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Assessment of *Pituranthos* species' adaptation to edaphic and climatological factors in Algerian Sahara

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Dedication

I dedicate this work to the people of Gaza, whose unwavering spirit was a constant companion during the writing of this thesis, a period marked by the Israeli bombardment. Their profound resilience served as both a guiding light and a sobering reminder of the struggle for justice. It is my hope that this academic pursuit honors their endurance and contributes to a future of peace and freedom.

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Abstract

Assessment of *Pituranthos* species' adaptation to edaphic and climatological factors in Algerian Sahara

Background and Objective: This research investigates the adaptation of the *Pituranthos* genus to the edaphic and climatic conditions of the Algerian Sahara. The study presents a comprehensive analysis of two medicinal species; *Pituranthos chloranthus* (Ghardaïa) and *Pituranthos scoparius* (Adrar) to determine how seasonal variations and geographical origins modulate their phytochemical profiles and biological potential.

Methodology: Plant materials were collected across distinct seasons (autumn and spring) and analyzed alongside soil and climate data. The physiological response was assessed through the quantification of osmolytes, while secondary metabolites were characterized using UPLC/MS-MS for ethanolic extracts and GC-MS for essential oils. Furthermore, the study utilized the reducing potential of these extracts to green-synthesize a novel silver-doped magnesium oxide (Ag/MgO) nanocomposite, characterized via UV-Vis, XRD, FTIR, SEM, and EDX.

Results: Analytical data revealed highly specific, environment-driven chemoprofiles. *P. scoparius* (Adrar) exhibited a monoterpene-rich profile (β -Phellandrene, D-Limonene), while *P. chloranthus* (Ghardaïa) was dominated by phenylpropanoids, specifically Myristicin and Safrole. Biological assays (DPPH, FRAP, and albumin denaturation) confirmed potent antioxidant and anti-inflammatory activities. Notably, the synthesized Ag/MgO nanocomposite demonstrated superior antibacterial efficacy against seven pathogenic strains compared to crude extracts.

Conclusion: The findings elucidate the sophisticated biochemical adaptation mechanisms of *Pituranthos* species to arid environments. The study concludes that species selection, geographic origin, and harvest season are critical factors for optimizing bioactive yields. This work validates the traditional medicinal utility of these species and highlights their significant potential in pharmacology and green nanotechnology.

Keywords: *Pituranthos*, Phytochemical variability, Algerian Sahara, Green synthesis, Ag/MgO nanocomposites, Biological activity.

Abstract in Arabic (الملخص)

تقييم تكيف أنواع جنس *Pituranthos* مع العوامل التربوية والمناخية في الصحراء الجزائرية

الأهداف: تهدف هذه الدراسة إلى تقييم آليات تكيف أنواع جنس *Pituranthos* مع العوامل التربوية والمناخية في الصحراء الجزائرية. ويركز العمل على استقصاء التباين الكيميائي النباتي والإمكانات البيولوجية لنوعين طبيين: *P. chloranthus* (ولاية غرداية) و *P. scoparius* (ولاية أدرار)، مع تحديد دور التغيرات الموسمية والأصل الجغرافي في تعديل خصائص المركبات النشطة بيولوجياً.

المنهجية: تضمن الإطار المنهجي جمع المادة النباتية من المنطقتين خلال فصلي الخريف والربيع، مع تحليل دقيق لظروف المناخ والتربة. شملت الدراسة تقدير الأوزموليات وتحليلاً كيميائياً للمستخلصات الإيثانولية والزيوت العطرية باستخدام تقنيات UPLC/MS-MS و GC-MS. وعلاوة على ذلك، تم توظيف قدرة المستخلصات على الاختزال لتصنيع مركب نانوي أخضر هجين من أكسيد المغنيسيوم المطعم بالفضة (Ag/MgO)، والذي جرى توصيفه بتقنيات متقدمة مثل EDX و SEM و FTIR و XRD.

النتائج: أظهرت النتائج تبايناً كيميائياً دقيقاً مرتبطاً بالبيئة؛ حيث تميزت عينات أدرار بغناها بالكاتشين والريسفيراتول وزيوت عطرية أحادية التربين (مثل الفيلاندرين)، بينما تميزت عينات غرداية بالريبوفلافين والكرامين وزيوت غنية بالفينيل بروبانويد (الميريستيسين والسافورول). أثبتت الاختبارات البيولوجية فعالية عالية كمضادات للأكسدة والالتهابات، كما أظهر المركب النانوي (Ag/MgO) كفاءة مضادة للبكتيريا تفوق بشكل ملحوظ المستخلصات الخام.

الاستنتاج: خلصت الدراسة إلى أن اختيار النوع، الموقع الجغرافي، وموسم الحصاد عوامل حاسمة في تحديد جودة وكمية المركبات النشطة. توضح هذه النتائج آليات التكيف المعقدة لنباتات *Pituranthos* مع البيئات القاحلة، وتؤكد صحة استخداماتها التقليدية كمصدر طبيعي للمركبات العلاجية المعززة بتقنيات النانو الخضراء.

الكلمات المفتاحية: التكيف، البوليفينول، العوامل المناخية، الأنشطة البيولوجية، الجسيمات النانوية، الزيوت العطرية،

أكسيد المغنيسيوم، الميريستيسين، GC-MS

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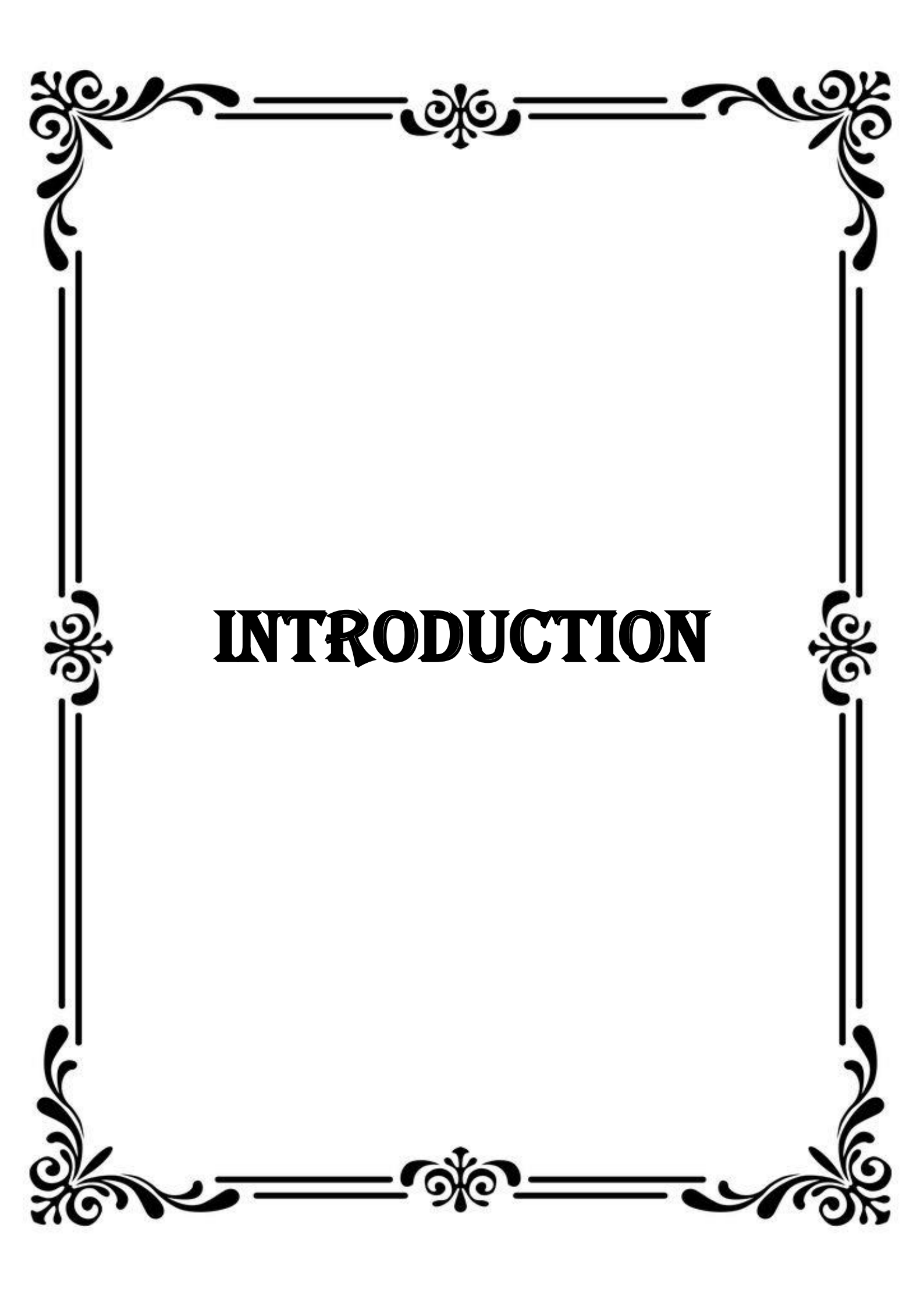
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INTRODUCTION

Since time immemorial, humanity's quest for healing has been intrinsically linked to the botanical world (Davis & Choisy, 2024). Long before the advent of modern pharmacology, ancient civilizations across the globe turned to the forest, the meadow, and the field for remedies, weaving a rich tapestry of knowledge that formed the foundation of all medicine (Zhang, 2019).

Traditional medicine across the globe relies on foundational medicinal plants. Key examples include turmeric and ashwagandha from India's Ayurveda, ginseng and artemisia from Traditional Chinese Medicine, black seed and senna from the Arab world, and cinchona from the Amazon. These plants form a shared herbal heritage that continues to inform modern medicine (James *et al.*, 2018).

This deep-rooted relationship is not merely a historical relic but a living tradition, with the World Health Organization estimating that up to 80% of the world's population still relies primarily on plant-based medicines for their primary healthcare (James *et al.*, 2019). Furthermore, the modern pharmaceutical industry itself stands upon this botanical foundation, with a substantial proportion of its drugs from the aspirin derived from willow bark to the potent anticancer agent paclitaxel from the Pacific yew tree being plant-derived or plant-inspired (Mahdi, 2010; Slichenmyer & Von Hoff, 1991). This enduring reliance underscores the plant kingdom's indispensable role as a source of therapeutic agents, a role that continues to be critical for global health, cultural heritage, and scientific discovery.

A potent antimalarial compound extracted from sweet wormwood (*Artemisia annua* L.), a discovery for which the Nobel Prize was awarded in 2015 (Weathers *et al.*, 2017). This dual role serving as both direct sources of medicine for the vast majority of the world's population and as invaluable lead compounds for the pharmaceutical industry cements the status of medicinal plants as an irreplaceable global resource for present and future health security (Mishra *et al.*, 2025).

Underpinning this profound medicinal value is a significant socio-economic dimension that further solidates the importance of these botanical resources (Pergola *et al.*, 2024). The global market for medicinal plants, encompassing herbal supplements, botanicals, and raw materials for pharmaceuticals, is a multi-billion-dollar industry that fuels local economies and international trade (Asigbaase *et al.*, 2023). However, this economic narrative is twofold. For millions of rural households and indigenous communities, particularly in biodiversity-rich regions, the collection, cultivation, and trade of medicinal plants is not merely a commercial activity but a vital source of livelihood and a pillar of cultural heritage (Vallejo *et al.*, 2024). This reliance creates a complex interplay between conservation and economic necessity, where

the sustainable management of these species is directly linked to human well-being (Fajinmi *et al.*, 2023). Consequently, any factor that threatens the availability, quality, or potency of medicinal plants such as environmental degradation and climate change carries not only biological implications but also direct and often severe socio-economic consequences. Despite their indispensable role in both traditional and modern contexts, this vast natural pharmacy is now under unprecedented threat (Volenzo & Odiyo, 2020). The planetary changes characterizing the Anthropocene epoch marked by profound human alteration of Earth's systems are directly undermining the ecological foundations upon which these medicinal plants depend.

The resilience of this natural pharmacy is inherently tethered to the stability of its environment. Medicinal plants, whether harvested from the wild or cultivated, are not static repositories of active compounds; rather, they are dynamic living systems whose physiological and biochemical profiles are profoundly shaped by their growing conditions (Avasiloaiei *et al.*, 2023).

Environmental fluctuations be they in water availability, temperature regimes, soil nutrient composition, or exposure to ultraviolet radiation act as powerful abiotic stressors that trigger a cascade of plant responses. To ensure their survival, plants undergo complex metabolic reprogramming, which frequently involves the altered production of secondary metabolites (Jangpangi *et al.*, 2025).

These compounds, which are the very source of their medicinal value, are integral to the plant's defense and adaptation mechanisms (El-Saadony *et al.*, 2025). Consequently, a shift in climate or a sudden drought is not merely a threat to the plant's survival, but a direct modulator of its therapeutic potency, creating a critical and volatile link between ecosystem health and medicinal efficacy (Solanki *et al.*, 2024)

The specific nature of these biochemical changes is dictated by the type and intensity of the abiotic stress encountered. Among the most influential environmental factors are light, water, temperature, and soil conditions. Light quality and photoperiod, particularly exposure to ultraviolet-B (UV-B) radiation, can act as an elicitor, stimulating the production of protective phenolic compounds like flavonoids (Yang *et al.*, 2018).

Water availability is another critical determinant; drought stress often induces oxidative stress within plant tissues, leading to an upregulation of the antioxidant defense system and a consequent accumulation of secondary metabolites such as terpenoids and alkaloids (Bhattacharya & Pal, 2025). Similarly, temperature extremes both heat and cold can disrupt

membrane stability and enzyme function, forcing metabolic shifts that alter the concentration and profile of bioactive molecules (Theodoridis *et al.*, 2023).

Soil characteristics, including salinity, pH, and the availability of essential macronutrients like nitrogen and phosphorus, directly influence biosynthetic pathways, as these elements are fundamental building blocks and co-factors for the synthesis of medicinal compounds (Pang *et al.*, 2025).

