Morphological, Chemical, and Biological Characterization of Species from the Cupressaceae Family

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By

Kmar M'barek

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To my parents and all those I love.

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ABSTRACT

The aim of this study is to determine the morphological, chemical, and biological characteristics of *Tetraclinis articulata* (Vahl) Mast., *Juniperus phoenicea L.,* and *Cupressus sempervirens* L. The phytotoxicity of their leaves was evaluated through the effects of their aqueous extracts (at concentrations of 10, 20, 30, and 40 g/l), leachates (at concentrations of 100, 200, 300, 400, and 500 g/l), and organic extracts (at concentrations of 2000, 4000, and 6000 ppm) on the germination and growth of four target species (lettuce, radish, barley, and tomato).

Additionally, pot cultures were conducted by incorporating leaf powder (at 50 and 100 g/kg) into soil, which was irrigated with their leachates and aqueous extracts at IC50 and MIC (concentrations inducing 50% and 100% inhibition of target plant growth observed during bioassays). Furthermore, the mode of action of allelochemicals in aqueous extracts was determined. The results indicate that both extracts and leachates significantly decreased germination index and germination rate, with leachates exhibiting higher toxicity.

Lettuce was the most sensitive species, with its germination almost completely inhibited at concentrations of 30 g/l and 300 g/l in all cases. Barley, on the other hand, was the most tolerant and continued to germinate even at higher concentrations. Similar trends were observed for growth inhibition, which increased with concentration. Regarding the organic extracts, chloroform was the most harmful, with the highest toxicity recorded at 6000 ppm for all three fractions.

Incorporating leaf powder into soil and irrigating with leachates and aqueous extracts demonstrated that the effects observed in bioassays were propagated in soil. The biomass of the different plants showed high toxicity towards the four target species, reducing their growth by an average of 90% at 100 g/kg. Similarly, irrigation with aqueous extracts and leachates induced growth inhibition in the target plants, with leachates showing greater toxicity.

Quantitative and qualitative analysis of the organic extracts was performed using gas chromatography–mass spectrometry (GC–MS). The analysis revealed that the leaves of the three plants are rich in terpene compounds, especially mono- and sesquiterpenes, which are known for their potential allelopathic effects. The content of these compounds varied with the organic solvent and species.

This analysis revealed the presence of major components that characterize each species. The compounds characterizing *T. articulata* (Vahl) Mast. include hexadecanoic acid, m-di-tert-butylbenzene, caryophyllene alcohol, camphor, α-pinene, totarol, and bornyl acetate. *C. sempervirens* L. is characterized by γ-cadinene, γ-muurolene, α-cedrol, γ-gurjunene, and germacrene D. *J. phoenicea* L. is characterized by α-pinene, borneol, manoyl oxide, and phytol. The inhibition of germination and growth of the target plants is attributed to the presence of these compounds. These disturbances are linked to the degradation of plasma membranes in both leaves and roots, as evidenced by electrolyte leakage and lipid peroxidation of the membranes.

In the roots, this membrane disintegration was accompanied by disruption of mitochondrial respiration, as indicated by a decrease in formazan content. The decrease in chlorophyll pigment content, which accompanied other disturbances, is believed to contribute to the inhibition of seedling growth. Concurrently, these seedlings attempted to mitigate this stress by initiating a defense strategy involving increased production of certain metabolites such as proline, polyphenols, flavonoids, and tannins. This accumulation results from the stimulation of lyase enzyme activity, particularly TAL, involved in their biosynthesis.

The results of this study suggest the potential use of biomass from the leaves of *T. articulata* (Vahl) Mast., *C. sempervirens* L., and *J. phoenicea* L. for managing agro-ecosystems as an alternative to highly detrimental industrial inputs.

Preface

In the current context of modern agriculture, the intensive use of synthetic pesticides has become a common practice for weed management. However, this increasing dependence on chemical pesticides has raised significant concerns about their impacts on human health and the environment. Faced with these challenges, it is imperative to develop weed management approaches that are not only effective but also sustainable and environmentally friendly.

