternal side.

Bibliography

1. Wheeler JA. Information, physics, quantum: The search for links. *Feynman Comput*. Published online 2018:309-336. doi:10.1201/9780429500459
2. Gaoue OG, Coe MA, Bond M, Hart G, Seyler BC, McMillen H. Theories and Major Hypotheses in Ethnobotany. *Econ Bot*. 2017;71(3):269-287. doi:10.1007/s12231-017-9389-8
3. Prance GT. Ethnobotany, the science of survival: a declaration from Kaua'i. *Econ Bot*. 2007;61(1):1-2. doi:10.1007/bf02862367
4. Rahman IU, Afzal A, Iqbal Z, et al. Historical perspectives of ethnobotany. *Clin Dermatol*. 2019;37(4):382-388. doi:10.1016/j.clindermatol.2018.03.018
5. Nolan JM, Turner NJ. Ethnobotany: The Study of People-Plant Relationships. In: Anderson EN, Pearsall D, Hunn E, Turner N, eds. *Ethnobiology*. Wiley-Blackwell; 2011:133-147. doi:10.1002/9781118015872.ch9
6. Albuquerque UP, Hurrell JA. Ethnobotany: one concept and many interpretations. In: Albuquerque UP HN, ed. *Recent Developments and Case Studies in Ethnobotany*. Vol 1. SBEE/NUPEEA; 2010:87-99.
7. Hernández XE. Carta firmada dirigida a Arturo Argueta. Published online 1990.
8. Villamar AA. Epistemología de las etnociencias. La construcción de las etnociencias de la naturaleza y el desarrollo de los saberes bioecológicos de los pueblos indígenas. Published online 1997.
9. Mazzocchi F. Western science and traditional knowledge: Despite their variations, different forms of knowledge can learn from each other. *EMBO Rep*. 2006;7(5):463-466. doi:10.1038/sj.embor.7400693
10. Ford R. *The Nature and Status of Ethnobotany.* Museum of Anthropology; 1978.
11. Heinrich M. Ethnobotany and its role in drug development. *Phyther Res*. 2000;14(7):479-488. doi:10.1002/1099-1573(200011)14:7<479::AID-PTR958>3.0.CO;2-2
12. Studley J. Dominant Knowledge Systems & Local Knowledge. 1998;(January 1998).
13. Gerique A. *An Introduction to Ethnoecology an Ethnobotany: Theory and Methods. Integrative Assessment and Planning Methods for Sustainable Agroforestry in Humid and Semiarid Regions*. Advanced Scientific Training; 2006.
14. Hoffman B, Gallaher T. Importance indices in ethnobotany. *Ethnobot Res Appl*. 2007;5:201-218. doi:10.17348/era.5.0.201-218
15. Hurrell JA. Las posibilidades de la etnobotánica y un nuevo enfoque a partir de la ecología y su propuesta cibernética. *Rev Española Antropol Arne,-ícana*. 1987;XVII(December 1987).
16. Boulding KE. The economics of the coming spaceship Earth. In: Jarrett H, ed. *Environmental Quality Issues in a Growing Economy*. Johns Hopkins University Press; 1966:3-14.
17. Pearce DW, Turner RK. *Economics of Natural Resources and the Environment*. Johns Hopkins University Press; 1989.
18. Geisendorf S, Pietrulla F. The circular economy and circular economic concepts—a literature analysis and redefinition. *Thunderbird Int Bus Rev*. 2018;60(5):771-782. doi:10.1002/tie.21924
19. Velenturf APM, Purnell P. Principles for a sustainable circular economy. *Sustain Prod Consum*. 2021;27:1437-1457. doi:10.1016/j.spc.2021.02.018
20. Sikdar S. Circular economy: Is there anything new in this concept? *Clean Technol Environ Policy*. 2019;21(6):1173-1175. doi:10.1007/s10098-019-01722-z
21. Ellen MacArthur Foundation. Circularity Indicators: An Approach to Measuring Circularity. *Ellen MacArthur Found*. 2015;(August 2021):12. doi:10.13140/RG.2.2.29213.84962
22. European Commission. Circular Economy Package : Questions & Answers. 2015;(December).
23. Ghisellini P, Cialani C, Ulgiati S. A review on circular economy: The expected transition to a balanced interplay of environmental and economic systems. *J Clean Prod*. 2016;114:11-32. doi:10.1016/j.jclepro.2015.09.007
24. Stahel WR. *History of the Circular Economy. The Historic Development of Circularity and the Circular Economy. in The Circular Economy in the European Union - An Interim Review.* (Eisenriegler S, ed.). Springer Nature Switzerland; 2020. https://doi.org/10.1007/978-3-030-50239-3_9
25. Du Pisani JA. Sustainable development – historical roots of the concept. *Environ Sci*. 2006;3(2):83-96. doi:10.1080/15693430600688831
26. Mebratu D. Sustainability and sustainable development: Historical and conceptual review. *Environ Impact Assess Rev*.

1998;18(6):493-520. doi:10.1016/S0195-9255(98)00019-5

27. Brundtland. Our Common Future ('The Brundtland Report'): World Commission on Environment and Development. *Top 50 Sustain Books*. Published online 1987:52-55. doi:10.9774/gleaf.978-1-907643-44-6_12

28. Mensah J. Sustainable development: Meaning, history, principles, pillars, and implications for human action: Literature review. *Cogent Soc Sci*. 2019;5(1). doi:10.1080/23311886.2019.1653531

29. Goodland R. The Concept of Environmental Sustainability. *Annu Rev Ecol Syst , Vol 26 (1995), pp 1-24 Publ by Annu Rev Stable URL http//www.jstor.org/stable/2097196 CONCEPT ENVIRO*. 2017;26(1995):1-24.

30. Peet R. *Theories of Development*. Guilford Press; 1999.

31. Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary metabolites of plants and their role: Overview. *Curr Trends Biotechnol Pharm*. 2015;9(3):293-304.

32. Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. *Med Plants Cent Asia Uzb Kyrg*. 2013;1(6):13-14. doi:10.1007/978-1-4614-3912-7_4

33. Wink M. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. *Medicines*. 2015;2(3):251-286. doi:10.3390/medicines2030251

34. Agrawal AA, Weber MG. On the study of plant defence and herbivory using comparative approaches: How important are secondary plant compounds. *Ecol Lett*. 2015;18(10):985-991. doi:10.1111/ele.12482

35. Divekar PA, Narayana S, Divekar BA, et al. Plant Secondary Metabolites as Defense Tools against Herbivores for Sustainable Crop Protection. *Int J Mol Sci*. 2022;23(5). doi:10.3390/ijms23052690

36. Cheng AX, Lou YG, Mao YB, Lu S, Wang LJ, Chen XY. Plant terpenoids: Biosynthesis and ecological functions. *J Integr Plant Biol*. 2007;49(2):179-186. doi:10.1111/j.1744-7909.2007.00395.x

37. Iason G. The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. *Proc Nutr Soc*. 2005;64(1):123-131. doi:10.1079/pns2004415

38. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol*. 2020;18(11):607-621. doi:10.1038/s41579-020-0412-1

39. Wink M. Plant secondary metabolites modulate insect behavior-steps toward addiction? *Front Physiol*. 2018;9(APR):1-9. doi:10.3389/fphys.2018.00364

40. Adeyemi Hassan MM. The potential of secondary metabolites in plant material as deterents against insect pests: A review. *African J Pure Appl Chem*. 2010;4(11):243-246. http://www.academicjournals.org/journal/AJPAC/article-abstract/B76CE2E2388

41. Hussain WS. Allelopathy : Allelochemicals a Brief Review. 2020;20(2):5556-5560.

42. Miraldi E, Baini G. Medicinal Plants and Health in Human History: From Empirical Use To Modern Phytotherapy. *J Siena Acad Sci*. 2019;10(1):7-12. doi:10.4081/jsas.2018.8529

43. Jütte R, Heinrich M, Helmstädter A, et al. Herbal medicinal products – Evidence and tradition from a historical perspective. *J Ethnopharmacol*. 2017;207(March):220-225. doi:10.1016/j.jep.2017.06.047

44. Makarska-białokoz M. 3 . The development of phytotherapy from the Middle Ages to modern times History and significance of phytotherapy in the human history. *Arch Physiother Glob Res*. 2020;24(2):17-22. doi:10.15442/apgr.24.2.2

45. Kolarsick P, Kolarsick MA, Goodwin C. Anatomy and physiology of the skin. *Dermatology Nurses' Assoc*. Published online 2011. doi:10.1007/978-3-319-78310-9_2

46. Yadav N, Parveen S, Chakravarty S, Banerjee M. *Skin Anatomy and Morphology*.; 2020. doi:10.1007/978-981-13-2541-0_1

47. Ribeiro AS, Estanqueiro M, Oliveira MB, Lobo JMS. Main benefits and applicability of plant extracts in skin care products. *Cosmetics*. 2015;2(2):48-65. doi:10.3390/cosmetics2020048

48. Abd Aziz A, Taher ZM, Muda R, Aziz R. Cosmeceuticals and Natural Cosmetics. *Recent Trends Malaysia Med Plants Res*. 2017;(December):126-175. https://www.researchgate.net/publication/313796813_Cosmeceuticals_and_Natural_Cosmetics

49. Aburjai T, Natsheh FM. Plants Used in Cosmetics. *Phyther Res*. 2003;17(9):987-1000. doi:10.1002/ptr.1363

50. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms. *J Invest Dermatol*. 2007;127(3):514-525. doi:10.1038/sj.jid.5700701

51. Fenibo EO, Ijoma GN, Matambo T. Biopesticides in Sustainable Agriculture: A Critical Sustainable Development Driver Governed by Green Chemistry Principles. *Front Sustain Food Syst*. 2021;5(June):1-6. doi:10.3389/fsufs.2021.619058

52. Hasan M, Ahmad-Hamdani MS, Rosli AM, Hamdan H. Bioherbicides: An eco-friendly tool for sustainable weed

management. *Plants*. 2021;10(6):1-21. doi:10.3390/plants10061212

53. Cordeau S, Triolet M, Wayman S, Steinberg C, Guillemin JP. Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. *Crop Prot*. 2016;87:44-49. doi:10.1016/j.cropro.2016.04.016

54. Soltys D, Krasuska U, Bogatek R, Gniazdowsk A. Allelochemicals as Bioherbicides — Present and Perspectives. *Herbic - Curr Res Case Stud Use*. 2013;(June). doi:10.5772/56185

55. Batish DR, Singh HP, Kohli RK, Kaur S. Eucalyptus essential oil as a natural pesticide. *For Ecol Manage*. 2008;256(12):2166-2174. doi:10.1016/j.foreco.2008.08.008

56. Verdeguer M, Sánchez-Moreiras AM, Araniti F. Phytotoxic effects and mechanism of action of essential oils and terpenoids. *Plants*. 2020;9(11):1-48. doi:10.3390/plants9111571

57. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: Low-risk products in a high-stakes world. *Annu Rev Entomol*. 2012;57(March 2015):405-424. doi:10.1146/annurev-ento-120710-100554

58. Kaur R, Singh S. Use of natural products for weed management in high-value crops: An Overview. *Am J Agric Res*. 2019;(October). doi:10.28933/ajar-2018-11-2808

59. Souza B, Vázquez LL, Marucci RC. *Natural Enemies of Insect Pests in Neotropical Agroecosystems. Biological Control and Functional Biodiversity*. Springer Nature Switzerland; 2019. doi:10.1007/978-3-030-24733-1_6

60. Haddi K, Turchen LM, Viteri Jumbo LO, et al. Rethinking biorational insecticides for pest management: unintended effects and consequences. *Pest Manag Sci*. 2020;76(7):2286-2293. doi:10.1002/ps.5837

61. Hossain L, Rizwanur R, Khan MS. Alternatives of Pesticides. *Pestic Residue Foods Sources, Manag Control*. 2017;(April):1-200. doi:10.1007/978-3-319-52683-6

62. Gnanavel I. Eco-Friendly Weed Control Options for Sustainable Agriculture. *Sci Int*. 2015;3(2):37-47. doi:10.17311/sciintl.2015.37.47

63. Green JM, Owen MDK. Herbicide-resistant crops: Utilities and limitations for herbicide-resistant weed management. *J Agric Food Chem*. 2011;59(11):5819-5829. doi:10.1021/jf101286h

64. Meftaul IM, Venkateswarlu K, Dharmarajan R, et al. Controversies over human health and ecological impacts of glyphosate: Is it to be banned in modern agriculture? *Environ Pollut*. 2020;263:114372. doi:10.1016/j.envpol.2020.114372

65. Lotina-Hennsen B, King-Diaz B, Aguilar MI, Hernandez Terrones MG. Plant secondary metabolites. Targets and mechanisms of allelopathy. In: *Allelopathy: A Physiological Process with Ecological Implications*. Springer; 2006:229-230.

66. Radhakrishnan R, Alqarawi AA, Abd Allah EF. Bioherbicides: Current knowledge on weed control mechanism. *Ecotoxicol Environ Saf*. 2018;158(April):131-138. doi:10.1016/j.ecoenv.2018.04.018

67. Duke SO, Pan Z, Bajsa-hirschel J. Proving the Mode of Action of Phytotoxic Phytochemicals. *Plants*. Published online 2020:1-20.