Far from being passive victims of their environment, medicinal plants are dynamic organisms equipped with sophisticated perceptual and defense systems (Yang *et al.*, 2018). This is governed by the field of plant stress physiology, which reveals that upon perceiving an abiotic stress signal, a plant initiates a complex cascade of interconnected responses. These occur at multiple levels: physiologically, through changes in stomatal conductance and photosynthetic rate; biochemically, through the synthesis of protective compounds; and molecularly, through the rapid expression of stress-responsive genes (Hasanuzzaman *et al.*, 1 C.E.). A key survival mechanism in this process is the accumulation of osmolytes small, highly soluble organic compounds like proline, glycine betaine, and soluble sugars (Ashraf & Foolad, 2007). These molecules act as biochemical buffers by maintaining cellular turgor pressure, stabilizing proteins and membranes, and scavenging reactive oxygen species, thereby protecting the delicate cellular machinery from stress-induced damage (Wang *et al.*, 2024). Crucially, the metabolic pathways involved in osmolyte production are often intertwined with those for secondary metabolite biosynthesis, positioning these compatible solutes at the heart of the link between environmental stress and medicinal potency (Kawale *et al.*, 2024).

the very defense mechanisms that ensure a plant's survival are often directly linked to the synthesis of the compounds we value as medicine. The biochemical precursors and energy resources a plant diverts to produce protective osmolytes and antioxidants are frequently drawn from the same pools used to create valuable secondary metabolites (Ouhaddou *et al.*, 2023). This means that an environmental stressor does not merely trigger a generic survival reaction; it can fundamentally reprogram the plant's metabolic factory (Bulut *et al.*, 2025). However, the outcome of this reprogramming is not straightforward. A stressor may enhance the production of a desired alkaloid while suppressing a vital flavonoid, or it may push the plant's resources to their limit, boosting medicinal compounds at the grave cost of stunted growth (Gaude & Jalmi, 2025). This creates a critical, yet poorly understood, interplay between environment and medicine, where deciphering the rules of this metabolic trade-off is essential to predicting, preserving, and harnessing the medicinal power of plants in a changing world.

To fully appreciate this interplay, it is essential to understand the specific class of compounds at its heart: secondary metabolites. Unlike primary metabolites, such as sugars, amino acids, and nucleic acids, which are universal to all plant life and indispensable for fundamental processes like growth, development, and reproduction, secondary metabolites are not directly involved in these core physiological functions. Instead, they are biosynthesized as specialized compounds that facilitate the plant's interaction with its environment (Alami *et al.*, 2024).

It is this very ecological role acting as defenses against herbivores, pathogens, and UV radiation, or as attractants for pollinators that makes them pharmacologically active (Wink, 2003). Consequently, the major classes of secondary metabolites, including the nitrogen-containing alkaloids (morphine, caffeine), the diverse phenolics and their subgroup flavonoids (quercetin, resveratrol), and the volatile terpenoids (artemisinin, menthol), are most often the bioactive constituents responsible for the (Seca & Pinto, 2019) medicinal properties of plants, transforming them from simple botanical specimens into reservoirs of therapeutic potential (Seca & Pinto, 2019).

The relationship between environmental stress and secondary metabolite production, however, is not a simple linear one, but rather a complex and context-dependent phenomenon (Gaude & Jalmi, 2025). Environmental stress can function as a "eustress" a positive stimulus that strategically enhances the concentration of desired medicinal compounds by activating specific defense-related biosynthetic pathways (Laftouhi *et al.*, 2023). For instance, moderate drought stress may significantly boost the production of certain valuable alkaloids or phenolics. Conversely, that same stressor can become a "distress" when its intensity or duration exceeds a critical threshold, ultimately inhibiting biosynthesis as the plant's metabolic resources are diverted exclusively to immediate survival, or causing irreversible damage to growth and physiology (Rahman *et al.*, 2023). This delicate balance means that the ultimate outcome whether a stressor acts as a beneficial elicitor or a detrimental inhibitor is highly species-specific, stress-specific, and critically, dose-dependent (Kumar & Sharma, 2018). A precise understanding of these thresholds and interactions is therefore paramount for harnessing the potential of eustress in cultivation without compromising plant viability or medicinal yield.

This intricate and contingent nature of the plant-stress relationship underscores a critical gap between theoretical understanding and practical application. To advance from ecological principle to actionable knowledge in conservation and phytochemistry, targeted studies on key medicinal species are urgently required. This thesis addresses this imperative by focusing on the *Pituranthos* genus as a model system. Selected for its rich ethnobotanical history and its

adaptation to harsh, fluctuating environments, *Pituranthos* provides an ideal subject to dissect the precise mechanisms and metabolic trade-offs that govern the production of bioactive compounds under abiotic stress.

This thesis positions the *Pituranthos* genus as a critical model to address this knowledge gap, justified by its ecological significance and its under-explored potential in stress physiology. While numerous studies have catalogued the general medicinal properties and ecological adaptations of various desert plants, a significant and specific void exists regarding the *Pituranthos* genus. In the context of the Saharan environment a region experiencing acute climatic shifts the specific physiological and biochemical mechanisms underlying its adaptive responses to abiotic stress remain largely unknown. Although the phytochemical composition and biological activities of *Pituranthos* have been reported in other regions, studies systematically linking these traits to environmental drivers are scarce. Crucially, the role of key edaphic factors, such as soil composition and nutrient availability, in shaping its unique profile of osmolyte accumulation and overall stress resilience has not been fully explored. Therefore, this research is necessary and timely, as it seeks to provide the first comprehensive analysis of how Saharan environmental conditions, particularly soil properties, orchestrate the survival strategies and medicinal compound synthesis in *Pituranthos*, delivering actionable insights for its conservation and sustainable use under the threat of climate change.

To address this critical knowledge gap, this thesis is designed to systematically investigate the impact of individual and combined environmental stressors on *Pituranthos chloranthus* and *Pituranthos scoparius*. The overarching aim of this thesis is to elucidate the physiological and phytochemical responses of these plants combined drought and heat stress, and to evaluate the implications for its medicinal quality and sustainable cultivation. This thesis investigates the climate of the two regions where each plant prospers, soil properties of these plants, the accumulation of key bioactive compounds, specifically osmolytes, the correlation of the changes in phytochemical yield with the in vitro antioxidant, anti-inflammatory and antibacterial activity of plant extracts to assess functional medicinal potency.

The present study was planned to include the following parts:

PART 1: Bibliographical part contains three chapters

- Chapter I: Environmental factors as the key to medicinal plant adaptation.
- Chapter II: Bibliographic revision of the studied medicinal plants and their secondary metabolites.

- Chapter III: nanoparticles.

PART 2: Experimental part of our work

- Chapter IV: Materials and Methods.
- Chapter VI: Results and Discussion.

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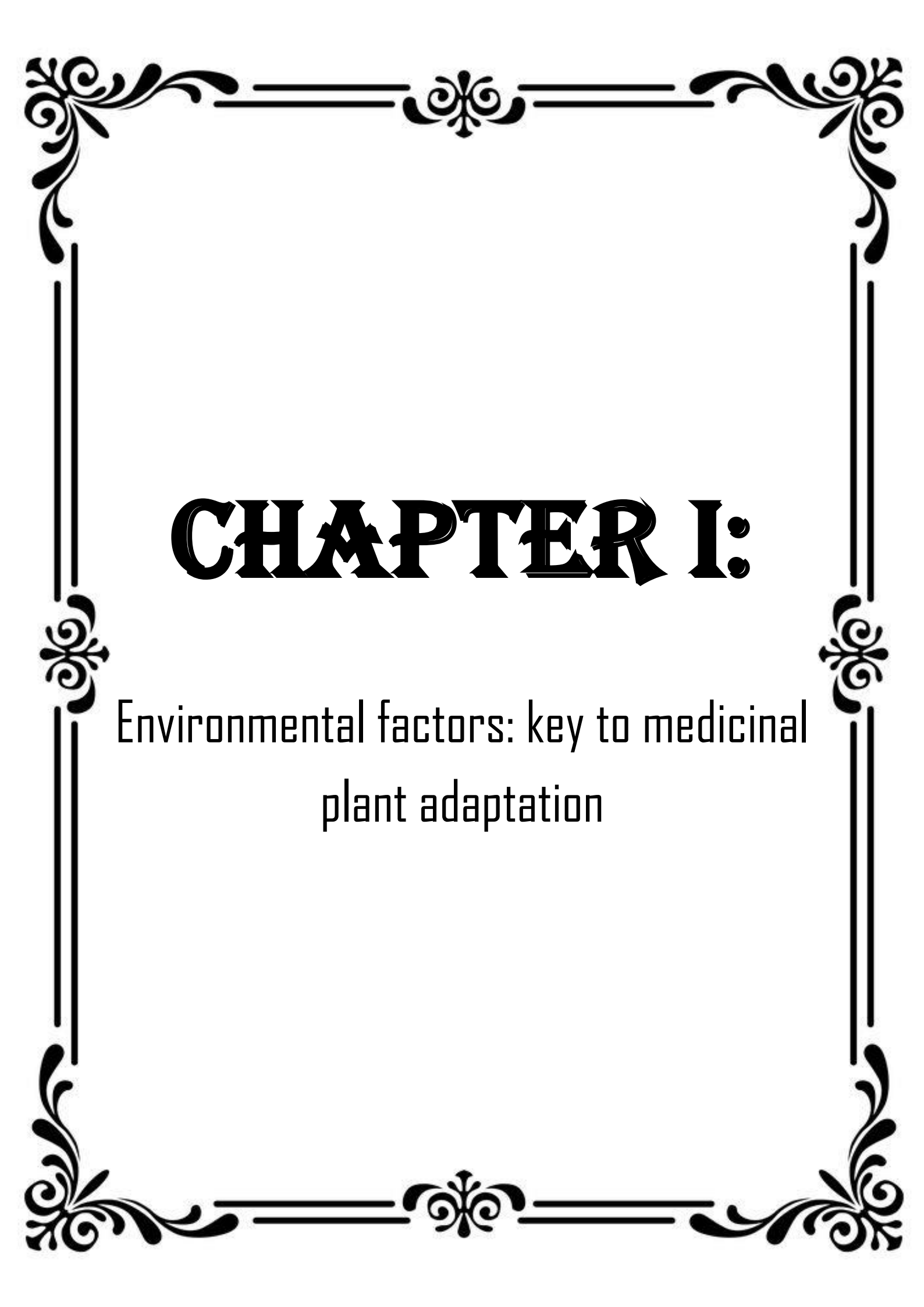
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First part

Bibliographic Synthesis



CHAPTER I:

Environmental factors: key to medicinal
plant adaptation



Introduction

Medicinal plants have been used for centuries in traditional and modern medicine due to their bioactive compounds, which possess therapeutic properties (Theodoridis *et al.*, 2023). Their secondary metabolites (alkaloids, flavonoids, terpenoids, phenolics, etc.) are crucial for pharmacological applications. However, the production and concentration of these compounds are highly influenced by environmental conditions, particularly climatic and edaphic (soil-related) factors (Yang *et al.*, 2018).

I. Abiotic factors

I.1. Definition

Abiotic factors are the non-living components of ecosystems such as climate and soil play a pivotal role in determining the survival, morphological traits, and biochemical profile of medicinal plants. Variations in temperature, light intensity, water availability, and soil composition can either enhance or suppress the synthesis of pharmacologically active compounds (Yang *et al.*, 2018), directly impacting the therapeutic value of these plants. As climate change exacerbates environmental stressors, understanding these relationships becomes critical for sustainable cultivation and conservation efforts (Q. Zhang *et al.*, 2021).

I.2. Classification

I.2.1. Climatic factors

Climatic factors are fundamental determinants of plant growth, distribution, and biochemical composition. For medicinal plants, these factors act as double-edged swords, while optimal conditions promote biomass accumulation and productivity, stressful climatic conditions (drought, extreme temperatures, UV radiation) often trigger the synthesis of pharmacologically active secondary metabolites as part of the plant's defense mechanisms (Porfirio *et al.*, 2017).

I.2.1.1. Temperature

Temperature is a critical climatic factor that profoundly influences the physiological and biochemical processes of medicinal plants. As a primary regulator of enzymatic activity, photosynthesis, and metabolic pathways, temperature extremes (both high and low) can induce stress responses that alter secondary metabolite production. For instance, elevated temperatures often accelerate oxidative stress, prompting plants to synthesize protective compounds like phenolics and terpenoids (Botha *et al.*, 2018). Conversely, cold stress may enhance alkaloid accumulation as a cryoprotective strategy. Optimal temperature ranges, however, vary by species; while tropical medicinal plants thrive at 25–30°C, temperate species require cooler conditions. With climate change driving erratic temperature fluctuations, understanding these



thermal thresholds becomes essential for predicting shifts in medicinal plant distribution, productivity, and phytochemical efficacy (El Gendy *et al.*, 2023).

I.2.1.2. Precipitation

Precipitation is a pivotal climatic factor that directly governs water availability, influencing the growth, survival, and synthesis of medicinal compounds in plants (Z. Chen *et al.*, 2020). Both water scarcity (drought) and excessive rainfall impose physiological stress, triggering adaptive responses that can alter the production of secondary metabolites. Drought conditions, for instance, often enhance the accumulation of osmolytes (proline, glycine betaine) and defense-related compounds (Adzigbe *et al.*, 2025). Conversely, waterlogged soils resulting from heavy precipitation can lead to hypoxia (oxygen deprivation), impairing root function and reducing the uptake of essential nutrients, thereby diminishing plant vigor and yields of bioactive compounds. The timing, intensity, and distribution of rainfall are equally critical; erratic precipitation patterns due to climate change may disrupt traditional growth cycles of medicinal species, threatening both wild populations and cultivated yields. Understanding these dynamics is crucial for developing sustainable cultivation strategies and predicting how future climate scenarios will affect the quality and availability of medicinal plants (Solanki *et al.*, 2024).