This book explores the promises of allelopathy as a viable and ecological alternative to synthetic pesticides. Allelopathy, a natural phenomenon where plants produce secondary metabolites affecting the growth and development of other organisms, offers a potential pathway for biological weed control. This practice could transform how we approach weed management in agriculture, reducing our reliance on synthetic chemicals while preserving biodiversity and soil health.

The book delves deeply into the allelopathic effects of leaf extracts from three species in the Cupressaceae family: *Cupressus sempervirens* L., *Tetraclinis articulata* (Vahl) Master, and *Juniperus phoenicea* L. These species, chosen for their allelopathic potential and abundant biomass, are studied for their impacts on four representative crops: *Lactuca sativa* (lettuce), *Raphanus sativus* (radish), *Hordeum vulgare* (barley), and *Solanum lycopersicum* (tomato).

In addition to examining the chemical composition of the leaf extracts from these species, the book analyzes their effects on various physiological and biochemical processes of the target plants. The results presented here highlight the richness of these extracts in terpene compounds, known for their strong inhibitory effects, and demonstrate their potential as effective natural herbicides.

The importance of this research lies not only in discovering new methods of weed management but also in contributing to a more sustainable agricultural model. By providing a solid scientific basis for the use of allelopathic compounds, this book paves the way for innovations in the development of natural herbicides, offering a viable alternative to conventional agricultural practices.

This work is dedicated to all researchers and farmers striving to find sustainable solutions for healthier and more environmentally friendly agriculture. By exploring the possibilities offered by allelopathy, we hope to inspire new approaches and practices that will benefit both nature and humanity.

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INTRODUCTION

The increase in the use of synthetic pesticides for weed management in recent years (Fig. 0-1) has triggered a veritable environmental and health crisis (Fig. 0-2).

Fig. 0-1: The danger of using synthetic pesticides.

Fig. 0-2: Future impact of continuous use of synthetic herbicides on agricultural ecosystems.

Alarming data reveals an exponential rise in soil, water, and air pollution by these toxic chemicals. The consequences are devastating; entire ecosystems are being ravaged (Fig. 0-3), with animal and plant species disappearing at an alarming rate, and entire human communities are being affected by severe, sometimes irreversible, diseases. It is time to take urgent action before this destructive spiral becomes irreversible, condemning our future and that of future generations to a poisoned and sterile world.

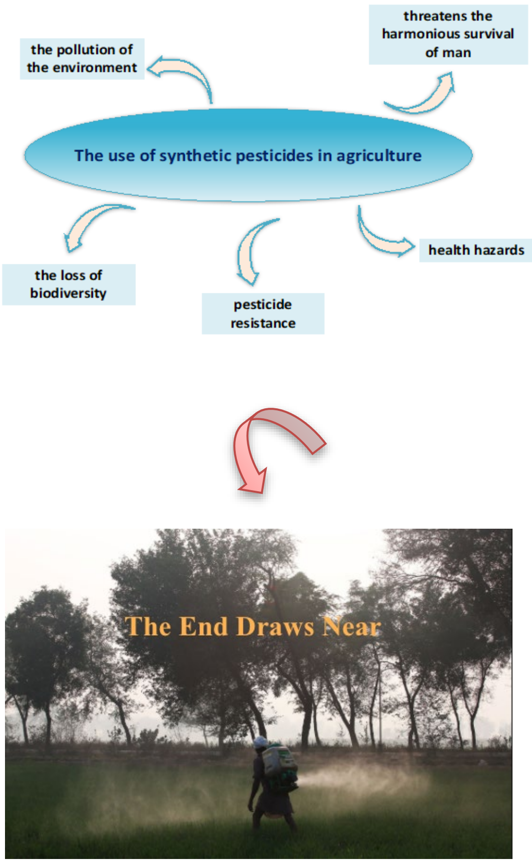

Fig. 0-3: Harmful effects of synthetic pesticides.