68. Laxmishree C, Nandita S. Botanical pesticides – a major alternative to chemical pesticides: A review. *Int J Life Sci*. 2017;5(4):722-729.

69. Ngegba PM, Cui G, Khalid MZ, Zhong G. Use of Botanical Pesticides in Agriculture as an Alternative to Synthetic Pesticides. *Agric*. 2022;12(5). doi:10.3390/agriculture12050600

70. Lengai GMW, Muthomi JW, Mbega ER. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Sci African*. 2020;7:e00239. doi:10.1016/j.sciaf.2019.e00239

71. El-Wakeil NE. Botanical Pesticides and Their Mode of Action. *Gesunde Pflanz*. 2013;65(4):125-149. doi:10.1007/s10343-013-0308-3

72. Paramasivam M. Laboratory bioassay methods to assess the insecticide toxicity against insect pests-A review. *~ 1441 ~ J Entomol Zool Stud*. 2017;5(3):1441-1445.

73. Guarrera PM. *Usi e Tradizioni Della Flora Italiana. Medicina Popolare Ed Etnobotanica*. Aracne editrice S.r.l.; 2006.

74. Barthel S, Crumley C, Svedin U. Bio-cultural refugia-Safeguarding diversity of practices for food security and biodiversity. *Glob Environ Chang*. 2013;23(5):1142-1152. doi:10.1016/j.gloenvcha.2013.05.001

75. La Rosa A, Cornara L, Saitta A, et al. Ethnobotany of the Aegadian Islands: safeguarding biocultural refugia in the Mediterranean. *J Ethnobiol Ethnomed*. 2021;17(1):1-19. doi:10.1186/s13002-021-00470-z

76. Pieroni, Andrea; Pawera, Lukas; Mujtaba Shah G. Gastronomic Ethnobiology. *Introd to Ethnobiol*. Published online 2016:1-310. doi:10.1007/978-3-319-28155-1

77. Pieroni A, Giusti ME. Alpine ethnobotany in Italy: Traditional knowledge of gastronomic and medicinal plants among the Occitans of the upper Varaita valley, Piedmont. *J Ethnobiol Ethnomed*. 2009;5:1-14. doi:10.1186/1746-4269-5-32

78. Mattalia G, Quave CL, Pieroni A. Traditional uses of wild food and medicinal plants among Brigasc, Kyé, and Provençal communities on the Western Italian Alps. *Genet Resour Crop Evol*. 2013;60(2):587-603. doi:10.1007/s10722-012-9859-x

79. Bellia G, Pieroni A. Isolated, but transnational: The glocal nature of Waldensian ethnobotany, Western Alps, NW Italy. *J Ethnobiol Ethnomed*. 2015;11(1). doi:10.1186/s13002-015-0027-1

80. Vitalini S, Tomè F, Fico G. Traditional uses of medicinal plants in Valvestino (Italy). *J Ethnopharmacol*. 2009;121(1):106-116. doi:10.1016/j.jep.2008.10.005

81. Vitalini S, Iriti M, Puricelli C, Ciuchi D, Segale A, Fico G. Traditional knowledge on medicinal and food plants used in Val San Giacomo (Sondrio, Italy) - An alpine ethnobotanical study. *J Ethnopharmacol*. 2013;145(2):517-529. doi:10.1016/j.jep.2012.11.024

82. Vitalini S, Puricelli C, Mikerezi I, Iriti M. Plants, people and traditions: Ethnobotanical survey in the Lombard Stelvio National Park and neighbouring areas (Central Alps, Italy). *J Ethnopharmacol*. 2015;173:435-458. doi:10.1016/j.jep.2015.05.036

83. Dei Cas L, Pugni F, Fico G. Tradition of use on medicinal species in Valfurva (Sondrio, Italy). *J Ethnopharmacol*. 2015;163:113-134. doi:10.1016/j.jep.2014.12.054

84. Bottoni M, Milani F, Colombo L, et al. Using Medicinal Plants in Valmalenco (Italian Alps): From Tradition to Scientific Approaches. *Molecules*. 2020;25(18):1-27. doi:10.3390/molecules25184144

85. Cornara L, La Rocca A, Terrizzano L, Dente F, Mariotti MG. Ethnobotanical and phytomedical knowledge in the North-Western Ligurian Alps. *J Ethnopharmacol*. 2014;155(1):463-484. doi:10.1016/j.jep.2014.05.046

86. Cavalloro V, Robustelli della Cuna FS, Quai E, et al. Walking around the Autonomous Province of Trento (Italy): An Ethnobotanical Investigation. *Plants*. 2022;11(17):1-18. doi:10.3390/plants11172246

87. Mattalia G, Graetz F, Harms M, et al. Temporal Changes in the Use of Wild Medicinal Plants in Trentino–South Tyrol, Northern Italy. *Plants*. Published online 2023.

88. Barinotto L. Piante medicinali e di uso tradizionale in Valle Orco. Published online 2013.

89. Binel B. Piante medicinali della Valle d'Aosta nella tradizione popolare. *Société la flore Vald*. 1972;bulletin n.

90. Chimenti Signorini RFM. Indagine etnofarmacologica nella Valtournanche (Valle d'Aosta). *Webbia, J Plant Taxon Geogr*. 1983;37(1):69-94.

91. Poggio L. *Piante Officinali Della Valle d'Aosta*. Neos edizi. (Edizioni N, ed.). Neos edizioni; 2006.

92. Denarier N. *Venti Erbe per Stare Bene. Piante Medicinali Della Valle d'Aosta*. Edizioni v.; 2006.

93. Pieller C. Guaritori e rimedi, la medicina tradizionale in Valle d'Aosta. Published online 2011.

94. Armand H. *Vivere e Guarire in Montagna*. End.; 2014.

95. Bétemps A. *Erbario, Erbe Misteriose e Flora Generosa Nello Spazio Selvatico o Antropizzato Della Montagna Valdostana e Dintorni*. (Verlucca P and, ed.).; 2012.

96. Colliard L. *La Culture Valdôtaine Au Cours Des Siècles*. (ITLA Editrice, ed.).; 1976.

97. Ascoli GI. *Schizzi Franco-Provenzali*. (Attinger V, ed.).; 1952.

98. Caneva G, Pieroni A, Guarrera PM. *Etnobotanica. Conservazione Di Un Patrimonio Culturale Come Risorsa per Uno Sviluppo Sostenibile*. (EDIPUGLIA, ed.).; 2013.

99. Danna C, Poggio L, Smeriglio A, Mariotti M, Cornara L. Ethnomedicinal and Ethnobotanical Survey in the Aosta Valley Side of the Gran Paradiso National Park (Western Alps, Italy). *Plants*. 2022;11(2). doi:10.3390/plants11020170

100. Alpiche E. La Flora vascolare del PNGP in numeri. Published online 2020.

101. Bovio M, Broglio M, Poggio L. *Guida Alla Flora Della Valle d'Aosta*. Blu edizio. (Edizioni B, ed.).; 2012.

102. Flora VDA. http://www.floravda.it/it/schede/

103. Plants of the World Online | Kew Science. Accessed November 22, 2021. http://www.plantsoftheworldonline.org/

104. Pignatti S. *Flora d'Italia*. (Media E-NB, ed.).; 2017.

105. IPFI: Index Plantarum. Accessed November 16, 2021. https://www.actaplantarum.org/flora/flora.php

106. Index Fungorum. Accessed November 16, 2021. http://www.indexfungorum.org/names/Names.asp

107. Portères R. *Ethnobotanique Générale. Laboratoire d'Ethnobotanique et Ethnozoologie.*; 1970.

108. Bonet MÀ, Parada M, Selga A, Vallès J. Studies on pharmaceutical ethnobotany in the regions of L'Alt Emporda and Les Guilleries (Catalonia, Iberian Peninsula). *J Ethnopharmacol*. 1999;68(1-3):145-168. doi:10.1016/S0378-8741(99)00083-5

109. Tardío J, Pardo-De-Santayana M. Cultural importance indices: A comparative analysis based on the useful wild plants of southern Cantabria (northern Spain). *Econ Bot*. 2008;62(1):24-39. doi:10.1007/s12231-007-9004-5

110. Heinrich M, Ankli A, Frei B, Weimann C, Sticher O. Medicinal plants in Mexico: Healers' consensus and cultural importance. *Soc Sci Med*. 1998;47(11):1859-1871. doi:10.1016/S0277-9536(98)00181-6

111. Friedman J, Yaniv Z, Dafni A, Palewitch D. A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethnopharmacological field survey among Bedouins in the Negev Desert, Israel. *J Ethnopharmacol*. 1986;16(2-3):275-287. doi:10.1016/0378-8741(86)90094-2

112. Andrade-Cetto A, Heinrich M. From the field into the lab: Useful approaches to selecting species based on local knowledge. *Front Pharmacol*. 2011;APR(April):1-5. doi:10.3389/fphar.2011.00020

113. Guarrera P, Lucchese F, Medori S. Ethnophytotherapeutical research in the high Molise region (Central-Southern Italy). *J Ethnobiol Ethnomed*. 2008;4:1-11. doi:10.1186/1746-4269-4-7

114. Petelka J, Plagg B, Säumel I, Zerbe S. Traditional medicinal plants in South Tyrol (northern Italy, southern Alps): biodiversity and use. *J Ethnobiol Ethnomed*. 2020;16(1):1-15. doi:10.1186/s13002-020-00419-8

115. International Classification of Diseases (ICD). Accessed November 16, 2021. https://www.who.int/classifications/classification-of-diseases

116. Apetrei CL, Tuchilus C, Aprotosoaie AC, Oprea A, Malterud KE, Miron A. Chemical, antioxidant and antimicrobial investigations of pinus cembra L. bark and needles. *Molecules*. 2011;16(9):7773-7788. doi:10.3390/molecules16097773

117. Süntar I, Tumen I, Ustün O, Keleş H, Küpeli Akkol E. Appraisal on the wound healing and anti-inflammatory activities of the essential oils obtained from the cones and needles of Pinus species by in vivo and in vitro experimental models. *J Ethnopharmacol*. 2012;139(2):533-540. doi:10.1016/j.jep.2011.11.045

118. Mitić ZS, Jovanović B, Jovanović S, et al. Comparative study of the essential oils of four Pinus species: Chemical composition, antimicrobial and insect larvicidal activity. *Ind Crops Prod*. 2018;111(October 2017):55-62. doi:10.1016/j.indcrop.2017.10.004

119. Basholli-Salihu M, Schuster R, Hajdari A, et al. Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three Pinus spp. *Pharm Biol*. 2017;55(1):1553-1560. doi:10.1080/13880209.2017.1309555

120. Lamaison J. Comparative study of viola lutea Huds, V. Calcarata L and V. odorata L. *Plantes médicinales et phytothérapie*. Published online 1991:6.

121. Brito JCM, Lima WG, Cordeiro LPB, da Cruz Nizer WS. Effectiveness of supplementation with quercetin-type flavonols for treatment of viral lower respiratory tract infections: Systematic review and meta-analysis of preclinical studies. *Phyther Res*. 2021;35(9):4930-4942. doi:10.1002/ptr.7122

122. Olafsdottir ES, Ingólfsdottir K. Polysaccharides from lichens: Structural characteristics and biological activity. *Planta Med*. 2001;67(3):199-208. doi:10.1055/s-2001-12012

123. Freysdottir J, Omarsdottir S, Ingolfsdottir K, Vikingsson A, Olafsdottir ES. In vitro and in vivo immunomodulating effects of traditionally-prepared extract and purified compounds from Cetraria islandica. *Proc Nutr Soc*. 2008;67(OCE):2021. doi:10.1017/S0029665108006228

124. Vouillamoz JF, Carlen C, Taglialatela-Scafati O, Pollastro F, Appendino G. The génépi Artemisia species. Ethnopharmacology, cultivation, phytochemistry, and bioactivity. *Fitoterapia*. 2015;106:231-241. doi:10.1016/j.fitote.2015.09.007

125. Petrova M, Zayova E, Vassilevska-Ivanova R, Vlahova M. Biotechnological approaches for cultivation and enhancement of secondary metabolites in Arnica montana L. *Acta Physiol Plant*. 2012;34(5):1597-1606. doi:10.1007/s11738-012-0987-x

126. Sharma S, Arif M, Nirala RK, Gupta R, Thakur SC. Cumulative therapeutic effects of phytochemicals in Arnica montana flower extract alleviated collagen-induced arthritis: Inhibition of both pro-inflammatory mediators and oxidative stress. *J Sci Food Agric*. 2016;96(5):1500-1510. doi:10.1002/jsfa.7252

127. Kriplani P, Guarve K, Baghael US. Arnica montana L. – a plant of healing: review. *J Pharm Pharmacol*. 2017;69(8):925-945. doi:10.1111/jphp.12724

128. Adom MB, Taher M, Mutalabisin MF, et al. Chemical constituents and medical benefits of Plantago major. *Biomed Pharmacother*. 2017;96(May):348-360. doi:10.1016/j.biopha.2017.09.152

129. Batiha GES, Beshbishy AM, Wasef LG, et al. Chemical constituents and pharmacological activities of garlic (Allium sativum L.): A review. *Nutrients*. 2020;12(3):1-21. doi:10.3390/nu12030872

130. Godinho LS, Aleixo De Carvalho LS, Barbosa De Castro CC, et al. Anthelmintic activity of crude extract and essential oil of tanacetum vulgare (Asteraceae) against adult worms of schistosoma mansoni. *Sci World J*. 2014;2014. doi:10.1155/2014/460342

131. Cout F. *Secret, Formule Di Guarigione in Uso in Valle d'Aosta*. (Verlucca P and, ed.).; 2005.

132. Abbet C, Mayor R, Roguet D, Spichiger R, Hamburger M, Potterat O. Ethnobotanical survey on wild alpine food plants in Lower and Central Valais (Switzerland). *J Ethnopharmacol*. 2014;151(1):624-634. doi:10.1016/j.jep.2013.11.022

133. Weleda Naturals GmbH - Biodynamic Research. http://www.biodynamic-research.net/ras/pl/medical-plants/rpwel/

134. Azienda Agricola Da Emy. https://www.daemy.it/

135. Valverbe. https://valverbe.it/28a-peucedanum-ostruthium-imperatoria

136. Danna C, Bazzicalupo M, Ingegneri M, et al. Anti-Inflammatory and Wound Healing Properties of Leaf and Rhizome Extracts from the Medicinal Plant Peucedanum ostruthium (L.) W. D. J. Koch. *Molecules*. 2022;27(13). doi:10.3390/molecules27134271

137. Coulter, J.M.; Rose JN. *Monograph of the North American Umbelliferae*. Contributions from the United States National Herbarium: Washington, DC, USA, 1900; Volume 7, pp. 9–256.