I.2.1.3. Light

Light is a fundamental climatic factor that regulates medicinal plant growth, development, and secondary metabolite production through its intensity, quality (wavelength), and photoperiod (day length). As the primary energy source for photosynthesis, light directly influences biomass accumulation, while specific wavelengths (UV-B, blue, red) act as signaling cues to modulate biochemical pathways (Seyedi *et al.*, 2024). For instance, UV-B radiation often stimulates the synthesis of phenolic compounds as a protective response against oxidative damage (Yang *et al.*, 2018). Similarly, blue light enhances the production of volatile terpenes in *Mentha piperita*, while red light can promote alkaloid biosynthesis in *Catharanthus roseus*. Photoperiodism further dictates phenological events such as flowering and dormancy. However, excessive light can cause photoinhibition, reducing photosynthetic efficiency, whereas insufficient light leads to etiolation and lowered metabolite production. With changing cloud cover and UV exposure due to climate change, understanding light-mediated responses is vital for optimizing cultivation conditions and ensuring consistent medicinal plant quality (Joshi *et al.*, 2025).



I.2.2. Edaphic factors

I.2.2.1. Soil pH

Soil pH is a master variable in medicinal plant cultivation, governing nutrient solubility, microbial activity, and metal toxicity. Slightly acidic to neutral soils (pH 5.5–7.0) optimize the availability of critical macronutrients (N, P, K) for most medicinal species, while extreme pH levels induce stress responses that alter secondary metabolite profiles. For example, *Camellia sinensis* (tea) thrives in acidic soils (pH 4.5–5.5), which enhance aluminum uptake to stimulate catechin synthesis (Pang *et al.*, 2025), whereas alkaline conditions (pH >7.5) may induce iron chlorosis in *Matricaria chamomilla*, reducing its essential oil yield. Soil pH also modulates rhizosphere microbiomes: acidic soils favor mycorrhizal associations that improve phosphorus acquisition in *Panax ginseng*, while alkaline soils promote actinobacteria beneficial for *Glycyrrhiza glabra* (licorice) growth. Notably, pH-dependent metal solubility (aluminum, manganese) can trigger defensive phenolic compound production in *Hypericum perforatum*. As soil acidification escalates due to agricultural practices and climate change, understanding pH-mediated phytochemical shifts becomes vital for maintaining medicinal plant quality and developing targeted soil amendments (Klein *et al.*, 2025).

I.2.2.2. Texture

Soil texture, determined by the relative proportions of sand, silt, and clay, critically influences water retention, root penetration, and nutrient availability for medicinal plants. Sandy soils (large particles) provide excellent aeration and drainage, reducing waterlogging risks but requiring frequent irrigation and fertilization due to low nutrient-holding capacity ideal for drought-tolerant species like *Aloe vera* (Alotaibi & Abd-Elgawad, 2023). In contrast, clay-rich soils (small particles) retain water and nutrients effectively but may impede root growth and oxygen diffusion, challenging species such as *Panax ginseng* that demand well-aerated substrates. Loamy soils, with balanced sand-silt-clay ratios, offer optimal conditions for most medicinal plants, combining adequate drainage with nutrient retention (Hakimzadeh Ardakani *et al.*, 2023). Soil texture also modulates microbial activity; clay particles harbor more bacteria that aid nitrogen fixation in *Glycyrrhiza glabra*. Furthermore, texture affects thermal properties: sandy soils warm faster in spring, benefiting early-growth species like *Calendula officinalis*, whereas clay soils buffer temperature fluctuations. Understanding these interactions is essential for site selection and soil amendment strategies to maximize medicinal compound yields under varying textural conditions (Benvenuti, 2023).

I.2.2.3. Nutrients

Soil nutrients are fundamental to medicinal plant growth, directly influencing biomass production and the synthesis of bioactive compounds. Macronutrients like nitrogen (N),



phosphorus (P), and potassium (K) play distinct roles: N promotes leaf growth and alkaloid accumulation (e.g., nicotine in *Nicotiana tabacum*), P enhances root development and energy transfer (critical for *Panax ginseng* saponins), while K regulates osmotic balance and flavonoid production (Miransari *et al.*, 2025). Micronutrients such as iron (Fe), zinc (Zn), and manganese (Mn) act as enzyme cofactors; for instance, Fe is essential for chlorophyll synthesis in *Mentha piperita*, and Zn boosts terpenoid yields in *Artemisia annua*. However, nutrient imbalances excess N or deficient Mg can disrupt secondary metabolism (Zhou *et al.*, 2022). Organic matter decomposition further mediates nutrient release, fostering symbiotic relationships. Precision nutrient management is thus vital to optimize both plant health and medicinal compound quality, especially in soils degraded by intensive agriculture or climate variability.

I.2.2.4. Salinity

Soil salinity, characterized by excessive soluble salts (NaCl , Na_2SO_4), disrupts medicinal plant growth through osmotic stress, ion toxicity, and nutrient imbalance. High salt concentrations reduce water uptake by roots, triggering physiological drought even in well-irrigated soils and force plants to allocate energy to osmolyte synthesis (proline or glycine betaine) to maintain cellular homeostasis. Concurrently, sodium (Na^+) and chloride (Cl^-) ions inhibit enzyme activity and photosynthesis (Joshi *et al.*, 2025). However, moderate salinity may enhance secondary metabolites as a defense response; for example, *Rosmarinus officinalis* increases antioxidant phenolics under salt stress, while *Catharanthus roseus* elevates vindoline alkaloids. Halophytic medicinal plants (*Portulaca oleracea*) thrive in saline conditions through specialized adaptations like salt glands or succulence. With global soil salinization escalating due to irrigation practices and sea-level rise, understanding these mechanisms is critical for breeding salt-tolerant varieties and saline agriculture strategies to sustain medicinal plant productivity (Dou *et al.*, 2025).

I.2.2.5. Organic matter

Soil organic matter (SOM), derived from decomposed plant and animal residues, serves as a cornerstone of medicinal plant productivity by enhancing soil structure, nutrient cycling, and microbial symbiosis. Rich in humus, SOM improves water retention in sandy soils while preventing compaction in clayey soils, creating optimal root conditions for species like *Panax ginseng* and *Echinacea purpurea*. It acts as a slow-release fertilizer, providing essential nutrients (N, P, S) through mineralization, which supports the synthesis of bioactive compounds for instance, higher SOM correlates with increased artemisinin in *Artemisia annua* and hypericin in *Hypericum perforatum*. Additionally, SOM fosters beneficial microbial communities (mycorrhizae and nitrogen-fixing bacteria) that enhance nutrient uptake and plant resilience (Rostaei *et al.*, 2024). In *Glycyrrhiza glabra*, SOM-rich soils boost glycyrrhizin production by



promoting rhizobial associations. However, excessive SOM can lead to waterlogging or nitrogen immobilization, stressing plants like *Lavandula angustifolia*, which prefer well-drained soils. With sustainable farming practices like cover cropping and compost application, maintaining balanced SOM levels is vital for optimizing both medicinal plant yield and phytochemical quality in the face of soil degradation and climate change(Xing *et al.*, 2025).

II. Abiotic stress and osmolyte accumulations in medicinal plants

II.1. Abiotic stress

II.1.1. Drought stress

Drought stress is a significant environmental challenge that affects agriculture, ecosystems, and water resources worldwide. As climate change exacerbates weather extremes, the frequency and severity of droughts are increasing, posing threats to food security and biodiversity (Nazari *et al.*, 2023).

Drought stress occurs when water availability falls below the demand required for normal physiological functions in plants, animals, and ecosystems. In agriculture, it refers to the condition where crops experience water deficiency, leading to reduced growth, yield losses, and even plant death(El Gendy *et al.*, 2023). Drought stress arises from a combination of natural and human-induced factors, including:

- climate change, which increases temperatures and evaporation rates, reducing soil moisture.
- Irregular rainfall patterns and prolonged dry spells disrupt water availability, while poor soil quality, such as sandy or degraded soils, fails to retain sufficient moisture.
- Human activities, such as deforestation, overgrazing, and excessive groundwater extraction.

further deplete water resources, exacerbating drought conditions. Together, these factors create water scarcity, negatively impacting agriculture, ecosystems, and water supplies (Solanki *et al.*, 2024).

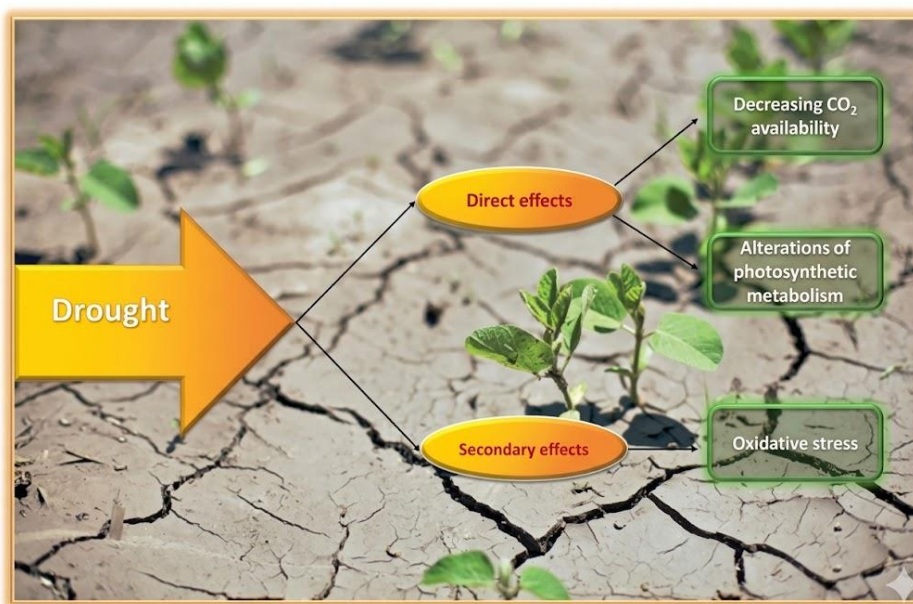


Fig 1. Effects of drought stress on plants(Nazari *et al.*, 2023)

Drought stress severely impacts plants by reducing photosynthesis, stunting growth, and causing oxidative damage, leading to lower crop yields and even plant death(Nazari *et al.*, 2023). On an ecosystem level, it threatens biodiversity by degrading habitats, increasing wildfire risks due to dry vegetation, and accelerating soil degradation and desertification. These effects disrupt agricultural productivity, water availability, and ecological balance, posing significant challenges to food security and environmental sustainability(Solanki *et al.*, 2024).

Drought stress is a growing concern due to climate change and unsustainable land use. Its impacts on agriculture, ecosystems, and water supplies necessitate urgent action through innovative farming techniques, improved water management, and global climate policies. By adopting resilient practices, societies can mitigate the adverse effects of drought and ensure sustainable resource use for future generations(Adzigbe *et al.*, 2025).

II.1.2. High Temperature Stress

High temperature stress, also known as heat stress, occurs when temperatures rise beyond a plant's optimal range, disrupting its physiological and biochemical processes. With global temperatures rising due to climate change, heat stress has become a significant threat to biodiversity, agriculture, reducing crop yields, and impacting food security(X. Zhang *et al.*, 2025).

II.1.2.1. Effects of High Temperature Stress on Plants

High temperature stress is a growing challenge for global agriculture, threatening both food crops and medicinal plants, many of which contain heat-sensitive bioactive compounds essential for pharmaceuticals(Shruti *et al.*, 2024). High temperature impacts the physiologically and biochemically plants:



- **Reduced Photosynthesis:** High temperatures damage chloroplasts and enzymes involved in photosynthesis (Singh *et al.*, 2021).
- **Membrane Damage:** Heat disrupts cell membrane integrity, causing leakage of ions and nutrients.
- **Protein Denaturation:** Enzymes and structural proteins lose functionality under extreme heat.
- **Oxidative Stress:** Increased production of reactive oxygen species (ROS) damages cells (Zulfiqar *et al.*, 2022).
- **Poor Pollination:** Heat stress during flowering reduces pollen viability, leading to lower fruit and seed set.
- **Accelerated Senescence:** Plants mature too quickly, shortening the grain-filling period in crops like wheat and rice.
- **Yield Losses:** Staple crops (maize, soybeans) can suffer 10-50% yield declines under severe heat stress (X. Zhang *et al.*, 2025).

II.1.3. Salinity stress

Salinity stress occurs when excessive salt concentrations in the soil disrupt plant water uptake and ion balance, leading to physiological drought, nutrient imbalances, and cellular damage. High sodium (Na^+) and chloride (Cl^-) ions interfere with enzyme activity, photosynthesis, and membrane integrity, while the osmotic stress reduces turgor pressure and stomatal opening (Dou *et al.*, 2025). Plants respond through osmotic adjustment (accumulating solutes like proline and glycine betaine), ion exclusion (limiting Na^+ uptake via root membranes), and compartmentalization (sequestering salts in vacuoles). Additionally, antioxidant systems combat salt-induced oxidative stress. Halophytes naturally tolerate salinity through specialized adaptations, whereas glycophytes (most crops) suffer growth inhibition or death. Understanding these mechanisms is critical for improving crop resilience in saline soils, which affect over 20% of irrigated farmland worldwide (Joshi *et al.*, 2025).

2.1.4. Heavy metal stress

Heavy metal stress occurs when plants are exposed to toxic metals (e.g., Cd, Pb, As), which disrupt cells by inhibiting enzymes, generating ROS, and competing with nutrients (Sulaiman *et al.*, 2024). Plants defend themselves through exclusion (root exudates, mycorrhizae), detoxification (phytochelatins, antioxidant enzymes), and hyperaccumulation.

II.2. Osmolytes Definition

Osmolytes are small, highly soluble organic molecules (proline, glycine betaine, sugars, and polyols) produced by plants under abiotic stress (drought, salinity, temperature extremes) to maintain cellular osmotic balance, protect macromolecular structures (proteins, membranes),



and scavenge reactive oxygen species (ROS) (Hasanuzzaman *et al.*, 1 C.E.). Unlike inorganic ions, osmolytes are compatible solutes they accumulate at high concentrations without disrupting enzyme function or cellular metabolism (Ashraf & Foolad, 2007). Their synthesis is a key adaptive strategy to stabilize hydration, prevent protein denaturation, and sustain metabolic activity under environmental stress, often indirectly enhancing the production of stress-induced secondary metabolites in plants (Nazari *et al.*, 2023).