The researchers are now looking for alternate ways, based on natural products, for weed management in agricultural crops (Jamil et al. 2009, 475-

482). The application of allelopathy (Fig. 0-4) has shown tremendous potential in agricultural pest management (Farooq et al. 2011, 493-506).

Fig. 0-4: Allelopathy protects human health and environment.

It can be utilized for suppressing weeds in field crops and has a pertinent significance for ecological, sustainable, and integrated weed management systems (Jabran et al. 2015, 57-65).

The term 'allelopathy' refers to any process induced by secondary metabolites produced by bacteria, fungi, algae and plants that affects the growth and development of biological and agricultural systems (International Allelopathy Society 1996). Molisch (1937) coined the term allelopathy to include both harmful and beneficial biochemical interactions between all types of plant including microorganisms. Allelopathy is a natural phenomenon which occurs both in aquatic and terrestrial environments (Żak, Musiewicz et Kosakowska 2012, 4-10). It is one approach that can be used as an alternative method to combat pests in cropping systems (Inderjit et Keating 1999, 141-231; Rice 1995, 439).

From the 1990s, allelopathic research shifted from merely laboratory work to pot culture and field studies. New biologically active substances with allelopathic activity usually affect other living organisms (Garima et Meera 2017, 686-688). Many plant species have allelopathic effects on other plant species (Dhima et al. 2006, 345-352). Information on the chemical nature and mode of action of allelochemicals is expanding (M'barek et al. 2019, 29-37). Defense agents, such as allelochemicals or allelopathins, are largely classified as secondary plant metabolites that play an important role in allelopathic interactions or plant defense and act as important ecological mechanisms (Rice 1995, 439).

Various research efforts have been made in the identification of allelochemicals from living plants (Wu et al. 2001, 1691-1700), testing the biological activities of identified allelochemicals (An, Pratley et Haig 2001, 383-394), detecting their dynamics in plants and the environment (Wolfson et Murdock 1990, 257-264), characterizing their modes of action (Czarnota et al. 2001, 813-825), determining the effect of abiotic (inorganic and organic components) and biotic factors (Einhellig 1989, 101-18), and identifying their genetic make-up, etc. (Quader et al. 2001, 747-60). The most common effect observed is the growth inhibition of target species (Żak, Musiewicz et Kosakowska 2012, 4-10). Allelopathic substances may cause morphological and ultrastructural changes, generate reactive oxygen species and oxidative stress, inhibit photosystem II, and affect photosynthesis, induce cell lysis, and plant death (Fistarol et al. 2004, 45-56).

An equally promising way is to use allelopathy for weed control by utilizing the bioactive compounds of allelopathic plants as herbicides, as these ecological herbicides are easily biodegradable and much safer than the current synthetic herbicides. Thus, a better understanding of allelopathy can help in developing more sustainable agroforestry systems (Garima et Meera 2017, 686-688).

In an attempt to reduce the use of synthetic pesticides, extensive investigations into the possible exploitation of plant compounds as natural commercial products, that are safe for humans and the environment were made. Indeed, the search of natural compounds and alternative management methods to classical pesticides has become an intense and productive research field (Žanić et al. 2008, 161-66). In this regard, greater attention is towards the use of allelopathic plants and their products for pest management in a sustainable manner. Therefore, it is worthwhile to explore the plants as sources of biological active compounds.

In nature, plants are frequently exposed to various environmental stresses that cause a negative impact on their survival, development and productivity to which plants' response depends on the duration, severity and the extent of the imposed stresses (Munne-Bosch et Alegere, 2004, 203). Under such stresses, plants produce a wide variety of natural products or secondary metabolites providing key functions in resistance to both biotic and abiotic constraints (Ballhorn et al. 2009, 743-45).