138. Gökay O, Kühner D, Los M, Götz F, Bertsche U, Albert K. An efficient approach for the isolation, identification and evaluation of antimicrobial plant components on an analytical scale, demonstrated by the example of Radix imperatoriae. *Anal Bioanal Chem*. 2010;398(5):2039-2047. doi:10.1007/s00216-010-4153-2

139. Adams M, Berset C, Kessler M, Hamburger M. Medicinal herbs for the treatment of rheumatic disorders-A survey of European herbals from the 16th and 17th century. *J Ethnopharmacol*. 2009;121(3):343-359. doi:10.1016/j.jep.2008.11.010

140. Cisowski W, Sawicka U, Mardarowicz M, Asztemborska M, Luczkiewicz M. Essential oil from herb and rhizome of Peucedanum ostruthium (L. Koch.) ex DC. *Zeitschrift fur Naturforsch - Sect C J Biosci*. 2001;56(11-12):930-932. doi:10.1515/znc-2001-11-1202

141. Hiermann A, Schantl D. Antiphlogistic and antipyretic activity of Peucedanum ostruthium. *Planta Med, 64, 400–403*. Published online 1998:6.

142. Sarkhail P. Traditional uses, phytochemistry and pharmacological properties of the genus Peucedanum: A review. *J Ethnopharmacol*. 2014;156:235-270. doi:10.1016/j.jep.2014.08.034

143. Lammel C, Zwirchmayr J, Seigner J, Rollinger JM, de Martin R. Peucedanum ostruthium inhibits e-selectin and VCAM-1 expression in endothelial cells through interference with nf-κb signaling. *Biomolecules*. 2020;10(9):1-22. doi:10.3390/biom10091215

144. Urbain A, Marston A, Hostettmann K. Coumarins from Peucedanum ostruthium as inhibitors of acetylcholinesterase. *Pharm Biol*. 2005;43(8):647-650. doi:10.1080/13880200500382720

145. Schinkovitz A, Gibbons S, Stavri M, Cocksedge MJ, Bucar F. Ostruthin: An antimycobacterial coumarin from the roots of Peucedanum ostruthium. *Planta Med*. 2003;69(4):369-371. doi:10.1055/s-2003-38876

146. Cousyn G, Dalfrà S, Scarpa B, Geelen J. Project BELFRIT Harmonizing the Use of Plants in Food Supplements in the European. *Eur Food Feed Law Rev*. Published online 2013:187-196.

147. Gahan PB. *Plant Histochemistry and Cytochemistry. An Introduction.* Academic Press; 1984.

148. Chieco C, Rotondi A, Morrone L, Rapparini F, Baraldi R. An ethanol-based fixation method for anatomical and micro-morphological characterization of leaves of various tree species. *Biotech Histochem*. 2013;88(2):109-119. doi:10.3109/10520295.2012.746472

149. Smeriglio A, Denaro M, Barreca D, et al. In vitro evaluation of the antioxidant, cytoprotective, and antimicrobial properties of essential oil from Pistacia vera L. Variety Bronte Hull. *Int J Mol Sci*. 2017;18(6). doi:10.3390/ijms18061212

150. Monforte MT, Smeriglio A, Germanò MP, Pergolizzi S, Circosta C, Galati EM. Evaluation of antioxidant, antiinflammatory, and gastroprotective properties of Rubus fruticosus L. fruit juice. *Phyther Res*. 2018;32(7):1404-1414. doi:10.1002/ptr.6078

151. Smeriglio A, Denaro M, De Francesco C, et al. Feijoa fruit peel: Micro-morphological features, evaluation of phytochemical profile, and biological properties of its essential oil. *Antioxidants*. 2019;8(8). doi:10.3390/antiox8080320

152. Smeriglio A, Bonasera S, Germanò MP, et al. Opuntia ficus-indica (L.) Mill. fruit as source of betalains with antioxidant, cytoprotective, and anti-angiogenic properties. *Phyther Res*. 2019;33(5):1526-1537. doi:10.1002/ptr.6345

153. Smeriglio A, De Francesco C, Denaro M, Trombetta D. Prickly Pear Betalain-Rich Extracts as New Promising Strategy for Intestinal Inflammation: Plant Complex vs. Main Isolated Bioactive Compounds. *Front Pharmacol*. 2021;12(September):1-11. doi:10.3389/fphar.2021.722398

154. Smeriglio A, Denaro M, Di Gristina E, et al. Pharmacognostic approach to evaluate the micromorphological, phytochemical and biological features of Citrus lumia seeds. *Food Chem*. 2022;375(December 2021):131855. doi:10.1016/j.foodchem.2021.131855

155. Teramachi F, Koyano T, Kowithayakorn T, Hayashi M, Komiyama K, Ishibashi M. Collagenase inhibitory quinic acid esters

from Ipomoea pes-caprae. *J Nat Prod*. 2005;68(5):794-796. doi:10.1021/np0500631

156. Ohtsuki T, Yokosawa E, Koyano T, et al. Quinic Acid Esters from Pluchea indica with Collagenase, MMP-2 and MMP-9 Inhibitory Activities. *Phyther Res*. 2008;22:264-266. doi:10.1002/ptr.2290

157. Hartmann A, Gostner J, Fuchs JE, et al. Inhibition of Collagenase by Mycosporine-like Amino Acids from Marine Sources. *Planta Med*. 2015;81(10):813-820. doi:10.1055/s-0035-1546105

158. Boukamp P. Normal Keratinization in a Spontaneously Immortalized. *J Cell Biol*. 1988;106(March):761-771.

159. Bazzicalupo M, Cornara L, Burlando B, et al. Carpobrotus edulis (L.) N.E.Br. extract as a skin preserving agent: From traditional medicine to scientific validation. *J Integr Med*. 2021;19(6):526-536. doi:10.1016/j.joim.2021.09.002

160. Muniandy K, Gothai S, Tan WS, et al. In Vitro Wound Healing Potential of Stem Extract of Alternanthera sessilis. *Evidence-based Complement Altern Med*. 2018;2018(2011). doi:10.1155/2018/3142073

161. Vogl S, Zehl M, Picker P, et al. Identification and quantification of coumarins in Peucedanum ostruthium (L.) Koch by HPLC-DAD and HPLC-DAD-MS. *J Agric Food Chem*. 2011;59(9):4371-4377. doi:10.1021/jf104772x

162. Palmioli A, Bertuzzi S, De Luigi A, et al. bioNMR-based identification of natural anti-Aβ compounds in Peucedanum ostruthium. *Bioorg Chem*. 2019;83(October 2018):76-86. doi:10.1016/j.bioorg.2018.10.016

163. Vitalini S, Palmioli A, Orlando F, et al. Phytotoxicity, nematicidal activity and chemical constituents of Peucedanum ostruthium (L.) W.D.J.Koch (Apiaceae). *Ind Crops Prod*. 2021;166(April):113499. doi:10.1016/j.indcrop.2021.113499

164. McCardell JH, Heritier J, Simonnet X, Carlen CP. Peucedanum ostruthium (L.) Koch: Morphological and phytochemical variability of twelve accessions from the Swiss alpine region. *Julius-Kuhn-Arch 2016, 453, 121–123*. 2016;36:20. doi:10.5073/jka.2016.453.043

165. Nani M, Leone A, Bom VP, et al. Evaluation and Comparison of Wound Healing Properties of an Ointment (AlpaWash) Containing Brazilian Micronized Propolis and Peucedanum ostruthium Leaf Extract in Skin Ulcer in Rats. *Int J Pharm Compd*. 2018;22(2):154-163.

166. Nguyen VT, Sakoff JA, Scarlett CJ. Physicochemical Properties, Antioxidant and Anti-proliferative Capacities of Dried Leaf and Its Extract from Xao tam phan (Paramignya trimera). *Chem Biodivers*. 2017;14(6). doi:10.1002/cbdv.201600498

167. Yang HB, Gao HR, Ren YJ, et al. Effects of isoimperatorin on proliferation and apoptosis of human gastric carcinoma cells. *Oncol Lett*. 2018;15(5):7993-7998. doi:10.3892/ol.2018.8303

168. Liu W, Li J, Zhang X, et al. Current advances in naturally occurring caffeoylquinic acids: Structure, bioactivity, and synthesis. *J Agric Food Chem*. 2020;68(39):10489-10516. doi:10.1021/acs.jafc.0c03804

169. Beken B, Serttas R, Yazicioglu M, Turkekul K, Erdogan S. Quercetin Improves Inflammation, Oxidative Stress, and Impaired Wound Healing in Atopic Dermatitis Model of Human Keratinocytes. *Pediatr Allergy, Immunol Pulmonol*. 2020;33(2):69-79. doi:10.1089/ped.2019.1137

170. Kim HP, Mani I, Iversen L, Ziboh VA. Effects of naturally-occurring flavonoids and biflavonoids on epidermal cyclooxygenase and lipoxygenase from guinea-pigs. *Prostaglandins Leukot Essent Fat Acids*. 1998;58(1):17-24. doi:10.1016/S0952-3278(98)90125-9

171. Tomé M, Almeida MH, Barreiro S, et al. Opportunities and challenges of Eucalyptus plantations in Europe: the Iberian Peninsula experience. *Eur J For Res*. 2021;140(3):489-510. doi:10.1007/s10342-021-01358-z

172. Dhakad AK, Pandey V V., Beg S, Rawat JM, Singh A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *J Sci Food Agric*. 2018;98(3):833-848. doi:10.1002/jsfa.8600

173. Floris I, Cocco A, Buffa F, Mannu R, Satta A. Insect pests of eucalyptus plantations in Sardinia (Italy). *Redia*. 2018;101(August):61-71. doi:10.19263/REDIA-101.18.09

174. Campisi E, Mancianti F, Pini G, Faggi E, Gargani G. Investigation in Central Italy of the Possible Association between Cryptococcus neoformans var . Gattii and Eucalyptus camaldulensis Author (s): E . Campisi , F . Mancianti , G . Pini , E . Faggi and G . Gargani Published by : Springer Stable URL : http. *Eur J Epidemiol*. 2021;18(4):357-362.

175. Forrest M. The performance of a Eucalyptus gunnii cut foliage plantation over 7 years. *Irish J Agric Food Res*. 2002;41(2):235-245.

176. Pacifici S, Ferrante A, Mensuali-sodi A, Serra G. Postharvest physiology and technology of cut eucalyptus branches : a review. *Agric Mediterr*. 2007;137(2007):124-131. https://www.researchgate.net/publication/255910152

177. Wirthensohn MG, Collins G, Jones GP, Sedgley M. Variability in waxiness of Eucalyptus gunnii foliage for floriculture. *Sci Hortic (Amsterdam)*. 1999;82(3-4):279-288. doi:10.1016/S0304-4238(99)00050-3

178. García C, Montero G, Coronado MA, et al. Valorization of Eucalyptus Leaves by Essential Oil Extraction as an Added Value Product in Mexico. *Waste and Biomass Valorization*. 2017;8(4):1187-1197. doi:10.1007/s12649-016-9695-x

179. Mediavilla I, Guillamón E, Ruiz A, Esteban LS. Essential oils from residual foliage of forest tree and shrub species: Yield and antioxidant capacity. *Molecules*. 2021;26(11):1-17. doi:10.3390/molecules26113257

180. Smeriglio A, Trombetta D, Cornara L, Valussi M, De Feo V, Caputo L. Characterization and phytotoxicity assessment of essential oils from plant byproducts. *Molecules*. 2019;24(16):1-16. doi:10.3390/molecules24162941

181. Pauzer MS, Borsato TDO, de Almeida VP, et al. Eucalyptus cinerea: Microscopic Profile, Chemical Composition of Essential Oil and its Antioxidant, Microbiological and Cytotoxic Activities. *Brazilian Arch Biol Technol*. 2021;64:1-19. doi:10.1590/1678-4324-75years-2021200772

182. Barbosa LCA, Filomeno CA, Teixeira RR. Chemical variability and biological activities of Eucalyptus spp. essential oils. *Molecules*. 2016;21(12):1-33. doi:10.3390/molecules21121671

183. Lemke N, Murawski A, Schmied-Tobies MIH, et al. Glyphosate and aminomethylphosphonic acid (AMPA) in urine of children and adolescents in Germany – Human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). *Environ Int*. 2021;156. doi:10.1016/j.envint.2021.106769

184. Ibáñez MD, Blázquez MA. Phytotoxicity of essential oils on selected weeds: Potential hazard on food crops. *Plants*. 2018;7(4). doi:10.3390/plants7040079

185. Setia N, Batish DR, Singh HP, Kohli RK. Phytotoxicity of volatile oil from Eucalyptus citriodora against some weedy species. *J Environ Biol*. 2007;28(1):63-66.