2.2.1. Glycine betaine

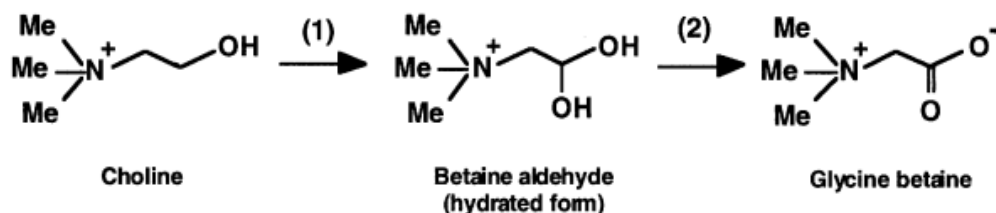
2.2.1.1. Definition

Glycine betaine (GB), chemically known as trimethylglycine, is a quaternary ammonium compound and a key osmolyte synthesized by plants, bacteria, and animals in response to abiotic stresses (e.g., drought, salinity, extreme temperatures). It is derived from choline oxidation in the chloroplasts and mitochondria via a two-step enzymatic pathway involving choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) (Annunziata *et al.*, 2019).

II.2.1.2. Synthesis of Glycine Betaine:

Glycine betaine (trimethylglycine) is synthesized through two primary pathways: (1) choline oxidation and (2) glycine methylation. In animals and humans, choline is first oxidized to betaine aldehyde via choline dehydrogenase (CHDH), followed by further oxidation to glycine betaine by betaine aldehyde dehydrogenase (BADH). Alternatively, in plants and bacteria, glycine betaine can be synthesized by the stepwise methylation of glycine using S-adenosylmethionine (SAM) as the methyl donor, catalyzed by glycine sarcosine methyltransferase (GSMT) and sarcosine dimethylglycine methyltransferase (SDMT). Some halophilic bacteria and archaea also produce glycine betaine from exogenously acquired choline or via de novo synthesis under osmotic stress (T. H. H. Chen & Murata, 2011). Industrially, glycine betaine is extracted from sugar beet molasses or produced synthetically for agricultural, pharmaceutical, and cosmetic applications (Figueroa-Soto & Valenzuela-Soto, 2018). Its biosynthesis is tightly regulated by osmotic stress signals, particularly in organisms adapting to high salinity or drought (Sakamoto & Murata, 2002).

(a)



(b)

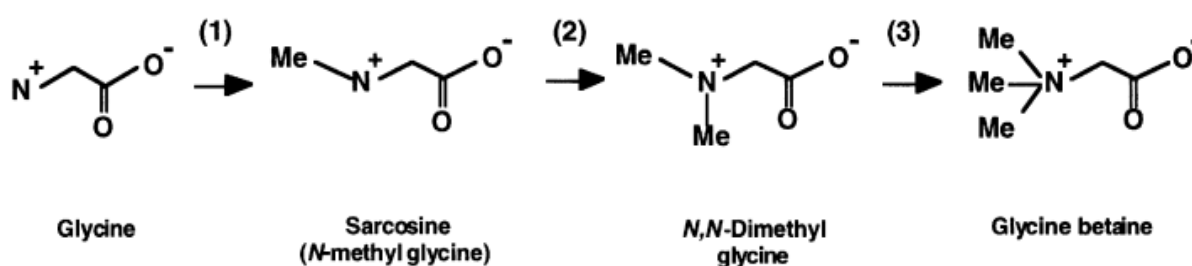


Fig 2. Two main pathways for the synthesis of GB(Sakamoto & Murata, 2002).

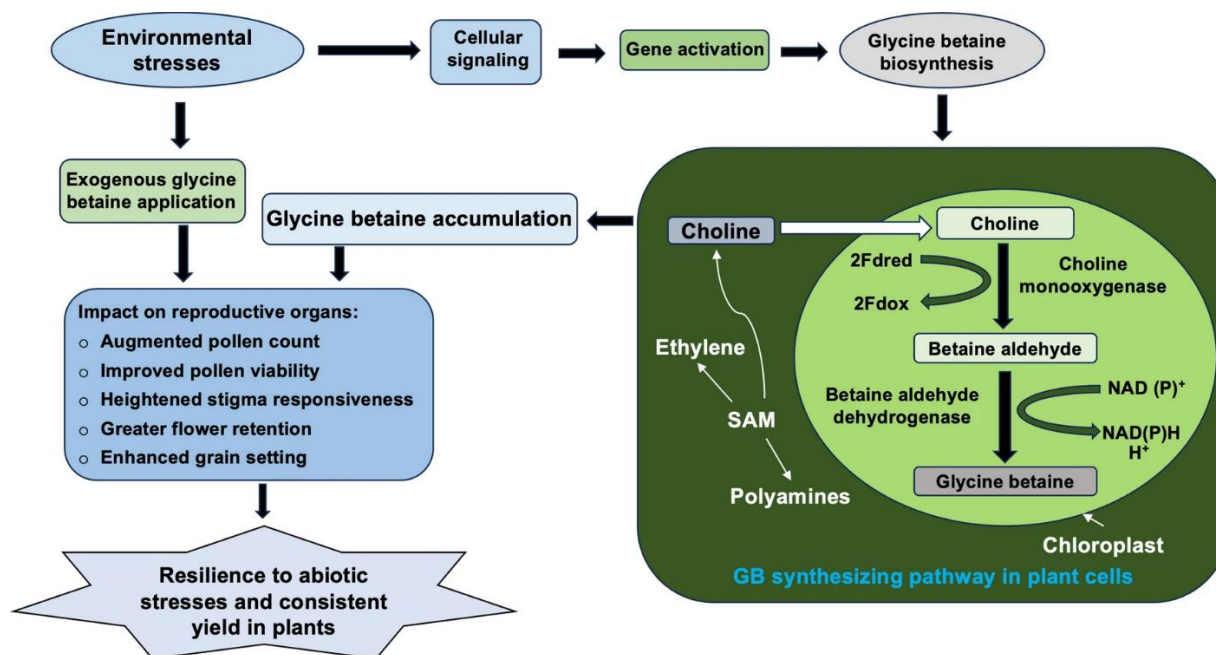


Fig 3. Schematic model of glycine betaine biosynthesis and accumulation in plants under abiotic stress(Jarin *et al.*, 2024)

II.2.1.3. General functions:

Glycine betaine is a crucial organic compound renowned in the biological world as a powerful osmoprotectant. Its primary function is to safeguard cells against environmental stress, particularly osmotic stress caused by drought, salinity, or extreme temperatures.

**a. Osmoprotection from drought and salinity stress:**

- Acts as a compatible solute, maintaining cell turgor and water balance under drought or high salinity by stabilizing osmotic potential.
- Reduces ion toxicity (Na^+/Cl^-) by balancing cytoplasmic solute concentrations (Annunziata *et al.*, 2019).

b. Protein and enzyme stabilization:

- Prevents denaturation of proteins and enzymes under heat, cold, or dehydration stress.
- Protects Rubisco (key photosynthetic enzyme) and other metabolic proteins (Sakamoto & Murata, 2002).

C. Photosynthesis protection:

- Safeguards photosystem II (PSII) from damage caused by oxidative stress (high light intensity + drought).
- Maintains chlorophyll content and electron transport efficiency (Figueroa-Soto & Valenzuela-Soto, 2018).

d. Antioxidant defense:

- Indirectly reduces reactive oxygen species (ROS) by stabilizing membranes and proteins, lowering oxidative damage.
- Enhances activity of antioxidant enzymes (SOD, CAT) in some plants (Li *et al.*, 2025).

E. Membrane integrity

- Prevents lipid peroxidation in cell membranes under stress (freezing, salinity).
- Maintains fluidity and functionality of thylakoid membranes in chloroplasts.

g. Cold and heat Tolerance:

- Buffers cells against freezing injury by cryoprotection.
- Reduces thermal aggregation of proteins during heat stress (Zulfiqar *et al.*, 2022)

h. Agricultural applications:

- ✓ Exogenous application of GB (via foliar sprays or seed priming) enhances crop resilience (wheat, maize, tomatoes).
- ✓ Engineered GB-accumulating plants show improved yields under abiotic stress (Lamlom *et al.*, 2025).

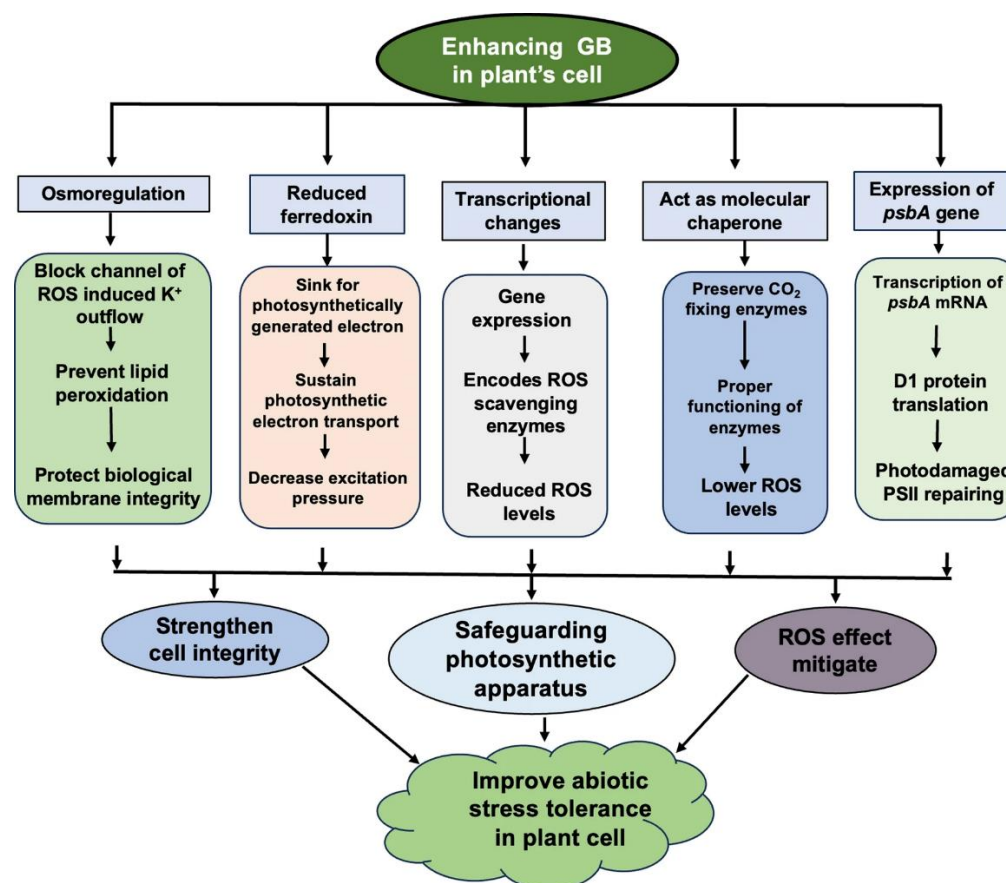


Fig 4. Function of glycine betaine (GB) in enhancing plant adaptation to abiotic stresses.

GB acts as a multifaceted protector by activating stress response genes, maintaining enzyme activity, safeguarding photosynthesis, preserving membrane integrity, and promoting reproductive stability. ROS, reactive oxygen species; PSII, Photosystem II(Jarin *et al.*, 2024)

II.2.2. Proline

II.2.2.1. Definition

Proline, as an organic nitrogen-rich compound, plays a subtle but important role in nutrient cycling within ecosystems. When plants die or shed tissues (leaves, roots), their accumulated proline is released into the soil. Microbial decomposers, such as bacteria and fungi, break down proline, converting its nitrogen into ammonium (NH_4^+) through mineralization (Mujtaba *et al.*, 2009). Soil bacteria can then nitrify this ammonium into nitrate (NO_3^-), a key plant-available nitrogen form. Since nitrogen is often a limiting nutrient in ecosystems, proline's decomposition helps sustain soil fertility and supports primary productivity (Hemadri Reddy *et al.*, 2024). Furthermore, in stressed environments (arid or saline soils), where proline-rich plant litter is more common, its efficient recycling by microbes ensures nitrogen retention rather than loss via leaching or volatilization. Thus, proline acts as a biochemical bridge between plant stress responses and soil nutrient dynamics, highlighting its ecological significance beyond cellular protection(Wei *et al.*, 2022).



II.2.2.2. Synthesis of proline

Proline synthesis in plants occurs primarily through two interconnected pathways: the glutamate pathway (dominant under stress) and the ornithine pathway (more active during growth and development). Both pathways contribute to proline accumulation as a response to environmental challenges (Wei *et al.*, 2022).

II.2.2.3. Ecological implications of proline synthesis

The synthesis and accumulation of proline, a well-known osmoprotectant, has significant ecological implications for plant survival and distribution. In arid, saline, or otherwise environmentally stressed ecosystems, the ability of a species to synthesize proline is a key determinant of its fitness and competitive ability:

- **Stress Adaptation:** Plants in arid or saline environments (halophytes, xerophytes) often exhibit higher proline synthesis capacity (Alvarez *et al.*, 2022).
- **Soil-Microbe Interactions:** Proline exuded from roots or released in litter influences microbial communities involved in nitrogen cycling.
- **Species Survival:** Variability in proline synthesis efficiency can determine a plant's resilience to climate extremes, shaping ecosystem composition (Kavi Kishor *et al.*, 2022).

Plants have evolved remarkable strategies to combat abiotic stresses like salinity, drought, and heavy metals, utilizing osmolytes for osmotic balance, antioxidants to mitigate oxidative damage, and secondary metabolites for defense and signaling. These adaptive mechanisms not only enhance survival under harsh conditions but also elevate the production of bioactive compounds in medicinal plants, enriching their therapeutic potential. Understanding these responses offers transformative applications from engineering stress-resilient crops to harnessing plant-derived pharmaceuticals bridging agricultural sustainability, ecological restoration, and medical innovation in the face of global environmental challenges.