Such response of plants, either of crops or weeds, to the exudates secreted from the neighboring plants' organs has helped in finding biopesticides to reduce the harmful effects of weeds by impairing germination, growth, development, and physiological functions such as membrane permeability (Galindo et al. 1999, 805-13), stomatal closure (Barkosky, Einhellig et Butler 2000, 2095-2109), respiration (Abrahim et al. 2000, 611-24), cell division (Anaya et Pelayo-Benavides 1997, 57-68), absorption of nutrients (Baar et al. 1994, 1073-79), photosynthesis (Baziramakenga, Simard et Leroux 1994, 2821-33), synthesis of ATP, gene expression, perspiration, and other metabolic processes (Blum et Gerig 2005, 1907-32).

The present study takes into consideration the evaluation of allelopathic activity of extracts of *Cupressus sempervirens* L., *Tetraclinis articulata* (Vahl) Master and *Juniperus phoenicea* L. leaves against four crops: *Lactuca sativa, Raphanus sativus, Hordeum vulgare* and *Solanum lycopersicum.*

Species of *Cupressus* genus (*Cupressaceae* family) are coniferous trees with worldwide distribution, comprising twelve species which are distributed in North America, the Mediterranean region and subtropical Asia at high altitudes (Rawat et al. 2010, 162-66). Many plants of this cypress family are important as timber sources or ornamentals and they are also considered to be medicinal trees (Nehdi 2013, 381-85; M'barek, Zribi et Haouala 2018, 187-202), they reduce risks of cancer and cardiovascular disease (Ozkan et al. 2016, 257).

In Tunisia, only one species of the genus *Cupressus*, *Cupressus sempervirens* L. (Cuénod 1954) was native. *C. sempervirensis* L. is a monoecious and evergreen tree (Emami et al. 2006, 103-8). It is considered to be a medicinal tree (Nehdi 2013, 381-85), with antidiabetic, anti-inflammatory (Mascolo et al. 1987, 28-31), antispasmodic, antiseptic (Keller, 1991), antimicrobial (Kassem et al. 1991, 205-7) and antifungal activities (Alfazairy 2004, 554-60).

Tetraclinis articulata (Vahl) Master (also known as *Thuya articulata* or *Callitris quadrivalvis*, *Cupressaceae* family) is a monoic species that flowers in spring without leaf fall. It is endemic to North Africa, Malta, Spain Morocco, Algeria and Tunisia (Bourkhiss et al. 2010, 4-11). In Tunisia, it is found in the north-east region. It is included in the IUCN (International Union for Conservation of Nature) red list, and protected by law in several countries. Its parts are used in folk medicine mainly against respiratory, intestinal infections, gastric pains, diabetes, hypertension, antidiarrheal, antipyretic, diuretic, antirheumatic, oral hypoglycemic and to treat eyes inflammation (Bourkhiss et al. 2010, 4-11). Its macerated leaves are used to prepare the herbal tea, the aqueous extract of its wood is used as natural biocides and the decoction of their leaves heals wounds and bruises (Djouahri, Boudarene et Sabaou 2013, 45-50). Previous studies reported that *T. articulata* (Vahl) Master exhibited antibacterial, antifungal, cytotoxic, anti-oxidant and anti-inflammatory properties (Bourkhiss et al. 2010, 4-11).

The genus *Juniperus* consists of approximately 60 species growing in the Northern Hemisphere (Rezzi et al. 2001, 179-188), including *Juniperus phoenicea* L. (*Cupressaceae* family). This species was first described by the renowned botanist Carolus Linnaeus and published in his seminal work, Species Plantarum, Volume 2, page 1040, in the year 1753. *Juniperus phoenicea* is a coniferous evergreen shrub or small tree native to the Mediterranean region (Aymonin 1990, 243-44). Screening plant essential oils and plant extracts for their biological activity can lead to the discovery of new pest control agents. Several essential oils have been reported as having herbicidal effects against weed germination and seedling growth (Amri et al. 2012, 199-207).

Lactuca sativa L. (lettuce) is a major leafy salad vegetable and is commonly used as an indicator species in most bioassay studies for allelopathy.

Radish is a nutritious root vegetable, used both in raw salads and as an ingredient in main dishes. It is also commonly used as an indicator species in most bioassay studies for allelopathy.