186. Kordali S, Cakir A, Sutay S. Inhibitory effects of monoterpenes on seed germination and seedling growth. *Zeitschrift fur Naturforsch - Sect C J Biosci*. 2007;62(3-4):207-214. doi:10.1515/znc-2007-3-409

187. Rasaeifar M, Hosseini N, Haji N, Asl H, Zandi P, Aghdam AM. Allelopathic effect of Eucalyptus globulus essential oil on seed germination and seedling establishment of Amaranthus blitoides and Cyndon dactylon. *Trakia J Sci*. 2013;11(1):73-81. http://www.uni-sz.bg

188. Kanatas P. Potential role of eucalyptus spp. And acacia spp. allelochemicals in weed management. *Chil J Agric Res*. 2020;80(3):452-458. doi:10.4067/S0718-58392020000300452

189. Puig CG, Revilla P, Barreal ME, Reigosa MJ, Pedrol N. On the suitability of Eucalyptus globulus green manure for field weed control. *Crop Prot*. 2019;121(May 2018):57-65. doi:10.1016/j.cropro.2019.03.016

190. Verdeguer M, Blázquez MA, Boira H. Phytotoxic effects of Lantana camara, Eucalyptus camaldulensis and Eriocephalus africanus essential oils in weeds of Mediterranean summer crops. *Biochem Syst Ecol*. 2009;37(4):362-369. doi:10.1016/j.bse.2009.06.003

191. Batish DR, Setia N, Singh HP, Kohli RK. Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide. *Crop Prot*. 2004;23(12):1209-1214. doi:10.1016/j.cropro.2004.05.009

192. Batish DR, Singh HP, Setia N, Kaur S, Kohli RK. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of Eucalyptus citriodora. *Zeitschrift fur Naturforsch - Sect C J Biosci*. 2006;61(7-8):465-471. doi:10.1515/znc-2006-7-801

193. Vaid S, Batish DR, Singh HP, Kohli RK. Phytotoxic Effect of Eugenol Towards Two Weedy Species. *The Bioscan*. 2010;5(1):339-341. http://www.thebioscan.in/Journals_PDF/5301- S. VAID.pdf

194. Ibáñez MD, Blázquez MA. Phytotoxic Effects of Commercial Eucalyptus citriodora, Lavandula angustifolia, and Pinus sylvestris Essential Oils on Weeds, Crops, and Invasive Species. *Molecules*. 2019;24(15):1-15. doi:10.3390/molecules24152847

195. Singh HP, Batish DR, Kaur S, Arora K, Kohli RK. α-Pinene inhibits growth and induces oxidative stress in roots. *Ann Bot*. 2006;98(6):1261-1269. doi:10.1093/aob/mcl213

196. Zhang J, An M, Wu H, Liu DL, Stanton R. Chemical composition of essential oils of four Eucalyptus species and their phytotoxicity on silverleaf nightshade (Solanum elaeagnifolium Cav.) in Australia. *Plant Growth Regul*. 2012;68(2):231-237. doi:10.1007/s10725-012-9711-5

197. Amini J, Farhang V, Javadi T, Nazemi J. Antifungal effect of plant essential oils on controlling Phytophthora species. *Plant Pathol J*. 2016;32(1):16-24. doi:10.5423/PPJ.OA.05.2015.0091

198. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. 2013;6(12):1451-1474. doi:10.3390/ph6121451

199. WHO. *Quality Control Methods for Herbal Materials*. WHO Press; 2011. https://apps.who.int/%0Airis/handle/10665/44479

200. Alamgir ANM. Microscopy in Pharmacognosy. In: *Therapeutic Use of Medicinal Plants and Their Extracts*. Springer, Cham; 2017. doi:https://doi.org/10.1007/978-3-319-63862-1_11

201. Council of Europe. *European Pharmacopeia*. 5th ed. Council of Europe; 2004.

202. Rogiers SY, Hardie WJ, Smith JP. Stomatal density of grapevine leaves (Vitis vinifera L.) responds to soil temperature and atmospheric carbon dioxide. *Aust J Grape Wine Res*. 2011;17(2):147-152. doi:10.1111/j.1755-0238.2011.00124.x

203. Santos LDT, Thadeo M, Iarema L, Meira RMSA, Ferreira FA. Foliar anatomy and histochemistry in seven species of Eucalyptus. *Rev Arvore*. 2008;32(4):769-779. doi:10.1590/s0100-67622008000400019

204. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671-675. doi:10.1038/nmeth.2089

205. Albrechtová J, Kubínová Z, Soukup A. Plant Cell Morphogenesis. 2014;1080(November):67-76. doi:10.1007/978-1-62703-643-6

206. Badria FA, Aboelmaaty SW. Plant Histochemistry: A Versatile and Indispensible Tool in Localization of Gene Expression, Enzymes, Cytokines, Secondary Metabolites and Detection of Plants Infection and Pollution. *Acta Sci Pharm Sci*. 2019;3(7):88-100. doi:10.31080/asps.2019.03.0318

207. O'Brien, T.P.; McCully ME. *The Study of Plant Structure: Principles and Selected Methods*. Termocarphy; 1981.

208. Pathan AK, Bond J, Gaskin RE. Sample preparation for scanning electron microscopy of plant surfaces-Horses for courses. *Micron*. 2008;39(8):1049-1061. doi:10.1016/j.micron.2008.05.006

209. Danna C, Cornara L, Smeriglio A, et al. Eucalyptus gunnii and eucalyptus pulverulenta 'baby blue' essential oils as potential natural herbicides. *Molecules*. 2021;26(21):1-18. doi:10.3390/molecules26216749

210. Jennings, W.; Shibamoto T. *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography*. Academic Press; 1980.

211. Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. *J Chromatogr A*. 1990;503(C):1-24. doi:10.1016/S0021-9673(01)81487-4

212. Goodner KL. Practical retention index models of OV-101, DB-1, DB-5, and DB-Wax for flavor and fragrance compounds. *Lwt*. 2008;41(6):951-958. doi:10.1016/j.lwt.2007.07.007

213. Adams R. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. 4th ed. Allured Publishing Corporation; 2007.

214. McLafferty F. *Wiley Registry of Mass Spectral Data, with NIST Spectral Data CD Rom*. 7th ed. John Wiley & Sons; 1998.

215. Bewley JD. Seed germination and dormancy. *Plant Cell*. 1997;9(7):1055-1066. doi:10.1105/tpc.9.7.1055

216. Malaspina P, Papaianni M, Ranesi M, et al. Bioactive Compounds for Agricultural Applications. Published online 2022:1-17.

217. Xiao Z, Storms R, Tsang A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal Biochem*. 2006;351(1):146-148. doi:10.1016/j.ab.2006.01.036

218. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement Altern Med*. 2011;11. doi:10.1186/1472-6882-11-5

219. Caputo L, Smeriglio A, Trombetta D, et al. Chemical Composition and Biological Activities of the Essential Oils of Leptospermum petersonii and Eucalyptus gunnii. *Front Microbiol*. 2020;11(April):1-15. doi:10.3389/fmicb.2020.00409

220. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pac J Trop Biomed*. 2012;2(9):739-742. doi:10.1016/S2221-1691(12)60220-2

221. Montone AMI, Papaianni M, Malvano F, Capuano F, Capparelli R, Albanese D. Lactoferrin, quercetin, and hydroxyapatite act synergistically against pseudomonas fluorescens. *Int J Mol Sci*. 2021;22(17). doi:10.3390/ijms22179247

222. Vitale S, Di Pietro A, Turrà D. Autocrine pheromone signalling regulates community behaviour in the fungal pathogen Fusarium oxysporum. *Nat Microbiol*. 2019;4(9):1443-1449. doi:10.1038/s41564-019-0456-z

223. Murtaza G, Asghar R. α-amylase activities during seed development and germination in pea (Pisum sativum L.) treated with salicylic acid. *Pakistan J Bot*. 2012;44(6):1823-1829.

224. Danabas D, Ates M, Ertit Tastan B, et al. Effects of Zn and ZnO Nanoparticles on Artemia salina and Daphnia magna Organisms: Toxicity, Accumulation and Elimination. *Sci Total Environ*. 2020;711:134869. doi:10.1016/j.scitotenv.2019.134869

225. De Lillo E, Simoni S, Rapetti S, et al. Un nuovo acaro eriofide dannoso per l'Eucalipto da fronda in Italia e prime esperienze di Lotta. *ATTI Giornate Fitopatol*. Published online 2016:3-12.

226. De Brito PS, Sabedotti C, Flores TB, et al. Light and Scanning Electron Microscopy, Energy Dispersive X-Ray Spectroscopy, and Histochemistry of Eucalyptus tereticornis. *Microsc Microanal*. 2021;27(5):1295-1303. doi:10.1017/S1431927621012514

227. Migacz IP, Raeski PA, Almeida VP de, et al. Comparative leaf morpho-anatomy of six species of Eucalyptus cultivated in

Brazil. *Rev Bras Farmacogn*. 2018;28(3):273-281. doi:10.1016/j.bjp.2018.04.006

228. Soliman FM, Fathy MM, Salama MM, Saber FR. Botanical study of Eucalyptus Cinerea F . Muell . Ex Benth. cultivated in Egypt. *World J Pharm Pharm Sci*. 2015;4(10):310-328.

229. Bugarin D, Grbovic S, Orčič D, Mitić-Ćulafić D, Kneževic-Vukčević J, Mimica-Dukić N. Essential oil of eucalyptus gunnii hook. As a novel source of antioxidant, antimutagenic and antibacterial agents. *Molecules*. 2014;19(11):19007-19020. doi:10.3390/molecules191119007

230. Lucia A, Licastro S, Zerba E, Masuh H. Yield, chemical composition, and bioactivity of essential oils from 12 species of Eucalyptus on Aedes aegypti larvae. *Entomol Exp Appl*. 2008;129(1):107-114. doi:10.1111/j.1570-7458.2008.00757.x

231. Elaissi A, Medini H, Simmonds M, et al. Variation in volatile leaf oils of seven eucalyptus species harvested from Zerniza arboreta (Tunisia). *Chem Biodivers*. 2011;8(2):362-372. doi:10.1002/cbdv.201000103

232. Zrira S, Bessiere JM, Menut C, Elamrani A, Benjilali B. Chemical composition of the essential oil of nine Eucalyptus species growing in Morocco. *Flavour Fragr J*. 2004;19(2):172-175. doi:10.1002/ffj.1289

233. Brophy JJ, Lassak EV, Toia RF. The Steam Volatile Leaf Oil of Eucalyptus pulverulenta. *Planta Med*. 1985;(51):170-171.

234. Ieri F, Cecchi L, Giannini E, Clemente C, Romani A. GC-MS and HS-SPME-GC×GC-TOFMS determination of the volatile composition of essential oils and hydrosols (By-products) from four Eucalyptus species cultivated in Tuscany. *Molecules*. 2019;24(2):1-15. doi:10.3390/molecules24020226

235. Kahla Y, Zouari-Bouassida K, Rezgui F, Trigui M, Tounsi S. Efficacy of Eucalyptus cinerea as a Source of Bioactive Compounds for Curative Biocontrol of Crown Gall Caused by Agrobacterium tumefaciens Strain B6. *Biomed Res Int*. 2017;2017. doi:10.1155/2017/9308063

236. Soliman FM, Fathy MM, Salama MM, Saber FR. Chemical composition and bioactivity of the volatile oil from leaves and stems of Eucalyptus cinerea. *Pharm Biol*. 2014;52(10):1272-1277. doi:10.3109/13880209.2014.889177

237. Soltani A, Aouadi G, Yangui I, et al. Fumigant toxicity of two Cineole-rich essential oils and their combinations for the control of Carpophilus hemipterus Linnaeus and Sitophilus oryzae Linnaeus. *Ann l'INRGREF*. 2020;21:85-101.