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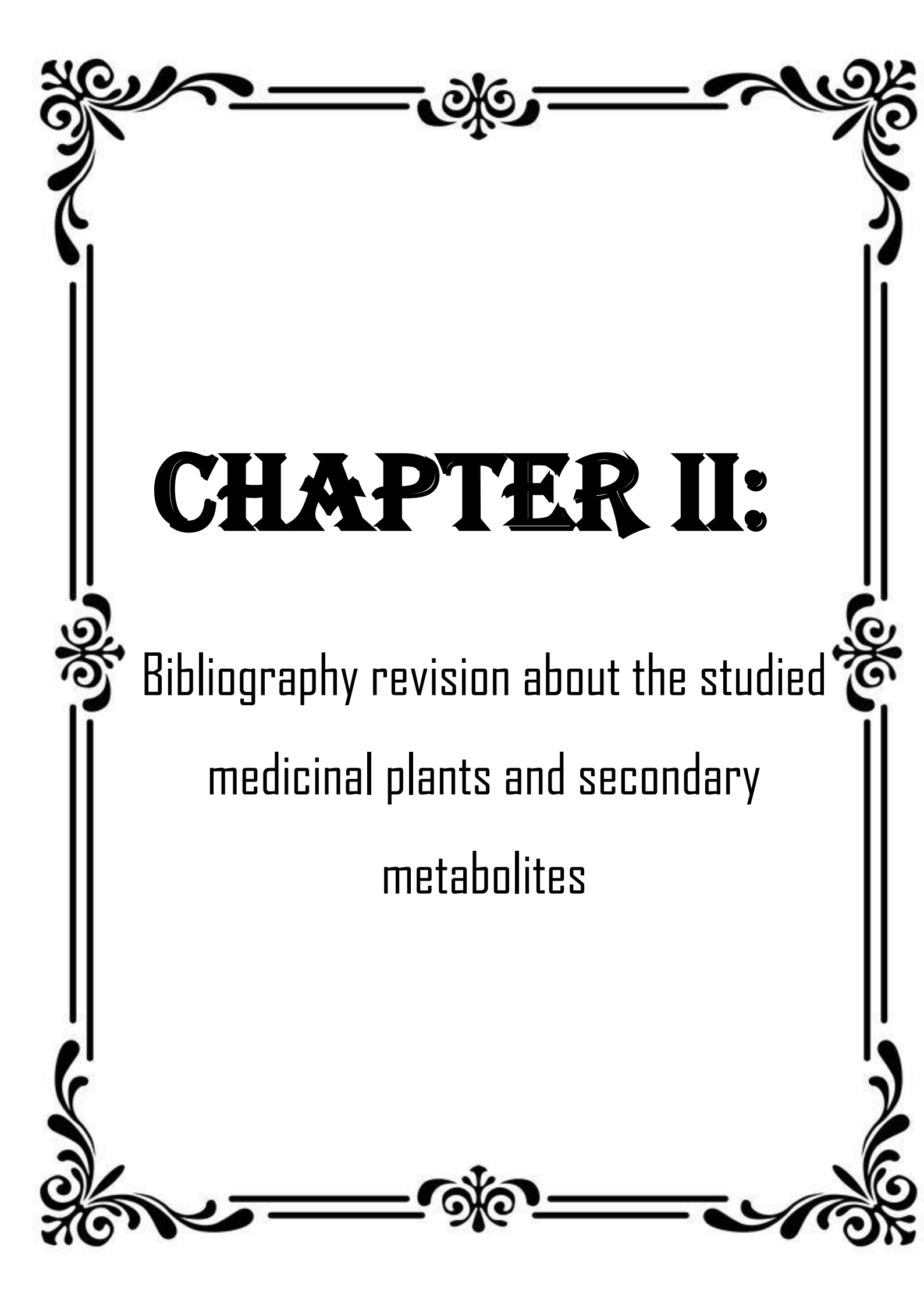
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CHAPTER II:

Bibliography revision about the studied
medicinal plants and secondary
metabolites



II.1. Generalities about apiaceae family

The Apiaceae family, known historically as Umbelliferae, holds a position of prime importance in the history of botany. It was not only the first family of flowering plants to be formally recognized by botanists in the late 16th century but also the subject of Robert Morison's seminal systematic study in 1672, which laid foundational principles for plant classification (Vilmorin, 1959). Taxonomically, this family, belonging to the Spermatophytes, encompasses a significant diversity of approximately 3,000 to 3,750 species spread across 300 to 455 genera. However, this very diversity presents a notable challenge: species within Apiaceae are notoriously difficult to distinguish from one another due to their often conserved floral morphology, a phenomenon that has perplexed botanists for centuries and is particularly evident in genera like *Pituranthos* (Spinozzi *et al.*, 2021)

This taxonomic complexity is inextricably linked to the family's remarkable global ecology. Apiaceae is a cosmopolitan family, with a distribution that spans temperate, subtropical, and tropical regions across all inhabited continents. Its center of diversity lies in the Northern Hemisphere, especially in the Mediterranean Basin, a global biodiversity hotspot, as well as across the varied landscapes of Asia, Europe, and North America (Sayed-Ahmad *et al.*, 2017). The family demonstrates extraordinary ecological plasticity, with species adapted to a stunning range of habitats from coastal cliffs and alpine meadows to arid deserts, wetlands, and temperate woodlands (Sousa *et al.*, 2021).

II.2. Importance of the Apiaceae family

The Apiaceae family holds profound ecological, economic, and medicinal significance, making it a focus of sustained research. Ecologically, its characteristic umbels are crucial for supporting pollinators and soil health, though some species can be invasive. Economically, the family is a cornerstone of global agriculture and industry, supplying essential root vegetables, leafy greens, spices, and high-value essential oils for perfumery and cosmetics (Sayed-Ahmad *et al.*, 2017). Medicinally, its species are treasure troves of bioactive compounds, with traditional uses as digestive aids and modern applications demonstrating anti-inflammatory, antispasmodic, and anticancer properties, underscoring their vital role in pharmaceutical discovery and human health (Ameur *et al.*, 2025).

II.3 *Pituranthos* genus

In the Arabian Ecoregion, plants belonging to the *Pituranthos* (Deverra) genus are referred to by various local names, including Shabbat al Jabel, Gozzah, Gazzoh, and Guezzah . These species are widely recognized in North African and Arabian traditional medicine. Numerous studies (Guetat, 2022; Hammiche & Maiza, 2006; Vérité *et al.*, 2004; Yanguì *et al.*,



2009) have documented their medicinal applications. Traditionally, *Deverra* species have been used to treat various ailments, such as asthma, rheumatism, fever, hepatitis, diabetes, and digestive disorders (Adida *et al.*, 2016; Guetat, 2022; Karbab *et al.*, 2021; Hammiche & Maiza, 2006).

The genus *Pituranthos* is one of the smallest genera in the family Apiaceae (Umbelliferae) comprises approximately 10–12 recognized species of perennial, aromatic shrubs or subshrubs. The genus has a disjunct distribution, with species occurring, mainly, in North Africa and Arabian ecoregion distributed in arid regions. These plants are characterized by finely dissected, pinnate leaves, often glaucous or covered with a waxy coating to reduce water loss. The stems are typically woody at the base and herbaceous toward the tips, bearing small, white or yellowish flowers arranged in compound umbels; a defining feature of the Apiaceae family (Benabderrahim *et al.*, 2019; Guetat, 2022; Guetat *et al.*, 2022; Kamel *et al.*, 2022; Oueslati *et al.*, 2021). The fruits are small, ribbed schizocarps that split into two mericarps upon maturity. *Pituranthos* species exhibit xerophytic adaptations, including deep root systems and reduced leaf surfaces, which enable them to survive in drought-prone environments. species, Common *Pituranthos* species are: *Pituranthos tortuosus*, *Pituranthos chloranthus*, *Pituranthos scoparius*, *Pituranthos triradiatus*, *Pituranthos battandieri*.

The genus *Pituranthos* is predominantly distributed across arid and semi-arid regions of North Africa and the Middle East, with its core range extending from the Maghreb (Morocco, Algeria, Tunisia, Libya) through Egypt and the Sinai Peninsula, into the Arabian Peninsula (Saudi Arabia, Yemen, Oman). Several species exhibit narrow endemism, such as *Pituranthos battandieri* in Morocco and *Pituranthos triradiatus* in southwestern Arabia, while others, like *Pituranthos tortuosus*, have broader ecological amplitudes, spanning the Sahara-Sindian phytogeographical zone. The genus thrives in rocky deserts, wadis, and sandy plains, typically in areas with minimal rainfall, reflecting its adaptation to xeric conditions (Guetat, 2022).

II.3.1. Medicinal use and biological, pharmacological effects

Different parts of *Pithranthos* species, such as leaf, stem, and fruit, have found different uses for a wide range of purposes. Among them, *Pituranthos* has been used for a long time as a useful remedy for different diseases: respiratory, treatment of cough, asthma, liver disorders, we summarized some scientific researches about this genus in the table x below.

Table 1. Biological and pharmacological effects of *Pituranthos* species

Species	Regions	Plant part used	Biological and pharmacological effects r	References
<i>Pituranthos chloranthus</i> ,	Tunisia	Aerial part, leaves (essential oil)	Antibacterial activity, antioxidant	(Lahmar <i>et al.</i> , 2018)
<i>Pituranthos chloranthus</i>	Tunisia	Aerial part (essential oil)	Reversal of resistance in bacteria underlies synergistic effect of essential oils with conventional antibiotics	(Lahmar <i>et al.</i> , 2017)
<i>Pituranthos chloranthus</i> ,	Tunisia	Aerial part (essential oil)	Bactericidal, fungicidal activities, natural disinfectant.	(Yangui <i>et al.</i> , 2009)
<i>Pituranthos chloranthus</i> ,	Tunisia	arial part (aqueous extract)	Antioxidant and anti-inflammatory activities using	(Rk, 2022)
<i>Pituranthos chloranthus</i> ,	Algeria	aerial parts (essential oil)	Antioxidant, Antibacterial, and antifungal activities.	(Lakhdari <i>et al.</i> , 2020)
<i>Pituranthos triradiatus</i>	Saudi Arabia	Each single part.	Antioxidant activity, antifungal	(Guetat <i>et al.</i> , 2022)
<i>Pituranthos chloranthus</i>	Tunisia	Stems and seeds (essential oils)	Antioxidant Activity	(Benissa <i>et al.</i> , 2022)
<i>Pituranthos chloranthus</i>	Tunisia	seeds and stems (essential oils)	Antioxidant Activities	(Aloui & and Zouari, 2019)
<i>Pituranthos tortuosus</i>	Tunisia	Stems, flowers and the seeds (essential oils)	Antioxidant Activities	(Aloui <i>et al.</i> , 2018)
<i>Deverra tortuosa</i> <i>Deverra triradiata</i>	Egypt.	Essential Oil Nanoemulsion	Anti-inflammatory, Antioxidant Activities	(Kamel <i>et al.</i> , 2022)
<i>Pituranthos tortuosus</i>	Tunisia	ethanol extracts of aerial parts	Antioxidant Activities	(Benabderrahim <i>et al.</i> , 2019)
<i>Pituranthos chloranthus</i>	Tunisia	essential oils obtained from aerial parts	antibacterial activities, Antioxidant Activities	(Neffati, Bouhlel, <i>et al.</i> , 2009)
<i>Pituranthos chloranthus</i>	Tunisia	essential oils obtained from aerial parts from different stations	antimutagenic activities, antibacterial activities, Antioxidant Activities	(Neffati <i>et al.</i> , 2009)
<i>Deverra tortuosa</i>	Saudi Arabia	Seeds	inhibitory activity, Antibacterial activities	(Oueslati <i>et al.</i> , 2021)
<i>Pituranthos triradiatus</i>	The Negev Desert	the shoots	Morphological and chemical variations	(Ashkenazy <i>et al.</i> , 1987)



<i>Deverra tortuosa</i>	Tunisia	stems and umbels	Antioxidant, antifungal, herbicidal activities essential Oils	(Khammassi <i>et al.</i> , 2023)
<i>Pituranthos scoparius</i>	Algeria	The aerial part	Essential Oil Chemical Variability	(Malti <i>et al.</i> , 2018)
<i>Pituranthos scoparius</i>	Algeria	The aerial part	antimicrobial and antioxidant activities of the <i>Pituranthos scoparius</i> essential oil.	(Ksouri <i>et al.</i> , 2017)
<i>pituranthos scoparius</i>	Algeria	The aerial part	oils composition	(Lograda <i>et al.</i> , 2013)
<i>Pituranthos tortuosus</i>	North Africa,	The aerial parts	ethnobotanical reports	(Najjaa <i>et al.</i> , 2017a)
<i>Pituranthos chloranthus</i> and <i>Pituranthos tortuosus</i>	Southern Tunisia	Fresh and dry aerial part (essential oils)	Chemical Composition and Antimicrobial Activity	(Mighri <i>et al.</i> , 2015)
<i>Pituranthos scoparius</i>	Tamanrasset	methanol extracts of the aerial part	Screening phytochimique, activités antioxydantes et pouvoir hémolytique	(Haddouchi <i>et al.</i> , 2016)
<i>Pituranthos chloranthus</i>	province of El Menia	Aqueous extract of the aerial part	Physicochemical and biological properties assessment	(Elhouda <i>et al.</i> , 2024)
<i>Pituranthos scoparius</i>	Bechar (Algeria)	aqueous extracts aerial	Phytochemical study and evaluation of the antiradical activity	(Adida <i>et al.</i> , 2016)
<i>Pituranthos scoparius</i>	Setif region,	The n-butanol extract(stem)	examine the ethnomedicinal uses, identify the chemical constituents, and to determine the toxicity and anti-inflammatory effects of these compounds in addition to the extract.	(Karbab <i>et al.</i> , 2021)
<i>pituranthos scoparius</i>	Algeria	Essential oil content of the flowers	Identification of essential oil content	(Smaili <i>et al.</i> , 2011)
<i>Deverra scoparia</i>	Algeria	stems and flowers	investigate at a large scale the variability of the chemical composition of the EOs	(Benarfa <i>et al.</i> , 2020)
<i>D. scoparia</i>	Algeria	Stems (essential oil)	Comparative chemical profiling and antioxidant properties of essential oil.	(Mechraoui <i>et al.</i> , 2024)
<i>Pituranthos scoparius</i>	Algeria	The essential oils of the aerial parts	Chemical Composition of the Essential Oil	(Gourine <i>et al.</i> , 2011)



<i>Pituranthos battandieri</i>	Bechar	The aerial parts	Bio-guided isolation of leishmanicidal and trypanocidal	(Mennai <i>et al.</i> , 2021)
<i>Pituranthos scoparius</i>	Algeria	aerial part	Assessment of antioxidant potential and phytochemical analysis	(Belyagoubi-Benhammou <i>et al.</i> , 2017)
<i>Pituranthos scoparius</i>	Algeria	The aerial parts (essential oil)	Antioxidant effect	(Chikhoune <i>et al.</i> , 2017)
<i>Pituranthos scoparius</i>	Ghardaia	The stems and leaves (essential oil) and ethanolic extract	Antioxidant and antibacterial properties	(Ameur <i>et al.</i> , 2025)

II.3.2. *Pituranthos chloranthus*

Pituranthos chloranthus (Coss. & Durieu) is a perennial, xerophytic shrub that typically reaches 30–80 cm in height, characterized by a highly branched, woody stem covered in striated, glabrous bark that becomes fibrous with age (Yanguì *et al.*, 2009). Its leaves are alternate, sessile, and finely dissected into linear or filiform segments (5–20 mm long), giving them a needle-like appearance; they are glaucous-green and emit a strong aromatic odor when crushed, a trait linked to their essential oil content (Hammiche & Maiza, 2006). *Pituranthos chloranthus* produces compound umbels (3–5 cm in diameter), a defining trait of the Apiaceae family. Each umbel consists of 5–12 unequal rays, bearing small (1.5–2 mm diameter), yellowish-green flowers with five petals and prominent stamens. Flowering occurs from March to June, with a peak in April, coinciding with the seasonal rainfall in its native arid habitats (Neffati, Bouhlel, *et al.*, 2009). Fruits are a schizocarp (3–4 mm long), splitting into two mericarps with five distinct, winged ribs, aiding wind dispersal. Its seeds are albuminous, with a thin, fibrous seed coat that aids in drought resistance (Benissa *et al.*, 2022).