Lettuce and radish were chosen for their high sensitivity to allelochemicals, while the other two species were selected because they are not widely studied. Tomato is a major vegetable crop used in many cooking recipes or as fresh salads. Barley is a major crop in dryland agriculture in Tunisia and other countries.

In the light of this work we have determined, in a first step, the chemical composition of the organic extracts obtained from the leaves of *T. articulata* (Vahl) Master, *C. sempervirens* L. and *J. phoenicea* L. for use in agriculture as natural pesticides. The morphological characterizations of the species have also been determined.

In a second step, their allelopathic potential was assessed against four crops: *Lactuca sativa* (lettuce), *Raphanus sativus* (radish), *Hordeum vulgare* (barley) and *Solanum lycopersicum* (tomato).

Tetraclinis articulata (Vahl) Mast., *Cupressus sempervirens* L. and *Juniperus phoenicea* L., three species of Cupressaceae (Fig. 0-4) possess a significant phytotoxicity and released some allelochemicals while tested in field and laboratory experiments. They exert strong inhibitory effects on seed germination and radicle elongation in test crops. The analysis of the chemical composition of their aqueous leaf extracts by GC / MS, showed the richness of these species in terpene compounds, particularly in mono and sesquiterpenes, known for their strong inhibitory effects.

In a third step, the present study takes into consideration also the determination of the mode of action of aqueous leaf extracts of *Tetraclinis articulata* (Vahl) Mast., *Cupressus sempervirens* L. and *Juniperus phoenicea* L. on lettuce. Cupressaceae species (Fig. 0-5) were chosen for the abundance of their biomass and their allelopathic potentials which have so far been little studied.

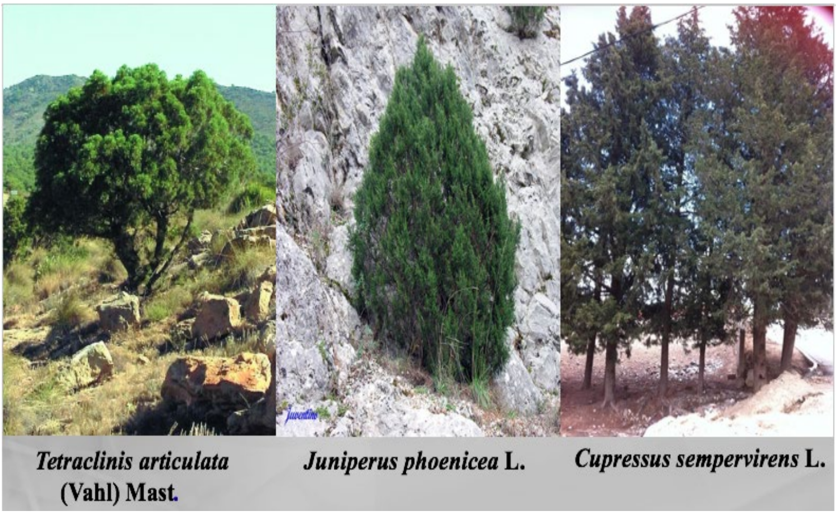

Fig. 0-5: Photographs of three species of Cupressaceae.

For that, their leaf extracts were sprayed on the seedlings of lettuce to examine their effect on some physiological and biochemical processes in lettuce such as the cell metabolic activity, leaf pigments, proline content, electrolyte leakage, lipid peroxidation, phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) activities and production of secondary metabolites, phenolic contents and proanthocyanidin in the roots and leaves. The acquisition of such knowledge may provide a scientific basis for the innovation of effective herbicides.

CHAPTER 1

BIBLIOGRAPHIC SYNTHESIS: ALLELOPATHY

1. Definition

The term allelopathy originates from the Greek words allelon, meaning "mutual" or "reciprocal," and pathos, meaning "suffering" (Rizvi 2012). Molisch (1937) coined this term to describe the set of biochemical interactions, both detrimental and beneficial, occurring among all types of plants, including microorganisms. Subsequently, American ecologist Elroy Leon Rice (1985) expanded the definition to encompass all direct or indirect, positive or negative effects of one plant on another or on microorganisms, through the release of biochemical substances into the natural environment. Presently, McCoy, Widhalm et McNickle (2022) have reported that allelopathy is a mechanism of interference, where chemical compounds produced by certain plant species can either enhance or suppress the growth of associated plants. These compounds can also play a significant role in intra- and interspecific competition, thereby determining the types of associations among plants.