238. Sadraoui-Ajmi I, Benali N, Soltani A, et al. Usage of agricultural DAP-fertilizer and Eucalyptus essential oils as potential attractants against the mediterranean fruit fly Ceratitis capitata (Tephritidae). *J Asia Pac Entomol*. 2022;25(1). doi:10.1016/j.aspen.2021.101857

239. Ahmadi E, Khajehali J, Jonckheere W, Van Leeuwen T. Biochemical and insecticidal effects of plant essential oils on insecticide resistant and susceptible populations of Musca domestica L. point to a potential cross-resistance risk. *Pestic Biochem Physiol*. 2022;184(May):105115. doi:10.1016/j.pestbp.2022.105115

240. Mann TS, Kiran Babu GD, Guleria S, Singh B. Comparison of Eucalyptus cinerea essential oils produced by hydrodistillation and supercritical carbon dioxide extraction. *Nat Prod Commun*. 2011;6(1):107-110. doi:10.1177/1934578x1100600125

241. Alzogaray RA, Lucia A, Zerba EN, Masuh HM. Insecticidal activity of essential oils from eleven Eucalyptus spp. and two hybrids: Lethal and sublethal effects of their major components on Blattella germanica. *J Econ Entomol*. 2011;104(2):595-600. doi:10.1603/EC10045

242. Palacios SM, Bertoni A, Rossi Y, Santander R, Urzúa A. Efficacy of essential oils from edible plants as insecticides against the house fly, musca domestica L. *Molecules*. 2009;14(5):1938-1947. doi:10.3390/molecules14051938

243. Silva SM, Abe SY, Murakami FS, Frensch G, Marques FA, Nakashima T. Essential oils from different plant parts of Eucalyptus cinerea F. Muell. ex Benth. (Myrtaceae) as a source of 1,8-Cineole and their bioactivities. *Pharmaceuticals*. 2011;4(12):1535-1550. doi:10.3390/ph4121535

244. Filomeno CA, Barbosa LCA, Teixeira RR, et al. Corymbia spp. and Eucalyptus spp. essential oils have insecticidal activity against Plutella xylostella. *Ind Crops Prod*. 2017;109(May):374-383. doi:10.1016/j.indcrop.2017.08.033

245. Heikal AAEM. Variation in the Essential Oil Content and its Composition in Eucalyptus cinerea Leaves and its Relation to Some Environmental Factors. *J Essent Oil-Bearing Plants*. 2017;20(4):995-1005. doi:10.1080/0972060X.2017.1351896

246. Maleknia SD, Vail TM, Cody RB, Sparkman DO, Bell TL, Adams MA. Temperature-dependent release of volatile organic compounds of eucalypts by direct analysis in real time (DART) mass spectrometry. *Rapid Commun Mass Spectrom*. 2009;24:1457-1466. doi:10.1002/rcm

247. Lee BH, Annis PC, Tumaalii F, Choi WS. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *J Stored Prod Res*. 2004;40(5):553-564. doi:10.1016/j.jspr.2003.09.001

248. Zhou L, Li J, Kong Q, et al. Chemical composition, antioxidant, antimicrobial, and phytotoxic potential of Eucalyptus grandis × E. urophylla leaves essential oils. *Molecules*. 2021;26(5). doi:10.3390/molecules26051450

249. Silva ER, Igartuburu JM, Overbeck GE, Soares GL, Macias FA. Are phytotoxic effects of Eucalyptus saligna (Myrtaceae) essential oil related to its major compounds? *Aust J Bot*. 2021;69:174-183.

250. Qiu X, YU S, Wang Y, Fang B, Cai C, Li S. Identification and allelopathic effects of 1,8-cineole from Eucalyptus urophylla on lettuce. *Allelopath J*. 2010;(26):255–264.

251. Zhou S, Wei C, Zhang C, Han C, Kuchkarova N, Shao H. Chemical composition, phytotoxic, antimicrobial and insecticidal activity of the essential oils of dracocephalum integrifolium. *Toxins (Basel)*. 2019;11(10):1-19. doi:10.3390/toxins11100598

252. Romagni JG, Duke SO, Dayan FE. Inhibition of plant asparagine synthetase by monoterpene cineoles. *Plant Physiol*. 2000;123(2):725-732. doi:10.1104/pp.123.2.725

253. De Martino L, Mancini E, De Almeida LFR, De Feo V. The antigerminative activity of twenty-seven monoterpenes. *Molecules*. 2010;15(9):6630-6637. doi:10.3390/molecules15096630

254. Barton AFM, Dell B, Knight AR. Herbicidal activity of cineole derivatives. *J Agric Food Chem*. 2010;58(18):10147-10155. doi:10.1021/jf101827v

255. Ramezani S, Saharkhiz MJ, Ramezani F, Fotokian MH. Use of essential oils as bioherbicides. *J Essent Oil-Bearing Plants*. 2008;11(3):319-327. doi:10.1080/0972060X.2008.10643636

256. Kohli RK, Batish DR, Singh HP. Eucalypt oils for the control of Parthenium (Parthenium hysterophorus L.). *Crop Prot*. 1998;17(2):119-122. doi:10.1016/S0261-2194(97)00095-1

257. Kaur S, Singh HP, Batish DR, Kohli RK. Chemical characterization and allelopathic potential of volatile oil of Eucalyptus tereticornis against Amaranthus viridis. *J Plant Interact*. 2011;6(4):297-302. doi:10.1080/17429145.2010.539709

258. Kohli RK, Singh D. Allelopathic impact of volatile components from Eucalyptus on crop plants. *Biol Plant*. 1991;33(6):475-483. doi:10.1007/BF02897723

259. Singh D, Kohli RK, Saxena DB. Effect of eucalyptus oil on germination and growth of Phaseolus aureus Roxb. *Plant Soil*. 1991;137(2):223-227. doi:10.1007/BF00011200

260. Miguel M, Gago C, Antunes M, et al. Antibacterial, Antioxidant, and Antiproliferative Activities of Corymbia citriodora and the Essential Oils of Eight Eucalyptus Species. *Medicines*. 2018;5(3):61. doi:10.3390/medicines5030061

261. Elaissi A, Salah KH, Mabrouk S, Larbi KM, Chemli R, Harzallah-Skhiri F. Antibacterial activity and chemical composition of 20 Eucalyptus species' essential oils. *Food Chem*. 2011;129(4):1427-1434. doi:10.1016/j.foodchem.2011.05.100

262. Aleksic Sabo V, Knezevic P. Antimicrobial activity of Eucalyptus camaldulensis Dehn. plant extracts and essential oils: A review. *Ind Crops Prod*. 2019;132(November 2018):413-429. doi:10.1016/j.indcrop.2019.02.051

263. Rivas da Silva AC, Lopes PM, Barros de Azevedo MM, Costa DC, Alviano CS, Alviano DS. Biological activities of α-pinene and β-pinene enantiomers. *Molecules*. 2012;17(6):6305–6316. doi:10.3390/molecules17066305

264. Cordeiro L, Figueiredo P, Souza H, et al. Terpinen-4-ol as an antibacterial and antibiofilm agent against staphylococcus aureus. *Int J Mol Sci*. 2020;21(12):1-14. doi:10.3390/ijms21124531

265. Sales A. Production, Properties, and Applications of α-Terpineol. Published online 2020:1261-1279.

266. Guimarães AC, Meireles LM, Lemos MF, et al. Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*. 2019;24(13):1-12. doi:10.3390/molecules24132471

267. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines*. 2017;4(3):58. doi:10.3390/medicines4030058

268. Hąc-Wydro K, Flasiński M, Broniatowski M, Sołtys M. Studies on the Behavior of Eucalyptol and Terpinen-4-ol - Natural Food Additives and Ecological Pesticides - In Model Lipid Membranes. *Langmuir*. 2017;33(27):6916-6924. doi:10.1021/acs.langmuir.7b00774

269. Połeć K, Wójcik A, Flasiński M, Wydro P, Broniatowski M, Hąc-Wydro K. The influence of terpinen-4-ol and eucalyptol – The essential oil components - on fungi and plant sterol monolayers. *Biochim Biophys Acta - Biomembr*. 2019;1861(6):1093-1102. doi:10.1016/j.bbamem.2019.03.015

270. Naithani HB. Botany of Genus Eucalyptus. In: Bhojvaid PP, Kaushik S, Singh YP, Kumar D, Thapliyal M, S. B, eds. *Eucalyptus in India*. ; 2014:1-20.

271. Myburg AA, Potts BM, Marques CM, Kirst M, Gion J, Grima-pettenatti J. Eucalypts. In: Kole C, ed. Vol 7. Springer-Verlag GmbH; 2007.

272. Parra-O C, Bayly M, Udovicic F, Ladiges P. ETS sequences support the monophyly of the eucalypt genus Corymbia (Myrtaceae). *Taxon*. 2006;55(3):653-663. doi:10.2307/25065641

273. Steane DA, Nicolle D, Sansaloni CP, et al. Population genetic analysis and phylogeny reconstruction in Eucalyptus (Myrtaceae) using high-throughput, genome-wide genotyping. *Mol Phylogenet Evol*. 2011;59(1):206-224. doi:10.1016/j.ympev.2011.02.003

274. Hager T, Benson D. The Eucalypts of the Greater Blue Mountains World Heritage Area: distribution, classification and

habitats of the species of Eucalyptus, Angophora and Corymbia (family Myrtaceae) recorded in its eight conservation reserves. *Cunninghamia a J plant Ecol East Aust*. 2010;11(4):425-444. https://bmnature.info/docs/documents/Cun114425Hag.pdf

275. Nicolle D. Classification of the Eucalypts (Angophora, Corymbia and Eucalytpus). 2021;Version 5:59. https://www.dn.com.au/Classification-of-the-Eucalypts.html

276. Rozefelds A. Eucalyptus phylogeny and history: a brief summary. *Tasforests-Hobart*. 1996;8(December):15-26. http://www.forestrytas.com.au/assets/0000/0377/vol_8_pages15-26.pdf

277. Trabado GI, Wilstermann D. Eucalyptus Global Map 2008: cultivated forests worldwide - EUCALYPTOLOGICS: GIT Forestry Consulting Information Resources on Eucalyptus Cultivation Worldwide. Published 2008. Accessed July 4, 2022. http://git-forestry-blog.blogspot.com/2008/09/eucalyptus-global-map-2008-cultivated.html

278. Zhang YX, Wang XJ. Geographical spatial distribution and productivity dynamic change of eucalyptus plantations in China. *Sci Rep*. 2021;11(1):1-15. doi:10.1038/s41598-021-97089-7

279. Polito F, Fratianni F, Nazzaro F, et al. Essential Oil Composition, Antioxidant Activity and Leaf Micromorphology of Five Tunisian Eucalyptus Species. *Antioxidants*. 2023;12(4). doi:10.3390/antiox12040867

280. Salehi B, Sharifi-Rad J, Quispe C, et al. Insights into Eucalyptus genus chemical constituents, biological activities and health-promoting effects. *Trends Food Sci Technol*. 2019;91(June):609-624. doi:10.1016/j.tifs.2019.08.003

281. Benelli G, Pavela R, Canale A, Mehlhorn H. Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? *Parasitol Res*. 2016;115(7):2545-2560. doi:10.1007/s00436-016-5095-1

282. Pavela R, Benelli G. Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors - A review. *Exp Parasitol*. 2016;167:103-108. doi:10.1016/j.exppara.2016.05.010

283. Benelli G, Pavela R. Beyond mosquitoes—Essential oil toxicity and repellency against bloodsucking insects. *Ind Crops Prod*. 2018;117(February):382-392. doi:10.1016/j.indcrop.2018.02.072

284. Manda RR, Addanki VA, Srivastava S. Microbial bio-pesticides and botanicals as an alternative to synthetic pesticides in the sustainable agricultural production. *Plant Cell Biotechnol Mol Biol*. 2020;21(61-62):31-48.

285. Diaz JH. Chemical and plant-based insect repellents: Efficacy, safety, and toxicity. *Wilderness Environ Med*. 2016;27(1):153-163. doi:10.1016/j.wem.2015.11.007

286. Nguyen QBD, Vu MAN, Hebert AA. Insect repellents: An updated review for the clinician. *J Am Acad Dermatol*. 2023;88(1):123-130. doi:10.1016/j.jaad.2018.10.053

287. Venkat Reddy A, Anusha C, Yashaswini C, Manasa K, Ramesh T. Botanical Insecticides: A Major Alternative to Synthetic Insecticides in Insect Pest Management. In: *Advances in Agricultural Entomology*. AkiNik Publications; 2020:37-60.

288. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol*. 2006;51(May):45-66. doi:10.1146/annurev.ento.51.110104.151146

289. Isman MB. Pesticides based on plant essential oils: Phytochemical and practical considerations. *ACS Symp Ser*. 2006;1218(January 2006):13-26. doi:10.1021/bk-2016-1218.ch002

290. Koul O, Walia S, Dhaliawal G. Essential Oils as Green Pesticides : Potential and Constraints. *Biopestic Int*. 2008;4(1):63-84.

291. Vecchio MG, Loganes C, Minto C. Beneficial and Healthy Properties of Eucalyptus Plants: A Great Potential Use. *Open Agric J*. 2016;10(1):52-57. doi:10.2174/1874331501610010052

292. Zhang J, An M, Wu H, Stanton R, Lemerle D. Chemistry and Bioactivity of Eucalyptus Essential Oils. *Allelopath J*. 2010;25(2):313-330.

293. Cerasoli S. Eucalyptus globulus and other eucalypts in Europe: distribution, habitat, usage and threats. 2016;(September).