Fig 5. *Pituranthos chloranthus* (original photos)

Pituranthos chloranthus is endemic to the arid and semi-arid regions of North Africa, with a distribution primarily concentrated in northeastern Algeria and northwestern Tunisia (Lakhdari *et al.*, 2020). It occurs in coastal and inland desert areas, particularly in the Tell Atlas and Saharan Atlas Mountain ranges, where it grows in rocky slopes, gravel plains (regs), and wadi systems. The species is notably associated with limestone and gypsum-rich substrates, reflecting its adaptation to alkaline soils. Its range coincides with the Mediterranean-Saharan transition zone, characterized by hot, dry summers and limited winter rainfall. Isolated populations have been recorded in northern Libya (Guetat, 2022).

II.3.2.1. Systematic classification

To summarize the systematic position, the species *Pituranthos chloranthus* can be classified according to Ozenda's Classification (Vilmorin, 1959).

Kingdom: Plantae (Plants)

Embranchement : Tracheophyta

Sub-Embranchement : Angiospermae (Angiosperms)

Class: Dicotyledons

Subclass: Rosidae

Order: Apiales

Family: Apiaceae

Genus: *Pituranthos*

Species: *Pituranthos chloranthus*



II.3.2.2. Ethnobotanical research and traditional use

Traditional medicine remains the primary resource for the majority of populations to address their health problems. Passed down orally from one generation to the next, this knowledge faces the risk of being lost over time. Today, ethnobotanical and ethno-medicinal studies are recognized as essential methods for documenting medicinal plants and their uses (Najjaa *et al.*, 2017b).

In Algeria and neighboring regions, medicinal plants serve as both natural food supplements and remedies in folk medicine. Various *Pituranthos* species have been documented for their therapeutic applications (Hamza *et al.*, 2019; Lakhdari, 2022; Malti *et al.*, 2018; Vérité *et al.*, 2004). For instance, *Pituranthos scoparius* stem and seed oils are traditionally administered to alleviate rheumatism and fever. Furthermore, *Pituranthos chloranthus* (locally called Guezzah) is prepared as a head poultice to combat migraines (Benissa *et al.*, 2022).

Pituranthos chloranthus serves two key purposes: its unique fragrance and taste are prized for enriching dried fruits, while its edible parts feature prominently in North African cuisine. Moroccan Berbers, for example, eat the tender shoots and raw root cores, combining the plant's above-ground portions with ash to flavor roasted meats and flatbreads (Ouabou *et al.*, 2025). Beyond culinary uses, it acts as a traditional seasoning and a natural preservative in cheese production, with water-based extracts extending shelf life (Lahmar *et al.*, 2018).

Traditionally, *P. chloranthus* stems have served as drying straw for figs and grapes in agriculture. In southern Tunisia, its flowers are placed on rainwater storage containers to purify the water for drinking (Yangui *et al.*, 2009) and to obtain sweet extracts. In Djannet, southern Algeria, locals apply this plant as a remedy for scorpion stings. Furthermore, *Pituranthos chloranthus* exhibits insecticidal effects (Ouabou *et al.*, 2025).

II.3.3. *Pituranthos Scoparius* (*Deverra Scoparia*)

P. scoparius (synonym *Deverra scoparia*) is a plant native to North Africa's Sahara. While scarce in the central Sahara, it thrives in the Tassili, the Saharan Atlas, and the northern Sahara (Lograda *et al.*, 2013). Locally, it is referred to as 'Tattayet' (Tamahaq/Tuareg) and 'Guezzah' (Arabic).

Pituranthos scoparius (Coss. & Durieu) Benth. & Hook.f., a member of the Apiaceae family, is a perennial Saharan shrub adapted to arid environments. Morphologically, it features slender, woody stems and finely dissected, needle-like leaves that reduce water loss. The plant produces small, white to yellowish umbel inflorescences, characteristic of the Apiaceae family, with compound flowers. Its root system is robust, often deep or extensive, aiding in drought resistance. *P. scoparius* emits a strong aromatic scent due to essential oils stored in glandular

trichomes, which contribute to its ecological and ethnobotanical significance (Ksouri *et al.*, 2017). The fruit is a schizocarp, splitting into mericarps upon maturity. Endemic to North Africa, it thrives in rocky deserts and high plateaus, notably in the Tassili n'Ajjer and Saharan Atlas regions (Vérité *et al.*, 2004).



Fig 6. *Pituranthos scoparius* (original photo)

II.3.3.1 Systematic classification

According to (Vilmorin, 1959), the classification of *Pituranthos scoparius* is:

Kingdom: Plantae (Plants)

Embranchement : Tracheophytes

Sub-Embranchement : Angiosperms

Class: dicotyledons

Subclass: Asterids

Order: Apiales

Family: Apiaceae

Genus: Pituranthos

Species: *Pituranthos scoparius* (Coss. & Durieu) Benth. & Hook

Synonyms: *Deverra scoparia* Coss. & Durieu

II.3.3.2 Traditional, medicinal uses of *Pituranthos scoparius*

Pituranthos scoparius (or *Deverra scoparia*) is a well-known herbal remedy in North African traditional medicine, particularly in Algeria, where it addresses a range of health conditions (Vérité *et al.*, 2004).

Beyond its medicinal applications, it serves as a culinary flavoring agent. Practitioners of folk medicine use it to manage rheumatic pain, respiratory issues like asthma, infectious diseases (measles, jaundice), gastrointestinal problems, postpartum recovery, and abdominal



discomfort (Malti *et al.*, 2018). It also aids in relieving headaches, muscle spasms, chronic pain, metabolic disorders like diabetes, liver diseases (hepatitis), and urinary tract infections (Guetat, 2022).

II.4. Secondary metabolites

Plants produce a vast array of organic compounds, traditionally classified as primary and secondary metabolites; however, the distinction between these categories isn't always clear. Primary metabolites, including phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, play crucial roles in fundamental processes such as photosynthesis, respiration, and growth (Najjaa *et al.*, 2017b).

In contrast, secondary metabolites are structurally diverse and often accumulate in surprisingly high concentrations in certain plants. Many of these compounds are species-specific, making them useful in chemotaxonomic studies. Once understudied, their ecological functions are now gaining recognition; they protect plants from herbivores and microbial infections, attract pollinators and seed-dispersing animals, act as allelopathic agents, shield against UV radiation, and even serve as signaling molecules in processes like nitrogen-fixing root nodule formation in legumes (Youssef *et al.*, 2023).

Plant secondary metabolites are diverse molecules that support environmental adaptation but are not essential for core cellular processes like growth and reproduction. Unlike primary metabolites, these specialized compounds are produced primarily for defense and are often restricted to particular plant species or genera (Singh *et al.*, 2023).

Based on their biosynthetic pathways, plant secondary metabolites are categorized into three major groups:

1. Phenolic compounds
2. Nitrogen-containing alkaloids
3. Terpenes.

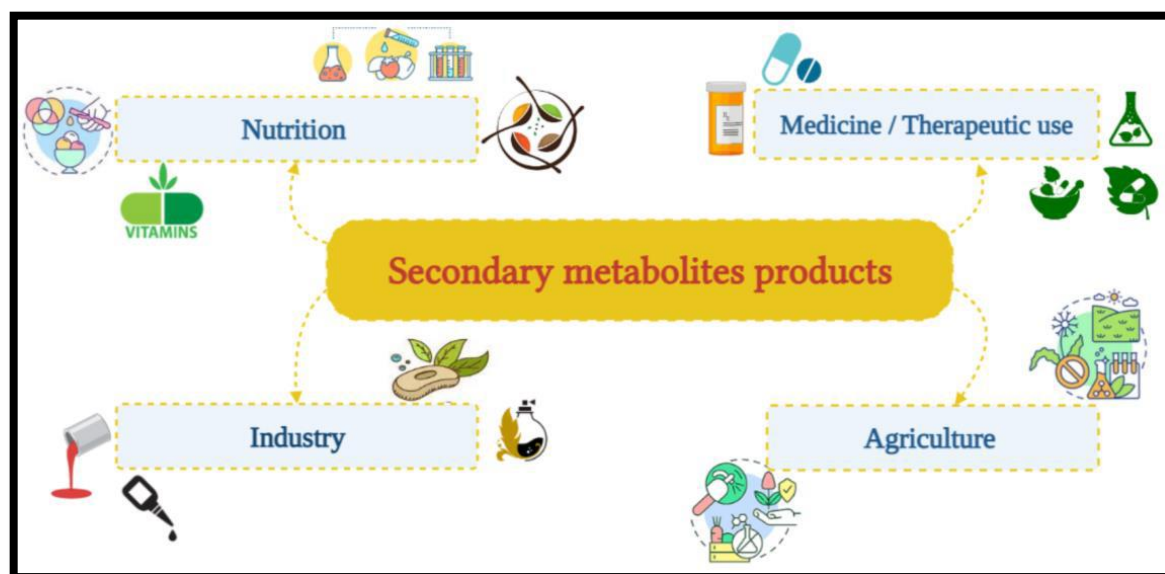


Fig 7. Fields of use of secondary metabolism products produced by plants

II.4.1. Chemical compounds in *Pituranthos chloranthus*

The documented biological activities of *Pituranthos chloranthus* are primarily attributed to its essential oil, a complex mixture rich in volatile compounds (Aloui *et al.*, 2018). The oil's composition is subject to variation based on geographic origin and plant part, but it is consistently dominated by monoterpene hydrocarbons (limonene, α -pinene, sabinene) and phenylpropenes, most notably myristicin, which confers a characteristic aroma (Lakhdari *et al.*, 2020). Beyond its volatile fraction, the plant also contains glycosylated flavonoids (Touil *et al.*, 2006). The combined action of these phytochemicals confers strong antioxidant, antimicrobial, and insecticidal properties, which directly support its traditional applications in treating ailments and preserving food (Neffati, Bouhlef, *et al.*, 2009).

II.4.2. Chemical compounds in *Pituranthos scoparius*

The Apiaceae family is known for its richness in coumarins, particularly furanocoumarins, which are considered responsible for numerous biological activities observed in plants of this family (Hamada *et al.*, 2004).

According to (Dahia *et al.*, 2009) the methanolic and aqueous extracts of *Pituranthos scoparius* (Coss. & Dur.) Benth. & Hook. have been analyzed and fifteen metabolites were identified: two cinnamic acids (5-O-caffeoyl quinic acid and 5-feruloyl quinic acid), and thirteen known flavonoids (vicenin-2, six quercetin and six isorhamnetin O-glycosylated derivatives). 5-O-caffeoylquinic acid was the main component, while, of the flavonoids, the isorhamnetin derivatives were present to a greater extent.

The essential oil and organic extracts of *Pituranthos scoparius* have been the subject of phytochemical investigations (Mechraoui *et al.*, 2024), revealing a diverse profile of bioactive

compounds. The volatile fraction is frequently characterized by a high abundance of monoterpene hydrocarbons. Notably, analyses have identified α -pinene, limonene, and β -pinene as major constituents, contributing to the plant's aromatic properties (Smaili *et al.*, 2011). Beyond its volatile components, research on the non-volatile fractions, particularly from the stems and roots, has led to the isolation of various coumarins and furocoumarins, such as scoparone, imperatorin, and xanthotoxin, which are known for their biological activities (Hamada *et al.*, 2004). Furthermore, phytochemical studies have also reported the presence of flavonoids and alkaloids, adding to the chemical complexity of this species and providing a basis for its traditional uses in folk medicine (Haddouchi *et al.*, 2016). The specific chemical composition can vary significantly depending on the plant part, geographical location, and period of harvest.

II.4.3. Terpenes

They are the primary constituents of essential oils and are responsible for the aromatic and flavor profiles of many herbs, flowers, and fruits. Structurally, terpenes are built from repeating units of isoprene (C_5H_8), forming chains or rings. They can be simple (monoterpenes, like limonene) or complex (triterpenes, like squalene). Terpenes play ecological roles in attracting pollinators, repelling herbivores, and aiding plant defense. Some terpenes have medicinal properties and are used in perfumes, food additives, and pharmaceuticals (menthol, beta-carotene, and taxol)(Ninkuu *et al.*, 2021a).