2. Historical background

Although the term allelopathy was first coined in 1937 (Putnam 1983, 34-45) by the Austrian professor Hans Molisch in the book "Der Einfluss einer Pflanze auf die andere," this phenomenon has been recognized for over 2000 years (Rice 1985). Indeed, since antiquity, humans have observed that certain plants impede the growth of neighboring species. The Greek botanist Theophrastus (300 BC) noted that chickpeas destroyed weeds, while Gaius Plinius Secundus (after AD) observed that the walnut tree prevented the growth of any plants under its foliage (Rizvi 2012). However, as early as 1832, Augustin Pyrame De Candolle suggested that soil fatigue could be attributed to exudates from crops (Rice 1985). This notion was later reinforced by Stickney and Hoy in 1881 when they observed the effect of black walnut (*Juglans nigra*) on surrounding vegetation (Friedman et

Waller 1985, 47-50).

In the 20th century, interest in allelopathy was reignited with the development of techniques for extraction, isolation, and identification of chemical compounds, as well as bioassay techniques (Willis 1997, 7-55). These advancements led to a deeper understanding of the underlying mechanisms of allelopathy and opened new avenues for exploring its potential in managing agricultural ecosystems.

3. Molecules involved

It is documented that the chemicals emitted by plants, which induce allelopathic effects, are referred to as allelochemicals (Parry 1982; Kruse, Strandberg et Strandberg 2000, 66). These intricate molecules play pivotal roles in interspecific dynamics, characterized as non-nutrient compounds synthesized by organisms, exerting biological or behavioral effects on individuals of distinct species (Regnault-Roger et Hamraoui 1997, 401-12). Notably, these compounds operate at minimal concentrations and predominantly arise from the realm of plant secondary metabolism.

Indeed, a plethora of allelochemicals are categorized as secondary metabolites, stemming primarily from pathways like acetate or the shikimic acid pathway (Fig. 1-1). This diverse array includes phenolic compounds, tannins, flavonoids, terpenes, alkaloids, steroids, quinones, and glycosides (Inderjit 2003, 625-26). Virtually all classes of secondary metabolites participate in allelopathic interactions (Weston et Duke 2003, 367-89), their effects often demonstrating synergistic, additive, or antagonistic properties (Bais et al. 2003, 1377-80).

As per Regnault-Roger, Philogène et Vincent (2008, 51-60), intricate connections exist between the physiological processes of plants, such as photosynthesis and respiration, and the synthesis of secondary metabolites, which may harbor allelopathic properties. Consequently, the same author posited that the emergence and accrual of these compounds frequently align with the stages of plant growth and development, further influenced by environmental factors. This dynamic interplay underscores the intricate relationship between plant physiology, secondary metabolism, and allelopathic potential, highlighting the nuanced responses of plants to their surroundings.

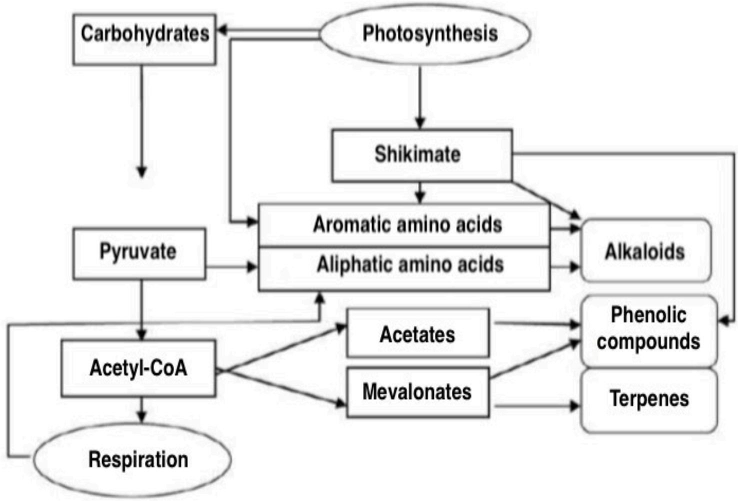

Fig. 1-1: Major pathways of secondary metabolite synthesis and their relationship with primary metabolism (Regnault-Roger, Philogène et Vincent 2008, 51-60).