294. Danna C, Malaspina P, Cornara L, et al. Eucalyptus essential oils in pest control: a review of chemical composition and applications against insects and mites. *Crop Prot*. 2023;176(April). doi:10.1016/j.cropro.2023.106319

295. Carson CF, Hammer KA. Chemistry and Bioactivity of Essential Oils. In: Thormar H, ed. ; 2010.

296. Southwell I. Eucalyptus leaf oils: Use, chemistry, distillation and marketing. *Phytochemistry*. 1992;31(6):2193. doi:10.1016/0031-9422(92)80403-2

297. Pandey A, Chattopadhyay P, Banerjee S, Pakshirajan K, Singh L. Antitermitic activity of plant essential oils and their major constituents against termite Odontotermes assamensis Holmgren (Isoptera: Termitidae) of North East India. *Int Biodeterior Biodegrad*. 2012;75:63-67. doi:10.1016/j.ibiod.2012.09.004

298. Slimane B Ben, Ezzine O, Dhahri S, Ben Jamaa ML. Essential oils from two Eucalyptus from Tunisia and their insecticidal action on Orgyia trigotephras (Lepidotera, Lymantriidae). *Biol Res*. 2014;47(1):1-8. doi:10.1186/0717-6287-47-29

299. Batista-Pereira LG, Fernandes JB., Silva MFGF da;, Vieira PC., Bueno OC., Corrêa AG. Electrophysiological Responses of Atta sexdens rubropilosa Workers. *Z Naturforsch*. 2006;61c:749-755.

300. Lucia A, Audino PG, Seccacini E, Licastro S, Zerba E, Masuh H. Larvicidal effect of Eucalyptus grandis essential oil and turpentine and their major components on aedes Aegypti larvae. *J Am Mosq Control Assoc*. 2007;23(3):299-303. doi:10.2987/8756-971X(2007)23[299:LEOEGE]2.0.CO;2

301. Toloza AC, Lucia A, Zerba E, Masuh H, Picollo MI. Interspecific hybridization of Eucalyptus as a potential tool to improve the bioactivity of essential oils against permethrin-resistant head lice from Argentina. *Bioresour Technol*. 2008;99(15):7341-7347. doi:10.1016/j.biortech.2007.12.067

302. Filomeno CA, Almeida Barbosa LC, Teixeira RR, et al. Chemical diversity of essential oils of Myrtaceae species and their insecticidal activity against Rhyzopertha dominica. *Crop Prot*. 2020;137(January). doi:10.1016/j.cropro.2020.105309

303. Cruz GS, Wanderley-Teixeira V, Oliveira J V., et al. Sublethal effects of essential oils from eucalyptus staigeriana (Myrtales: Myrtaceae), Ocimum gratissimum (Lamiales: Laminaceae), and foeniculum vulgare (Apiales: Apiaceae) on the biology of spodoptera frugiperda (Lepidoptera: Noctuidae). *J Econ Entomol*. 2016;109(2):660-666. doi:10.1093/jee/tow005

304. Gusmão NMS, de Oliveira J V., Navarro DM d. AF, Dutra KA, da Silva WA, Wanderley MJA. Contact and fumigant toxicity and repellency of Eucalyptus citriodora Hook., Eucalyptus staigeriana F., Cymbopogon winterianus Jowitt and Foeniculum vulgare Mill. essential oils in the management of Callosobruchus maculatus (FABR.) (Coleoptera: Chrysomeli. *J Stored Prod Res*. 2013;54:41-47. doi:10.1016/j.jspr.2013.02.002

305. Juan LW, Lucia A, Zerba EN, Harrand L, Marco M, Masuh HM. Chemical composition and fumigant toxicity of the essential oils from 16 species of Eucalyptus against Haematobia irritans (Diptera: Muscidae) adults. *J Econ Entomol*. 2011;104(3):1087-1092. doi:10.1603/EC09431

306. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules*. 2012;17(4):3989-4006. doi:10.3390/molecules17043989

307. Hikal WM, Baeshen RS, Said-Al Ahl HAH. Botanical insecticide as simple extractives for pest control. *Cogent Biol*. 2017;3(1):1404274. doi:10.1080/23312025.2017.1404274

308. Khursheed A, Rather MA, Jain V, et al. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. *Microb Pathog*. 2022;173(PA):105854. doi:10.1016/j.micpath.2022.105854

309. Giunti G, Benelli G, Palmeri V, et al. Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. *Biol Control*. 2022;176(October):105071. doi:10.1016/j.biocontrol.2022.105071

310. Pineda M, Alves EL de A, Antunes JA, Carvalho V de C, Haddi K. Low Concentrations of Eucalyptus Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in Drosophila suzukii. *Agric*. 2023;13(2). doi:10.3390/agriculture13020404

311. *Eucalyptus Oil Market Report, 2028*. https://www.gminsights.com/industry-analysis/eucalyptus-oil-market

312. Wendimu A, Tekalign W. Field efficacy of ethnomedicinal plant smoke repellency against Anopheles arabiensis and Aedes aegypti. *Heliyon*. 2021;7(7):e07373. doi:10.1016/j.heliyon.2021.e07373

313. Krzyżowski M, Baran B, Łozowski B, Francikowski J. The role of dilution mediums in studies of fumigant insecticidal activity of essential oils. *J Pest Sci (2004)*. 2020;93(4):1119-1124. doi:10.1007/s10340-020-01241-7

314. Adak T, Barik N, Patil NB, et al. Nanoemulsion of eucalyptus oil: An alternative to synthetic pesticides against two major storage insects (Sitophilus oryzae (L.) and Tribolium castaneum (Herbst)) of rice. *Ind Crops Prod*. 2020;143(September 2019):111849. doi:10.1016/j.indcrop.2019.111849

315. Galli GM, Roza LF, Santos RCV, et al. Low Dose of Nanocapsules Containing Eucalyptus Oil Has Beneficial Repellent Effect Against Horn Fly (Diptera: Muscidae). *J Econ Entomol*. 2018;111(6):2983-2987. doi:10.1093/jee/toy267

316. Lucia A, Toloza AC, Guzmán E, Ortega F, Rubio RG. Novel polymeric micelles for insect pest control: Encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control. *PeerJ*. 2017;2017(4):1-19. doi:10.7717/peerj.3171

317. Pavoni L, Pavela R, Cespi M, et al. Green micro-and nanoemulsions for managing parasites, vectors and pests. *Nanomaterials*. 2019;9(9). doi:10.3390/nano9091285

318. Velho MC, Cossetin LF, Godoi SN de, et al. Nanobiopesticides: development and inseticidal activity of nanoemulsions containing lemongrass or eucalyptus oils. *Nat Prod Res*. 2021;35(24):6210-6215. doi:10.1080/14786419.2020.1837809

319. Rani N, Duhan A, Pal A, et al. Are nano-pesticides really meant for cleaner production? An overview on recent developments, benefits, environmental hazards and future prospectives. *J Clean Prod*. 2023;411:137232. doi:10.1016/j.jclepro.2023.137232

320. Yang YC, Choi HY, Choi WS, Clark JM, Ahn YJ. Ovicidal and Adulticidal Activity of Eucalyptus globulus Leaf Oil Terpenoids against Pediculus humanus capitis (Anoplura: Pediculidae). *J Agric Food Chem*. 2004;52(9):2507-2511. doi:10.1021/jf0354803

321. Avello L M, Fernández P, Fernández M, et al. Pediculicide effect of a Eucaliptus globulus L formulation. *Rev Chil Infectol*. 2016;33(4):433-437.

322. Yones DA, Bakir HY, Bayoumi SAL. Chemical composition and efficacy of some selected plant oils against Pediculus humanus capitis in vitro. *Parasitol Res*. 2016;115(8):3209-3218. doi:10.1007/s00436-016-5083-5

323. Soonwera M, Wongnet O, Sittichok S. Ovicidal effect of essential oils from Zingiberaceae plants and Eucalytus globulus on eggs of head lice, Pediculus humanus capitis De Geer. *Phytomedicine*. 2018;47(April):93-104. doi:10.1016/j.phymed.2018.04.050

324. Toloza AC, Lucía A, Zerba E, Masuh H, Picollo MI. Eucalyptus essential oil toxicity against permethrin-resistant Pediculus humanus capitis (Phthiraptera: Pediculidae). *Parasitol Res*. 2010;106(2):409-414. doi:10.1007/s00436-009-1676-6

325. Sharififard M, Safdari F, Siahpoush A, Kassiri H. Evaluation of some plant essential oils against the brown-banded cockroach, Supella longipalpa (Blattaria: Ectobiidae): A mechanical vector of human pathogens. *J Arthropod Borne Dis*. 2016;10(4):528-537.

326. Rezaei M, Khaghani R, Moharramipour S. Insecticidal activity of Artemisia sieberi, Eucalyptus camaldulensis, Thymus persicus and Eruca sativa oils against German cockroach, Blattella germanica (L.). *J Asia Pac Entomol*. 2019;22(4):1090-1097. doi:10.1016/j.aspen.2019.08.013

327. Hamdi SH, Hedjal-Chebheb M, Kellouche A, Khouja ML, Boudabous A, Ben Jemâa JM. Management of three pests' population strains from Tunisia and Algeria using Eucalyptus essential oils. *Ind Crops Prod*. 2015;74:551-556. doi:10.1016/j.indcrop.2015.05.072

328. Aref SP, Valizadegan O, Farashiani ME. Eucalyptus dundasii Maiden essential oil, chemical composition and insecticidal values against Rhyzopertha dominica (F.) and Oryzaephilus surinamensis (L.). *J Plant Prot Res*. 2015;55(1):35-41. doi:10.1515/jppr-2015-0005

329. Ebadollahi A, Naseri B, Abedi Z, Setzer WN, Changbunjong T. Promising Insecticidal Efficiency of Essential Oils Isolated from Four Cultivated Eucalyptus Species in Iran against the Lesser Grain Borer, Rhyzopertha dominica (F.). *Insects*. 2022;13(6). doi:10.3390/insects13060517

330. Papachristos DP, Stamopoulos DC. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae). *J Stored Prod Res*. 2001;38(2):117-128. doi:10.1016/S0022-474X(01)00007-8

331. Bett PK, Deng AL, Ogendo JO, et al. Chemical composition of Cupressus lusitanica and Eucalyptus saligna leaf essential oils and bioactivity against major insect pests of stored food grains. *Ind Crops Prod*. 2016;82:51-62. doi:10.1016/j.indcrop.2015.12.009

332. Ebadollahi A, Sendi JJ, Setzer WN, Changbunjong T. Encapsulation of Eucalyptus largiflorens Essential Oil by Mesoporous Silicates for Effective Control of the Cowpea Weevil, Callosobruchus maculatus (Fabricius) (Coleoptera: Chrysomelidae). *Molecules*. 2022;27(11). doi:10.3390/molecules27113531

333. Negahban M, Moharramipour S. Fumigant toxicity of Eucalyptus intertexta, Eucalyptus sargentii and Eucalyptus camaldulensis against stored-product beetles. *J Appl Entomol*. 2007;131(4):256-261. doi:10.1111/j.1439-0418.2007.01152.x

334. Izakmehri K, Saber M, Mehrvar A, Hassanpouraghdam MB, Vojoudi S. Lethal and sublethal effects of essential oils from Eucalyptus camaldulensis and Heracleum persicum against the adults of callosobruchus maculatus. *J Insect Sci*. 2013;13:1-10. doi:10.1673/031.013.15201

335. Amiri A, Bandani AR. Callosobruchus embryo struggle to guarantee progeny production. *Sci Rep*. 2020;10(1):1-9. doi:10.1038/s41598-020-70178-9

336. Fouad HA. Effect of Five Essential Oils as Repellents against the Cowpea Beetle , Callosobruchus maculates (F .). *Bull Enviroonment, PPharmacology Life Sci*. 2013;2(April):23-27.

337. Ya-Ali P, Yarahmadi F, Mehrnia MA. Efficacies of Two Nano-Formulations of Tasmanian Blue Gum Essential Oil to Control Callosobruchus maculatus. *J Econ Entomol*. 2020;113(3):1555-1562. doi:10.1093/jee/toaa069

338. Harish G, Nataraja M V., Holajjer P, et al. Efficacy and insecticidal properties of some essential oils against Caryedon serratus (Oliver)—a storage pest of groundnut. *J Food Sci Technol*. 2014;51(11):3505-3509. doi:10.1007/s13197-012-0877-7

339. Demeter S, Lebbe O, Hecq F, et al. Insecticidal activity of 25 essential oils on the stored product pest, sitophilus granarius. *Foods*. 2021;10(2):1-13. doi:10.3390/foods10020200

340. Song JE, Kim JM, Lee NH, Yang JY, Lee HS. Acaricidal and insecticidal activities of essential oils against a stored-food mite and stored-grain insects. *J Food Prot*. 2016;79(1):174-178. doi:10.4315/0362-028X.JFP-15-109

341. Patil NB, Adak T, Pandi GGP, Gowda GB, Jena M. Ecofriendly approach for rice weevil (Sitophilus oryzae) (Coleoptera : Curculionidae) management using fumigant oils. Published online 2016:16-21.