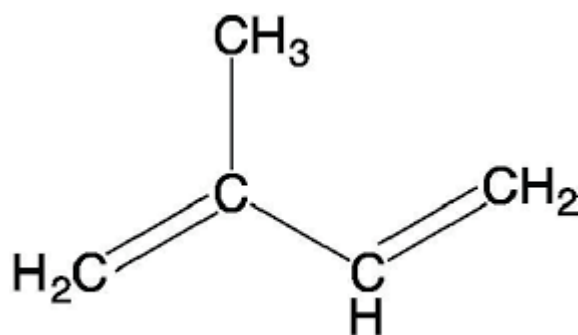


Fig 8. Structure of Isoprene

- **Classification of terpenes**

All molecules in this group are derived from isoprene units, each containing at least one 5-carbon isoprene structure. Their classification depends on the number of linked isoprene units, leading to categories such as hemi-, mono-, sesqui-, di-, tri-, and tetraterpenoid carotenoids, which contain 1, 2, 3, 4, 6, and 8 isoprenoid residues, respectively. Additionally, polyterpenes are formed by extended chains of multiple isoprene units (Ninkuu *et al.*, 2021b)

Table 2. Classification of terpenes

Terpene Class	Isoprene Units	Carbon Atoms (C)	Examples
Hemiterpenes	1	C ₅	Isoprene, Prenol
Monoterpenes	2	C ₁₀	Limonene, Myrcene, Pinene, Linalool
Sesquiterpenes	3	C ₁₅	β-Caryophyllene, Farnesene, Bisabolol
Diterpenes	4	C ₂₀	Taxol, Phytol, Cafestol
Sesterpenes	5	C ₂₅	Ophiobolin, Retinol (Vitamin A form)
Triterpenes	6	C ₃₀	Squalene, β-Sitosterol, Lanosterol
Tetraterpenoids	8	C ₄₀	Lycopene, β-Carotene, Lutein

• Biological Effects of Terpenoids

Terpenoids, a diverse class of naturally occurring organic compounds derived from isoprene units, exhibit a wide range of biological effects. Many terpenoids possess significant pharmacological properties, including anti-inflammatory, antimicrobial, anticancer, and antioxidant activities. For instance, monoterpenoids like limonene and menthol have demonstrated anticancer effects by inducing apoptosis in tumor cells, while sesquiterpenoids such as artemisinin are renowned for their potent antimalarial properties (Smaili *et al.*, 2011). Additionally, diterpenoids like taxol (paclitaxel) are widely used in chemotherapy due to their ability to inhibit cell division. Beyond their medicinal applications, terpenoids play crucial ecological roles, acting as defense compounds in plants against herbivores and pathogens, as well as attracting pollinators through their aromatic qualities. Their structural diversity and biological versatility make terpenoids valuable in both therapeutic and industrial contexts (Ninkuu *et al.*, 2021a).

Table 3. Major components reported in *Pituranthos scoparius* essential oils

Species	<i>Pituranthos scoparius</i>								
references	A						B	C	
localities	Ghardaïa	Ghardaïa	Algeria	Algeria	Laghout	Djelfa	Algeria	Djelfa	
Part of plant	Stem	seed	Stem	Seed	Aerial part	Aerial part	stem	Flower Season 1	Flower Season 2
α -pinene	8.2	6.8	11.0	34.0	35.1	26.7	0-33	35.14	37.70
Limonene	11.2	9.8	0.0	0.0	30.0	7.8	1.9-14.33	8.25	7.69
Germacrene D	1.0	12.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
myristicin	11.1	7.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dill apiole	12.2	1.1.	0.0	0.0	9.9	0.0	0.0	9.91	13.66
Bornyl acetate	0.0	0.0	21.0	0.2		9.6	0.0	0.0	0.0
Apiol	0.0	0.0	52.8	15.0	0.0	0.0	0.0	0.0	0.0
β -pinene	0.0	0.0	0.0	0.0	5.2	30.3	2.59-9.13	5.63	4.23
Sabinene	0.0	0.0	0.0	0.0	0.0	0.0	1.93-19.9	0.0	0.0
δ -3-carene	0.0	0.0	0.0	0.0	0.0	0.0	0.04-10.39	8.72	0.0
p-cymene	0.0	0.0	0.0	0.0	0.0	0.0	0.7-9.41	0.0	0.0
A-terpinene	0.0	0.0	0.0	0.0	0.0	0.0	0.2-10.46	0.0	0.0
γ - Muurolene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.52	3.91
A:(Gourine <i>et al.</i> , 2011), B :(Mechraoui <i>et al.</i> , 2024), C :(Benarfa <i>et al.</i> , 2020)									

Table 4. Major components of *P. chloranthus* essential oils

Species	<i>Pituranthos chloranthus</i>													
References	A	B	C		D		E		F					
Localities	Tunisia	Tunisia	Algeria	Algerie	Tunisia		Tunisia	Tunisia	Tunisia					
	Aerial part	Fresh Aerial part	Dry Aerial part	Fresh Aerial part	Stems	Stems	Fresh Aerial part	Dry Aerial part	stem	seed	stem	seed	stem	seed
Limonene	16.65	1.56	19.41	15.87	0.0	0.0	00	00	0.0	0.0	0.0	0.0.	0.0	0.0
Sabinene	29.6	0.18	13.05	4.89	0.0	0.0	15.0	12.6	0.0	0.0	0.0	0.0	0.0	0.0
Terpinene-4Ol	15.55	30.34	0.0	0.0	0.0	0.0	0.0	00	11.0	7.2	7.7	1.0	18.5	3.6
A-phellandrene	0.0	0.0	3.05	17.08	14.1	7.2	3.6	7.7	0.0	0.0	0.0	0.0	0.0	0.0
Myristicine	0.0	0.0	20.84	20.48	0.0	0.0	0.0	0.0	11.3	9.0	23.0	6.6	0.0	0.0
α -pinene	0.0	0.0	0.0	0.0	11.0	8.9	47.4	32.5	0.0	0.0	0.0	0.0	0.0	0.0
Elemicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	47.1	45.5	7.4	52.4	14.2	54.0
Apiole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8	19.3	25.3	28.0	20.3	25.3
B-pinene	0.0	0.0	0.0	0.0	8.1	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B-phellandrene	0.0	0.0	0.0	0.0	17.2	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A: (Lahmar <i>et al.</i> , 2017), B:(Yangui <i>et al.</i> , 2009), C:(Lakhdari <i>et al.</i> , 2020), D:(Aloui & and Zouari, 2019), E : (Mighri <i>et al.</i> , 2015), F:(Benissa <i>et al.</i> , 2022).														

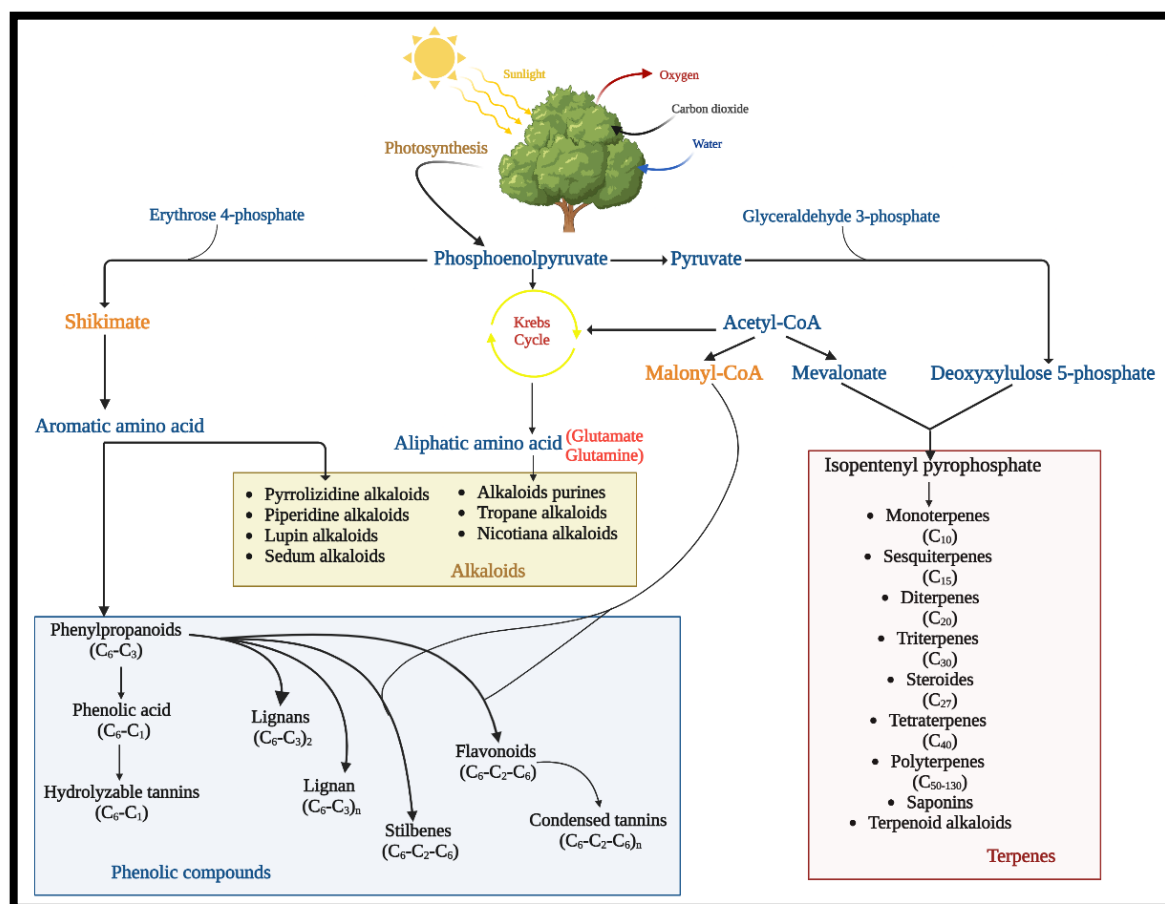


Fig 9. Schematic representation of the biosynthesis of secondary metabolites.

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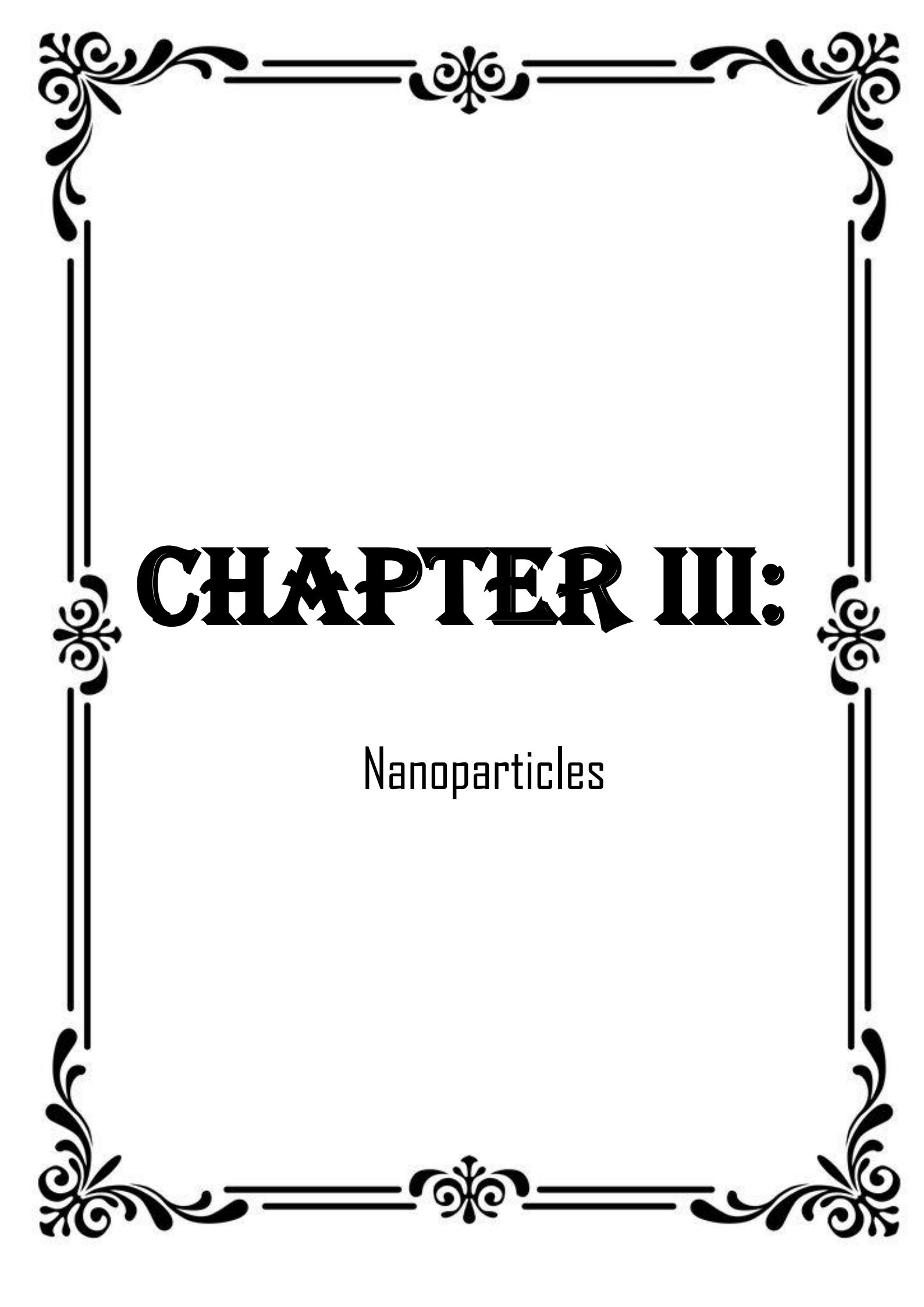
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CHAPTER III:

Nanoparticles

III. Nanotechnology

Nanotechnology is the science, engineering, and application of materials at the nanoscale, typically between 1 and 100 nanometers. At this scale, matter exhibits unique properties that differ from those at larger scales. This field involves manipulating atoms and molecules to create new materials, devices, and systems with enhanced functionalities. Nanotechnology spans multiple disciplines, including physics, chemistry, biology, and engineering, making it a highly interdisciplinary field (Alfei & Zuccari, 2025).