3.1 Chemical nature

Secondary metabolites often derive from the Shikimate synthesis pathway (Bouton 2005, 1-18). This biochemical metabolite holds significance in both plants and microorganisms, owing its name to the Japanese plant shikimi (Meyrer**,** Reeb et Bosdeveix 2004**,** 335-337). Nearly 50,000 secondary metabolites have been identified out of a total of 150,000 (Rosset, Jardy et Caude 1991, 370), with many known to possess phytotoxic properties (M'barek et Haouala 2024, 100-110).

According to Mazid, Khan et Firoz (2011, 232-49), three major categories of secondary metabolites are distinguished in plants: terpenoid compounds, phenolic compounds, and nitrogenous and sulfur compounds.

Terpenes and terpenoids constitute the largest class of secondary metabolites, united by their common biosynthetic origin from acetyl-CoA (Grayson 1998, 497-521). They are formed by the polymerization of isoprene (Damião 2011, 2233-52), a five-carbon unit (Fig. 1-2).

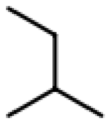

Fig. 1-2: Chemical structure of the isoprene unit C5 (Damião 2011, 2233-52).

They are classified based on the number of isoprene units (Damião 2011, 2233-52):

Hemiterpenes (C5): Hemiterpenes represent the smallest class of terpenoid compounds, consisting of only a single isoprene unit (C5). As the most basic building block, hemiterpenes form the foundation from which larger and more complex terpenoid structures are constructed. The general formula for hemiterpenes is (CH3)2C=CH2, with examples including isoprene, acetone, and 3-methyl-2-butene.

Despite their simplicity, hemiterpenes play an important role as precursors in the biosynthesis of more elaborate terpenoid molecules. Certain hemiterpenes also exhibit valuable biological activities, finding applications in perfumery, medicine, and other industries. Within the broader terpenoid family, hemiterpenes constitute the smallest subclass, followed by the increasingly larger monoterpenes (C10), sesquiterpenes (C15), and so on. Understanding the properties and functions of these fundamental C5 units provides key insights into the production and utilization of the diverse range of terpenoid natural products.

Monoterpenes (C10): A subgroup of terpenoids found in conifer resin (Bauce, Crépin, and Carisey 1994, 499-507), known as significant agents of toxicity towards insects (Mazid, Khan, and Firoz 2011, 232-49). Indeed, compounds such as α-pinene, β-pinene, limonene, and myrcene, which are present in conifers, are toxic to numerous insects, including bark beetles, which are significant conifer pests worldwide (Turlings et al. 1995, 4169-74). Nevertheless, some of these compounds may have positive effects on insect behavior (Cates, Henderson, and Redak 1987, 312-316).

Sesquiterpenes (C15): Several sesquiterpenes have been reported for their role in plant defense against herbivores and pathogens (Gershenzon and Dudareva 2007, 408-14; Mazid, Khan et Firoz 2011, 232-49).

These 15-carbon terpenoids are biosynthesized from farnesyl diphosphate (FPP) and display remarkable structural diversity, with over 5,000 different sesquiterpene skeletons identified to date (Degenhardt, Köllner and Gershenzon 2009, 1621-37). Many sesquiterpenes act as phytoalexins, antimicrobial compounds produced in response to pathogen attack, while others function as antifeedants or toxins that deter herbivores (Tholl 2006, 297-304). Sesquiterpenes also play important roles in plant-plant and plant-insect interactions, serving as airborne signals that attract natural enemies of herbivores or induce defenses in neighboring plants (Mumm and Dicke 2010, 628-667). The structural diversity and biological activities of sesquiterpenes make them attractive targets for engineering enhanced plant defenses and developing biopesticides.