342. Lee BH, Choi WS, Lee SE, Park BS. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, Sitophilus oryzae (L.). *Crop Prot*. 2001;20(4):317-320. doi:10.1016/S0261-2194(00)00158-7

343. Mossi AJ, Astolfi V, Kubiak G, et al. Insecticidal and repellency activity of essential oil of Eucalyptus sp. against Sitophilus zeamais Motschulsky (Coleoptera, Curculionidae). *J Sci Food Agric*. 2011;91(2):273-277. doi:10.1002/jsfa.4181

344. Karimi Karemu C, Ndung'U MW, Githua M. Repellent effects of essential oils from selected eucalyptus species and their major constituents against Sitophilus zeamais (Coleoptera: Curculionidae). *Int J Trop Insect Sci*. 2013;33(3):188-194. doi:10.1017/S1742758413000179

345. Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C. Bioactivities of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against Sitophilus zeamais Motschulsky and Tribolium confusum du Val. *J Stored Prod Res*. 2005;41(1):91-102. doi:10.1016/j.jspr.2004.01.004

346. Reyes EIM, Farias ES, Silva EMP, et al. Eucalyptus resinifera essential oils have fumigant and repellent action against Hypothenemus hampei. *Crop Prot*. 2019;116(May 2018):49-55. doi:10.1016/j.cropro.2018.09.018

347. Abd El-Salam AME, Salem SA elwahed, Abdel-Rahman RS. Fumigant and toxic activity of some aromatic oils for protecting dry dates from Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) in stores. *Bull Natl Res Cent*. 2019;43(1). doi:10.1186/s42269-019-0101-2

348. Nattudurai G, Paulraj MG, Ignacimuthu S. Fumigant toxicity of volatile synthetic compounds and natural oils against red flour beetle Tribolium castaneum (Herbst) (Coleopetera: Tenebrionidae). *J King Saud Univ - Sci*. 2012;24(2):153-159. doi:10.1016/j.jksus.2010.11.002

349. Pant M, Dubey S, Patanjali PK, Naik SN, Sharma S. Insecticidal activity of eucalyptus oil nanoemulsion with karanja and jatropha aqueous filtrates. *Int Biodeterior Biodegrad*. 2014;91:119-127. doi:10.1016/j.ibiod.2013.11.019

350. Mangang IB, Manickam L. Insect repellent pellets – an application of botanicals against red flour beetle – their antifungal activity during storage and use as potential fumigants. *J Sci Food Agric*. 2022;(May). doi:10.1002/jsfa.12037

351. Oviedo-Sarmiento JS, Bustos Cortes JJ, Delgado Ávila WA, et al. Fumigant toxicity and biochemical effects of selected essential oils toward the red flour beetle, Tribolium castaneum (Coleoptera: Tenebrionidae). *Pestic Biochem Physiol*. 2021;179(March). doi:10.1016/j.pestbp.2021.104941

352. Tunç I, Berger BM, Erler F, Dagli F. Ovicidal activity of essential oils from five plants against two stored-product insects. *J Stored Prod Res*. 2000;36(2):161-168. doi:10.1016/S0022-474X(99)00036-3

353. Russo S, Cabrera N, Chludil H, Yaber-Grass M, Leicach S. Insecticidal activity of young and mature leaves essential oil from eucalyptus globulus labill. Against tribolium confusum Jacquelin du val (Coleoptera: Tenebrionidae). *Chil J Agric Res*. 2015;75(3):375-379. doi:10.4067/S0718-58392015000400015

354. Ainane A, Khammour, Charaf S, et al. Chemical composition and insecticidal activity of five essential oils: Cedrus atlantica, Citrus limonum, Rosmarinus officinalis, Syzygium aromaticum and Eucalyptus globules. *Mater Today Proc*. 2019;13:474-485. doi:10.1016/j.matpr.2019.04.004

355. Zaka SM, Iqbal N, Saeed Q, et al. Toxic effects of some insecticides, herbicides, and plant essential oils against Tribolium confusum Jacquelin du val (Insecta: Coleoptera: Tenebrionidae). *Saudi J Biol Sci*. 2019;26(7):1767-1771. doi:10.1016/j.sjbs.2018.05.012

356. Lucia A, Juan LW, Zerba EN, Harrand L, Marcó M, Masuh HM. Validation of models to estimate the fumigant and larvicidal activity of Eucalyptus essential oils against Aedes aegypti (Diptera: Culicidae). *Parasitol Res*. 2012;110(5):1675-1686. doi:10.1007/s00436-011-2685-9

357. Lucia A, Licastro S, Zerba E, Audino PG, Masuh H. Sensitivity of Aedes aegypti adults (Diptera: Culicidae) to the vapors of Eucalyptus essential oils. *Bioresour Technol*. 2009;100(23):6083-6087. doi:10.1016/j.biortech.2009.02.075

358. Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresour Technol*. 2009;100(1):452-456. doi:10.1016/j.biortech.2008.02.038

359. Manh HD, Hue DT, Hieu NTT, Tuyen DTT, Tuyet OT. The mosquito larvicidal activity of essential oils from cymbopogon and eucalyptus species in vietnam. *Insects*. 2020;11(2):1-7. doi:10.3390/insects11020128

360. Cavalca PAM, de Assumpção Lolis MIG, Reis B, Bonato CM. Homeopathic and larvicide effect of eucalyptus cinerea essential oil against Aedes aegypti. *Brazilian Arch Biol Technol*. 2010;53(4):835-843. doi:10.1590/S1516-89132010000400012

361. Ríos N, Stashenko EE, Duque JE. Evaluation of the insecticidal activity of essential oils and their mixtures against Aedes

aegypti (Diptera: Culicidae). *Rev Bras Entomol*. 2017;61(4):307-311. doi:10.1016/j.rbe.2017.08.005

362. Soonwera M, Sittichok S. Adulticidal activities of Cymbopogon citratus (Stapf.) and Eucalyptus globulus (Labill.) essential oils and of their synergistic combinations against Aedes aegypti (L.), Aedes albopictus (Skuse), and Musca domestica (L.). *Environ Sci Pollut Res*. 2020;27(16):20201-20214. doi:10.1007/s11356-020-08529-2

363. de Brito GA, Rocha de Oliveira PF, de Andrade Silva CM, et al. Identification of Bioactive Compounds against Aedes aegypti (Diptera: Culicidae) by Bioassays and in Silico Assays. *Chem Biodivers*. 2021;18(9). doi:10.1002/cbdv.202100242

364. Costa AA, Naspi C V., Lucia A, Masuh HM, Barrera R. Repellent and Larvicidal Activity of the Essential Oil From Eucalyptus nitens Against Aedes aegypti and Aedes albopictus (Diptera: Culicidae). *J Med Entomol*. 2017;54(3):670-676. doi:10.1093/jme/tjw222

365. Costa AA, Gonzalez P V., Harburguer L V., Masuh HM. Effects of Temephos, Permethrin, and Eucalyptus nitens Essential Oil on Survival and Swimming Behavior of Aedes aegypti and Anopheles pseudopunctipennis (Diptera: Culicidae) larvae. *J Med Entomol*. 2018;55(5):1098-1104. doi:10.1093/jme/tjy086

366. Gillij YG, Gleiser RM, Zygadlo JA. Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresour Technol*. 2008;99(7):2507-2515. doi:10.1016/j.biortech.2007.04.066

367. Sheng Z, Jian R, Xie F, et al. Screening of larvicidal activity of 53 essential oils and their synergistic effect for the improvement of deltamethrin efficacy against Aedes albopictus. *Ind Crops Prod*. 2020;145(January):112131. doi:10.1016/j.indcrop.2020.112131

368. Bossou AD, Mangelinckx S, Yedomonhan H, et al. Chemical composition and insecticidal activity of plant essential oils from Benin against Anopheles gambiae (Giles). *Parasites and Vectors*. 2013;6(1). doi:10.1186/1756-3305-6-337

369. Medhi SM, Reza SA, Mahnaz K, et al. Phytochemistry and larvicidal activity of Eucalyptus camaldulensis against malaria vector, Anopheles stephensi. *Asian Pac J Trop Med*. 2010;3(11):841-845. doi:10.1016/S1995-7645(10)60203-9

370. Chauhan N, Malik A, Sharma S, Dhiman RC. Larvicidal potential of essential oils against Musca domestica and Anopheles stephensi. *Parasitol Res*. 2016;115(6):2223-2231. doi:10.1007/s00436-016-4965-x

371. Senthil Nathan S. The use of Eucalyptus tereticornis Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector Anopheles stephensi Liston (Diptera: Culicidae). *Bioresour Technol*. 2007;98(9):1856-1860. doi:10.1016/j.biortech.2006.07.044

372. Elzayyat E, Elleboudy N, Moustafa A, Ammar A. Insecticidal, Oxidative, and Genotoxic Activities of Syzygium aromaticum and Eucalyptus globulus on Culex pipiens Adults and Larvae. *Turkiye parazitolojii Derg*. 2018;42(3):213-222. doi:10.5152/tpd.2018.5626

373. Erler F, Ulug I, Yalcinkaya B. Repellent activity of five essential oils against Culex pipiens. *Fitoterapia*. 2006;77(7-8):491-494. doi:10.1016/j.fitote.2006.05.028

374. Tian Y, Dong F, Zhou X, Yang X. Repellent, Insecticidal and Antimicrobial Activities of Leaf Essential Oils from Three Eucalyptus Species. *Chem Biodivers*. 2020;17(2). doi:10.1002/cbdv.201900580

375. Sugumar S, Clarke SK, Nirmala MJ, Tyagi BK, Mukherjee A, Chandrasekaran N. Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. *Bull Entomol Res*. 2014;104(3):393-402. doi:10.1017/S0007485313000710

376. Yuan L, Yang X, Yu X, Wu Y, Jiang D. Resistance to insecticides and synergistic and antagonistic effects of essential oils on dimefluthrin toxicity in a field population of Culex quinquefasciatus Say. *Ecotoxicol Environ Saf*. 2019;169(November 2018):928-936. doi:10.1016/j.ecoenv.2018.11.115

377. Gemeda N, Mokonnen W, Lemma H, et al. Insecticidal activity of some traditionally used Ethiopian medicinal plants against sheep ked Melophagus ovinus. *J Parasitol Res*. 2014;2014. doi:10.1155/2014/978537

378. Kumar P, Mishra S, Malik A, Satya S. Compositional analysis and insecticidal activity of Eucalyptus globulus (family: Myrtaceae) essential oil against housefly (Musca domestica). *Acta Trop*. 2012;122(2):212-218. doi:10.1016/j.actatropica.2012.01.015

379. Khan HAA. Toxicity, repellent and oviposition deterrent effects of select essential oils against the house fly Musca domestica. *J Asia Pac Entomol*. 2021;24(1):15-20. doi:10.1016/j.aspen.2020.10.002

380. Rossi YE, Palacios SM. Insecticidal toxicity of Eucalyptus cinerea essential oil and 1,8-cineole against Musca domestica and possible uses according to the metabolic response of flies. *Ind Crops Prod*. 2015;63:133-137. doi:10.1016/j.indcrop.2014.10.019

381. Sinthusiri J, Soonwera M. Oviposition deterrent and ovicidal activities of seven herbal essential oils against female adults of housefly, Musca domestica L. *Parasitol Res*. 2014;113(8):3015-3022. doi:10.1007/s00436-014-3964-z

382. Maciel M V., Morais SM, Bevilaqua CML, et al. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. *Vet Parasitol*. 2010;167(1):1-7. doi:10.1016/j.vetpar.2009.09.053

383. Russo S, Yaber Grass MA, Fontana HC, Leonelli E. Insecticidal activity of essential oil from eucalyptus globulus against aphis nerii (Boyer) and gynaikothrips ficorum (Marchal). *AgriScientia*. 2018;35(1):63-67. doi:10.31047/1668.298x.v1.n35.20458

384. Ahmed Q, Agarwal M, Al-Obaidi R, Wang P, Ren Y. Evaluation of Aphicidal Effect of Essential Oils and Their Synergistic Effect against Myzus persicae (Sulzer) (Hemiptera: Aphididae). *Molecules*. 2021;26(3055). https://doi.org/10.3390/molecules26103055

385. Sharma A, Kaur I. By-product hydrosol of Eucalyptus globulus essential oil distillation as source of botanical insecticides: wealth from waste. *Not Sci Biol*. 2021;13(1).

386. Gomes SP, Favero S. Assessment of the Insecticidal Potential of Eucalyptus urograndis Essential Oil Against Rhodnius neglectus Lent (Hemiptera: Reduviidae). *Neotrop Entomol*. 2013;42(4):431-435. doi:10.1007/s13744-013-0144-2

387. Ribeiro AV, Farias E de S, Santos AA, et al. Selection of an essential oil from Corymbia and Eucalyptus plants against Ascia monuste and its selectivity to two non-target organisms. *Crop Prot*. 2018;110:207-213. doi:10.1016/j.cropro.2017.08.014

388. Szelényi MO, Erdei AL, Jósvai JK, et al. Box Tree Moth (Cydalima perspectalis) and Behaviour. *Insects*. 2020;11(465). doi:10.3390/insects11080465

389. Kobenan KC, Ochou GEC, Kouadio IS, et al. Chemical Composition, Antioxidant Activity, Cholinesterase Inhibitor and in Vitro Insecticidal Potentiality of Essential Oils of Lippia multiflora Moldenke and Eucalyptus globulus Labill. on the Main Carpophagous Pests of Cotton Plant in Ivory Coast. *Chem Biodivers*. 2022;19(4). doi:10.1002/cbdv.202100993

390. Batista-Pereira LG, Fernandes JB, Corrêa AG, Da Silva MFGF, Vieira PC. Electrophysiological responses of eucalyptus brown looper Thyrinteina arnobia to essential oils of seven Eucalyptus species. *J Braz Chem Soc*. 2006;17(3):555-561. doi:10.1590/S0103-50532006000300019

391. Boulamtat R, Mesfioui A, El-Fakhouri K, et al. Chemical composition, and insecticidal activities of four plant essential oils from Morocco against larvae of Helicoverpa armigera (Hub.) under field and laboratory conditions. *Crop Prot*. 2021;144(October 2020):105607. doi:10.1016/j.cropro.2021.105607

392. Ben Jemâa JM, Haouel S, Bouaziz M, Khouja ML. Seasonal variations in chemical composition and fumigant activity of five Eucalyptus essential oils against three moth pests of stored dates in Tunisia. *J Stored Prod Res*. 2012;48:61-67. doi:10.1016/j.jspr.2011.10.001

393. Mediouni Ben Jemâa J, Haouel S, Khouja ML. Efficacy of Eucalyptus essential oils fumigant control against Ectomyelois ceratoniae (Lepidoptera: Pyralidae) under various space occupation conditions. *J Stored Prod Res*. 2013;53:67-71. doi:10.1016/j.jspr.2013.02.007

394. Jesser EN, Werdin-González JO, Murray AP, Ferrero AA. Efficacy of essential oils to control the Indian meal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae). *J Asia Pac Entomol*. 2017;20(4):1122-1129. doi:10.1016/j.aspen.2017.08.004

395. Alitonou G, Avlessi F, Wotto VD, Ahoussi E, Dangou J, Sohounhloué DCK. Composition chimique, propriétés antimicrobiennes et activités sur les tiques de l'huile essentielle d'Eucalyptus tereticornis Sm. *Comptes Rendus Chim*. 2004;7(10-11):1051-1055. doi:10.1016/j.crci.2003.11.014

396. Hatem AN, AL-Asadi SAM. Evaluation of toxicity of Eucalyptus cammadelulensis essential oil in the hard tick Hyalommaanatolicum (Acari: Ixodidae) infested livestock in Basrah province, Iraq. *Ann Trop Med Public Heal*. 2020;23(13). doi:10.36295/ASRO.2020.231379

397. Pirali-Kheirabadi K, Razzaghi-Abyaneh M, Halajian A. Acaricidal effect of Pelargonium roseum and Eucalyptus globulus essential oils against adult stage of Rhipicephalus (Boophilus) annulatus in vitro. *Vet Parasitol*. 2009;162(3-4):346-349. doi:10.1016/j.vetpar.2009.03.015

398. Adenubi OT, Abolaji AO, Salihu T, Akande FA, Lawal H. Chemical composition and acaricidal activity of Eucalyptus globulus essential oil against the vector of tropical bovine piroplasmosis, Rhipicephalus (Boophilus) annulatus. *Exp Appl Acarol*. 2021;83(2):301-312. doi:10.1007/s10493-020-00578-z

399. Arafa WM, Aboelhadid SM, Moawad A, Shokeir KM, Ahmed O, Pérez de León AA. Control of Rhipicephalus annulatus resistant to deltamethrin by spraying infested cattle with synergistic eucalyptus essential oil-thymol-deltamethrin combination. *Vet Parasitol*. 2021;290(December 2020). doi:10.1016/j.vetpar.2021.109346

400. Madreseh-Ghahfarokhi S, Dehghani-Samani A, Pirali Y, Dehghani-Samani A. Zingiber officinalis and Eucalyptus globulus, potent lethal/repellent agents against Rhipicephalus bursa, probable carrier for zoonosis. *J Arthropod Borne Dis*. 2019;13(2):214-223. doi:10.18502/jad.v13i2.1248

401. Chagas AC de S, Passos WM, Prates HT, Leite RC, Furlong J, Fortes ICP. Efeito acaricida de óleos essenciais e concentrados emulsionáveis de Eucalyptus spp em Boophilus microplus. *Brazilian J Vet Res Anim Sci*. 2002;39(5). doi:10.1590/s1413-95962002000500006

402. Rodrigues L, Giglioti R, Gomes ACP, et al. In Vitro Effect of Volatile Substances from Eucalyptus Oils on Rhipicephalus microplus. *Rev Bras Farmacogn*. 2020;30(5):737-742. doi:10.1007/s43450-020-00091-9

403. Santos EGG Dos, Bezerra WADS, Temeyer KB, León AAP de, Costa-Junior LM, Soares AMDS. Effects of essential oils on native and recombinant acetylcholinesterases of Rhipicephalus microplus. *Rev Bras Parasitol Vet*. 2021;30(2):e002221. doi:10.1590/S1984-29612021024

404. George DR, Masic D, Sparagano OAE, Guy JH. Variation in chemical composition and acaricidal activity against Dermanyssus gallinae of four eucalyptus essential oils. *Exp Appl Acarol*. 2009;48(1-2):43-50. doi:10.1007/s10493-008-9225-z

405. Dehghani-Samani A, Madreseh-Ghahfarokhi S, Dehghani-Samani A, Pirali-Kheirabadi K. Acaricidal and repellent activities of essential oil of Eucalyptus globulus against Dermanyssus gallinae (Acari: Mesostigmata). *J HerbMed Pharmacol*. 2015;4(3):81-84.

406. Immediato D, Figueredo LA, Iatta R, et al. Essential oils and Beauveria bassiana against Dermanyssus gallinae (Acari: Dermanyssidae): Towards new natural acaricides. *Vet Parasitol*. 2016;229:159-165. doi:10.1016/j.vetpar.2016.10.018

407. Atmani-Merabet G, Fellah S, Belkhiri A. Comparative study of two Eucalyptus species from Algeria: Chemical composition, toxicity and acaricidal effect on Varroa destructor. *Curr Issues Pharm Med Sci*. 2020;33(3):144-148. doi:10.2478/cipms-2020-0026

408. Ghasemi V, Moharramipour S, Tahmasbi G. Biological activity of some plant essential oils against Varroa destructor (Acari: Varroidae), an ectoparasitic mite of Apis mellifera (Hymenoptera: Apidae). *Exp Appl Acarol*. 2011;55(2):147-154. doi:10.1007/s10493-011-9457-1

409. Ruffinengo S, Maggi M, Faverin C, et al. Essential oils toxicity related to Varroa destructor and Apis mellifera under laboratory conditions. *Zootec Trop*. 2007;25(1).

410. Calderone NW, Spivak M. Plant Extracts for Control of the Parasitic Mite Varroa jacobsoni (Acari: Varroidae) in Colonies of the Western Honey Bee (Hymenoptera: Apidae). *J Econ Entomol*. 1995;88(5):1211-1215. doi:10.1093/jee/88.5.1211

411. Perrucci S. Acaricidal activity of some essential oils and their constituents against Tyrophagus longior, a mite of stored food. *J Food Prot*. 1995;58(5):560-563. doi:10.4315/0362-028X-58.5.560

412. Saad EZ, Hussien R, Saher F, Ahmed Z. Acaricidal activities of some essential oils and their monoterpenoidal constituents against house dust mite, Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). *J Zhejiang Univ Sci B*. 2006;7(12):957-962. doi:10.1631/jzus.2006.B0957

413. Fazal S, Cheema KJ, Manzoor F, Maqbool A. Anti-parasitic efficacy of some essential oils/extracts against itch mite, Sarcoptes scabiei. *Asian J Chem*. 2015;27(4):1215-1218. doi:10.14233/ajchem.2015.17174

414. Roh HS, Lee BH, Park CG. Acaricidal and repellent effects of myrtacean essential oils and their major constituents against Tetranychus urticae (Tetranychidae). *J Asia Pac Entomol*. 2013;16(3):245-249. doi:10.1016/j.aspen.2013.03.001

415. Choi W Il, Lee SG, Park HM, Ahn YJ. Toxicity of plant essential oils to Tetranychus urticae (Acari: Tetranychidae) and Phytoseiulus persimilis (Acari: Phytoseiidae). *J Econ Entomol*. 2004;97(2):553-558. doi:10.1093/jee/97.2.553

416. Afify AEMMR, Ali FS, Turky AF. Control of Tetranychus urticae Koch by extracts of three essential oils of chamomile, marjoram and Eucalyptus. *Asian Pac J Trop Biomed*. 2012;2(1):24-30. doi:10.1016/S2221-1691(11)60184-6

417. Ali AD, Ior LD, Dogo GA, Joshua JI, Gushit JS. Ethnobotanical Survey of Plants Used as Biopesticides by Indigenous People of Plateau State, Nigeria. *Diversity*. 2022;14(10):1-18. doi:10.3390/d14100851

418. Moshi AP, Matoju I. The status of research on and application of biopesticides in Tanzania. Review. *Crop Prot*. 2017;92:16-28. doi:10.1016/j.cropro.2016.10.008

419. Havyarimana C, Nkengurutse J, Ngezahayo J, Cuni-Sanchez A, Masharabu T. Antimalarial and mosquito repellent plants: insights from Burundi. *Ethnobot Res Appl*. 2023;25(March):1-28. doi:10.32859/era.25.31.1-28

420. Maia MF, Moore SJ. Plant-based insect repellents : a review of their efficacy , development and testing PMD from lemon eucalyptus (Corymbia citriodora) extract. *Malar J*. 2011;10(1):1-15.

421. Nutanson I, Steen CJ, Schwartz RA, Janniger CK. Pediculus humanus capitis: An update. *Acta Dermatovenerologica Alpina, Pannonica Adriat*. 2008;17(4):147-159.

422. Leta S, Beyene TJ, De Clercq EM, Amenu K, Kraemer MUG, Revie CW. Global risk mapping for major diseases transmitted by Aedes aegypti and Aedes albopictus. *Int J Infect Dis*. 2018;67:25-35. doi:10.1016/j.ijid.2017.11.026

423. Iqbal W, Faheem MM, Kaleem SM, Iqra A, Iram N, Rashda A. Role of housefly (Musca domestica, Diptera; Muscidae) as a disease vector; a review. *J Entomol Zool Stud*. 2014;2(2):159-163.

424. Showler AT, Osbrink WLA, Lohmeyer KH. Horn Fly, Haematobia irritans irritans (L.), Overwintering . *Int J Insect Sci*. 2014;6:IJIS.S15246. doi:10.4137/ijis.s15246

425. Ahmad JR, Hassan S, Ahmad S, et al. Stored Grain Pests and Current Advances for their Management. In: Md Ahiduzzaman, ed. *Postharvest Technology: Recent Advances, New Perspectives and Applications*. ; 2022. https://doi.org/10.1007/s12559-

021-09926-6%0Ahttps://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttp://dx.doi.org/10.1016/j.compmedimag.2010.07.003

426. Attia S, Grissa KL, Lognay G, Bitume E, Hance T, Mailleux AC. A review of the major biological approaches to control the worldwide pest Tetranychus urticae (Acari: Tetranychidae) with special reference to natural pesticides: Biological approaches to control Tetranychus urticae. *J Pest Sci (2004)*. 2013;86(3):361-386. doi:10.1007/s10340-013-0503-0

427. Santos AA, Hogendoorn K, Ramos RS, Picanço MC. Distribution models for Ascia monuste and the host Brassica oleracea var. capitata. *J Appl Entomol*. 2019;143(9):1043-1051. doi:10.1111/jen.12675

428. Smith LB, Kasai S, Scott JG. Pyrethroid resistance in Aedes aegypti and Aedes albopictus: Important mosquito vectors of human diseases. *Pestic Biochem Physiol*. 2016;133:1-12. doi:10.1016/j.pestbp.2016.03.005

429. Naqqash MN, Gökçe A, Bakhsh A, Salim M. Insecticide resistance and its molecular basis in urban insect pests. *Parasitol Res*. 2016;115(4):1363-1373. doi:10.1007/s00436-015-4898-9

430. Isman MB. A renaissance for botanical insecticides? *Pest Manag Sci*. 2015;71(12):1587-1590. doi:10.1002/ps.4088

431. Mollison B, Holmgren D. *Permaculture One: A Perennial Agriculture for Human Settlements*. - Mollison B. and Holmgren D., 1978, Permaculture One: A Perennial Agriculture for Human Settlements, (Melbourne, Australia: Transworld Publishers), WCED.; 1978.

432. Steiner R. *Cycle of Conferences in Koberwitz and Dornach.*; 1924.

433. Howard SA. *An Agricultural Testament*. Oxford University Press; 1943.

434. Fukuoka M. *The One-Straw Revolution: An Introduction to Natural FarmingNo Title*. Rodale Press; 1975.

435. Hazelip E. *Synergistic Agriculture. The Origins, the Experience, the Practice*. Terra Nuov.; 2014.

436. Janker J, Mann S, Rist S. Social sustainability in agriculture – A system-based framework. *J Rural Stud*. 2019;65(June 2018):32-42. doi:10.1016/j.jrurstud.2018.12.010

437. Stahel W, Reday G. *The Potential for Substituting Manpower for Energy*.; 1976.