Diterpenes (C20): Phytol, an acyclic diterpene alcohol, is known for its ability to enhance chlorophyll efficiency during photosynthesis (Knaff 1991, 82-83), thereby maximizing CO2 fixation and biomass production (Jagendorf 1967, 1361-1369). Phytol is a key component of the chlorophyll molecule, and its presence optimizes the light-harvesting capacity of the photosynthetic apparatus (Tanaka and Tanaka 2006, 248-255). Additionally, the cyclic diterpenes known as gibberellins are reported to play various positive roles in numerous plant developmental processes such as seed germination, stem elongation, leaf expansion, flowering, and fruiting (Davies 1995, 13-38), as well as enhancing CO2 fixation and growth (Ouzounidou and Ilias 2005, 223-228). Gibberellins act by regulating the expression of key genes involved in cell division and expansion (Davière and Achard 2013, 1147-51). The diverse biological activities of diterpenes make them attractive targets for engineering improved photosynthetic efficiency and crop productivity.

Triterpenes (C30): Asclepiads are reported to produce several triterpenoid glycosides (sterols) that protect them against herbivores and ost insects (Agrawal and Konno 2009, 311-331). Another class of triterpenes, the limonoids, are abundant in citrus fruits and other plants of the Rutaceae family, where they act as antiherbivore agents (Mazid, Khan, et Firoz 2011, 232-49). For instance, azadirachtin, a complex limonoid from the neem tree (Azadirachta indica), exhibits various toxic and antifeedant effects against a wide range of insect pests (Mordue et Blackwell 1993, 903-24).

Polyterpenes (C5)n: Several high-molecular-weight polyterpenes occur in plants, including tetraterpenes, with carotenoids being the major representatives (Mazid, Khannet Foroz 2011, 232-249). Another example is natural rubber, a polyterpene polymer that acts as a defense mechanism against herbivores by forming a sticky, viscous barrier (Eisner and Jerrold 1995; Klein 1989, 610).

Phenolic compounds: Plants produce a wide variety of secondary metabolites containing a phenol group, which is a hydroxyl functional group (-OH) attached to an aromatic nucleus (Mazid, Khan et Firoz 2011, 232-49) (Fig. 1-3). These phenolic compounds can play an important role in the plant's defense system against pests and diseases, including protection against root-parasitic nematodes (Wuyts, De Waele et Swennen 2006, 308-14).

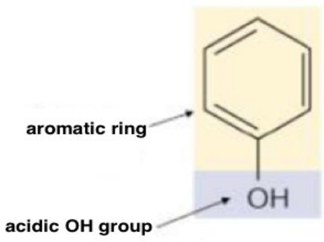

Fig. 1-3: Structure of phenolic molecules (Zagoskina 2023, 13874).

In this group of phenolic compounds, the following are distinguished:

- **Coumarins:** They originate from the shikimic acid pathway (Murray, Méndez et Stewart 1982, 702). These are simple phenolic compounds and appear to function in various plant defense mechanisms against herbivorous insects and fungi (Mazid, Khan et Firoz 2011, 232-249). However, coumarins also encompass a broad spectrum of antimicrobial activity against bacteria, fungi, and viruses (Brooker, Windorski et Blumi 2008, 81-89).

- **Lignin:** It is a highly branched phenolic polymer formed from three different alcohols, namely coniferyl, coumaryl, and sinapyl alcohols (Freudenberg et Neish 1968, 129). The proportions of these monolignol units vary among plant species and between different plant organs (Lewis et Yamamoto 1990, 455-96). Its chemical durability and resistance to degradation renders lignin relatively indigestible by many herbivores and pathogenic organisms such as fungi and bacteria (Mader et Amberg-Fisher 1982, 1128-31).