Nanotechnology has revolutionized various industries, from medicine to electronics, by enabling innovations that were previously unimaginable. It plays a crucial role in developing more efficient energy solutions, advanced medical treatments, and stronger, lighter materials. By working at the atomic level, scientists can design materials with precision, leading to breakthroughs in sustainability, healthcare, and technology. Its potential to address global challenges, such as disease, pollution, and energy shortages, makes it one of the most important scientific advancements of the 21st century (Ahire *et al.*, 2022).

Nanomaterials possess unique properties due to their high surface-area-to-volume ratio and quantum effects. These characteristics include increased strength, enhanced electrical conductivity, and improved chemical reactivity. Such properties make nanomaterials highly valuable for specialized applications in various fields (Srinivas *et al.*, 2010).

One of the most promising applications of nanotechnology is in medicine, particularly in drug delivery and diagnostics. Nanoparticles can be engineered to target specific cells, such as cancer cells, reducing side effects of treatments. Quantum dots are used for high-resolution imaging, while nanorobots are being researched for precision surgery. These advancements could lead to personalized medicine, where treatments are tailored to individual patients at the molecular level (Haleem *et al.*, 2023).

Nanotechnology offers innovative solutions for environmental challenges. Nanofilters can remove contaminants from water more effectively than conventional methods, providing clean drinking water in disaster-stricken or polluted areas. Nanoparticles are also used to break down toxic chemicals in soil and air, contributing to pollution control. These applications highlight nanotechnology's role in promoting environmental sustainability (Nishu & Kumar, 2023).

As nanotechnology advances, concerns about its environmental and health impacts have grown. Some nanoparticles may be toxic if released into ecosystems. Green nanotechnology focuses on developing eco-friendly methods for producing and using nanomaterials, minimizing waste and hazardous chemicals. Researchers are exploring plant-based synthesis of



nanoparticles and energy-efficient manufacturing processes to ensure sustainability (Chausali *et al.*, 2023).

This chapter explores the synthesis, properties, and applications of Biosynthesis, highlighting its factors and characteristics.

III.1. The synthesis of metal NPs

Metallic nanoparticles (MNPs) such as gold, silver, iron, and copper are synthesized through physical, chemical, or green methods, each with distinct advantages and drawbacks. Conventional physical and chemical approaches, including laser ablation and chemical reduction, often involve high energy consumption, toxic reagents, and hazardous byproducts, raising environmental and health concerns while increasing production costs. In contrast, green synthesis using plant extracts, microorganisms, or biomolecules as reducing agents offers a sustainable, cost-effective, and eco-friendly alternative by minimizing toxicity, lowering energy requirements, and aligning with global regulatory demands for safer nanomanufacturing. Despite their promising applications in medicine, catalysis, and electronics, MNPs pose potential risks such as cytotoxicity and environmental accumulation, necessitating rigorous safety assessments. The green route emerges as the preferred strategy, balancing performance with environmental responsibility, though further research is needed to enhance scalability and efficiency for industrial adoption (Jamkhane *et al.*, 2019).

III.1.1. Green synthesis of NPs (Biosynthesis)

The green synthesis of nanoparticles represents an eco-friendly and sustainable alternative to conventional chemical and physical methods, utilizing biological sources such as plant extracts, algae, fungi, and bacteria as reducing and stabilizing agents. This approach avoids toxic solvents, minimizes energy consumption, and reduces hazardous byproducts, aligning with the principles of green chemistry. Plant-mediated synthesis, for instance, leverages phytochemicals like flavonoids, terpenoids, and polyphenols to reduce metal ions into nanoparticles (Makarov *et al.*, 2014). While microbial synthesis employs enzymes and proteins for biologically controlled fabrication. The resulting nanoparticles often exhibit enhanced biocompatibility, making them suitable for biomedical, environmental, and catalytic applications. Additionally, green synthesis is cost-effective, scalable, and supports circular economy principles by utilizing renewable resources. Despite its advantages, optimizing reaction conditions, reproducibility, and nanoparticle uniformity remain key research challenges. Nevertheless, this method holds great promise for sustainable nanotechnology, combining environmental safety with innovative material science.



III.1.1.1. Green synthesis of Ag NPs by plants

The plant-mediated synthesis of silver nanoparticles (AgNPs) is a simple, eco-friendly, and cost-effective approach that utilizes phytochemicals present in plant extracts as natural reducing and stabilizing agents. Metabolites such as flavonoids, terpenoids, phenolic acids, and alkaloids facilitate the bio-reduction of silver ions (Ag^+) into nanoparticles (Ag^0) under ambient conditions, eliminating the need for toxic chemicals or high-energy processes. plant-derived AgNPs exhibit enhanced biocompatibility, making them ideal for biomedical applications, including antimicrobial, anticancer, and wound-healing therapies.

Silver nanoparticles have received increased attention as potential new agents, due to the believed broad-spectrum antimicrobial efficacy of silver, and its established use in burn and wound treatment. Some researchers have demonstrated increased bactericidal action of penicillin in combination with silver nanoparticles, raising the possibility of future clinical use (Fahim *et al.*, 2024).

Additionally, this green method minimizes environmental hazards and reduces production costs by utilizing renewable plant resources. However, challenges such as batch-to-batch variability and precise control over nanoparticle morphology require further optimization. Overall, plant-based synthesis offers a sustainable and scalable route for producing functional AgNPs with potential uses in medicine, agriculture, and environmental remediation.

III.1.1.2. Green synthesis of MgNPs by plants

Magnesium (Mg) is a promising material for orthopedic implants due to its density and elastic modulus, which closely match those of natural bone, as well as its biodegradability and adequate tensile strength. However, its rapid degradation in physiological environments leads to premature mechanical failure, and it lacks inherent antibacterial properties to combat bacterial biofilms. One effective strategy to enhance its performance is developing composite materials by combining Mg with reinforcing agents. Magnesium oxide nanoparticles (MgO NPs) are particularly suitable for this purpose due to their biocompatibility, bioactivity, strong antibacterial effects, and favorable mechanical properties. Despite these advantages, the limited research on MgO NPs as reinforcements in Mg-based composites, particularly regarding their impact on mechanical strength, corrosion resistance, and biological performance, remains a significant hurdle for their widespread use.

Various plant species contain bioactive phytochemicals act as natural reducing and capping agents, facilitating the conversion of magnesium ions (Mg^{2+}) into stable, nanoscale particles (Mg^0). This green approach occurs under mild reaction conditions (room temperature, neutral pH) without requiring toxic solvents or high-energy inputs, making it environmentally benign. The size, morphology, and stability of the resulting MgNPs can be modulated by

optimizing parameters such as plant extract concentration, metal precursor ratio, reaction time, and temperature.

Plant-synthesized MgNPs exhibit unique properties, including high surface reactivity, biocompatibility, and low toxicity, making them promising for biomedical (e.g., drug delivery, antimicrobial coatings), agricultural (nano-fertilizers), and environmental (pollutant degradation) applications. Compared to traditional synthesis, this method reduces production costs by using renewable plant biomass and minimizing waste. However, challenges such as controlling particle agglomeration, ensuring reproducibility, and scaling up production require further research. Future studies should focus on standardizing protocols, exploring novel plant sources, and evaluating the long-term stability and toxicity of MgNPs for safe commercial utilization (Silva *et al.*, 2022).

III.2. Factors for the Biosynthesis

The synthesis of silver nanoparticles (AgNPs) is highly dependent on key operational parameters that influence their size, shape, stability, and properties. Temperature plays a critical role, as higher temperatures typically accelerate reduction rates, leading to smaller particles, while lower temperatures favor controlled growth. The pH of the reaction medium affects nanoparticle stability and morphology, with alkaline conditions often enhancing reduction efficiency and preventing aggregation. Precursor concentration (silver nitrate, AgNO_3) determines nucleation density, where higher concentrations may increase yield but risk polydisperse particles. Reducing agents (sodium borohydride, citrate, or plant extracts) govern reduction kinetics, with stronger agents producing smaller NPs. Additionally, stabilizers or capping agents are essential to prevent agglomeration and ensure colloidal stability. Reaction time influences particle maturation, with prolonged durations potentially causing Ostwald ripening. Finally, mixing methods (magnetic stirring, sonication) ensure homogeneity, while light exposure can photo-reduce silver ions in some synthesis routes. Optimizing these parameters is crucial for tailoring AgNPs for specific applications, such as antimicrobials, sensors, or catalysis (Makarov *et al.*, 2014).

III.3. Characterization techniques of nanoparticles

Following the green synthesis of nanoparticles, characterization becomes essential to analyze their morphology, dimensions, surface properties, and uniformity. Multiple analytical techniques are utilized to evaluate these nanomaterials, as outlined in the subsequent sections.

1. UV-Visible spectrophotometer:

UV-visible spectroscopy serves as a highly reliable and efficient tool for the initial characterization of synthesized nanoparticles, including the monitoring of silver nanoparticle (AgNP) formation and stability. AgNPs exhibit strong interactions with specific light

wavelengths due to their unique optical properties. This technique offers several advantages, such as rapid analysis, simplicity, high sensitivity, and broad applicability to various nanoparticle types, all without requiring prior calibration. The absorption spectra are influenced by factors like particle size, surrounding dielectric medium, and chemical environment. In AgNPs, the proximity of conduction and valence bands allows free electron movement, while the collective oscillation of these electrons generates the distinctive surface plasmon resonance (SPR) absorption band.

2. Scanning electron microscopy (SEM) and Transmission

Electron microscopy, particularly transmission electron microscopy (TEM) and scanning electron microscopy (SEM), is widely employed for surface and morphological characterization of nanoparticles. While SEM provides elemental analysis and imaging at micron and submicron scales, TEM offers significantly higher resolution, enabling detailed examination of nanoscale features. These techniques are indispensable for assessing the surface morphology of newly synthesized nanoparticles, with TEM being especially valuable for precisely determining particle size and shape (Jagadeesh *et al.*, 2024).

3. Fourier transform infrared spectroscopy (FTIR)-

Fourier transform infrared spectroscopy (FTIR) is an essential analytical technique for identifying organic functional groups attached to nanoparticle surfaces, offering significant advantages in surface chemistry analysis. This method provides accurate, consistent results with excellent transmission ratios, enabling researchers to verify biomolecule involvement in nanoparticle synthesis. FTIR has proven particularly valuable for characterizing nanoscale materials, including the detection of functional molecules bound to silver nanoparticles. As shown in previous studies, this technique effectively reveals capping agents through their characteristic functional groups. The spectral peaks typically indicate nanoparticle coating by plant-derived secondary metabolites such as phenols, terpenoids, flavonoids, tannins, and glycosides, along with their associated functional groups, including carboxylic acids, ketones, and aldehydes.

4. X-ray diffraction (XRD)

is a widely used technique for characterizing crystalline and molecular structures. This method provides valuable information about particle size, crystallinity, and isomorphous substitution, serving as both a qualitative indicator of active components and a means to differentiate between compounds. When X-rays interact with crystalline materials, they produce distinct diffraction patterns that reveal the material's lattice properties. XRD's versatility enables the structural analysis of diverse materials, including biomolecules, polymers, glasses, superconductors, and inorganic catalysts. The technique's analytical power

lies in its diffraction peak patterns, which can be matched against standard references in the JCPDS database to identify material composition through their unique diffraction signatures (Jose Chirayil *et al.*, 2017).

The underlying principle of XRD is Bragg's law. Using the Scherrer formula, the size of nanoparticles is calculate.

Where D is the average crystallite size, k is the so-called shape factor (0.9), λ is the wavelength (0.15418 nm, $\text{CuK}\alpha$), $\Delta 2\theta$ is the Full Width at Half Maximum (FWHM), and θ is Bragg's angle.

5. Zeta potential measurement

Zeta potential measurement is a key analytical technique used to assess the stability and surface charge of colloidal dispersions, nanoparticles, and other fine particles in suspension. It quantifies the electrokinetic potential at the slipping plane of the particle-liquid interface, providing insights into repulsive forces that prevent aggregation. The measurement is typically performed using dynamic light scattering (DLS) or laser Doppler electrophoresis, where an electric field is applied to induce particle motion, and the velocity (electrophoretic mobility) is correlated to zeta potential. A high absolute zeta potential (generally above ± 30 mV) indicates strong electrostatic stabilization, while lower values suggest a higher risk of flocculation or sedimentation. This parameter is crucial in industries like pharmaceuticals, wastewater treatment, ceramics, and biotechnology, where colloidal stability significantly impacts product performance and shelf life.

6. Energy Dispersive Spectroscopy (EDS or EDX)

is an analytical technique used to determine the elemental composition of a material by detecting and measuring the characteristic X-rays emitted when the sample is bombarded with a high-energy electron beam, typically in a scanning electron microscope (SEM) or transmission electron microscope (TEM). Unlike wavelength-dispersive spectroscopy (WDS), EDS collects and sorts X-rays by energy using a semiconductor detector, such as a silicon drift detector (SDD). The resulting spectrum displays peaks corresponding to different elements, allowing for qualitative and semi-quantitative analysis. EDS is widely used in materials science, geology, metallurgy, and biological research due to its rapid data acquisition, non-destructive nature, and ability to analyze multiple elements simultaneously. However, its resolution for light elements (carbon, oxygen) is limited compared to WDS (Jagadeesh *et al.*, 2024).



III.4. Biological Applications of Nanoparticles

Nanoparticles (NPs) have revolutionized various fields due to their unique physicochemical properties, such as high surface area-to-volume ratio, tunable surface chemistry, and enhanced reactivity. In medicine, NPs are used for targeted drug delivery, cancer therapy, and diagnostic imaging (gold nanoparticles in biosensors and iron oxide NPs in MRI contrast agents). Agriculture benefits from nano-fertilizers (zinc and magnesium NPs for improved crop yield) and nano-pesticides (silver NPs for antifungal action). In industry, NPs enhance product performance, such as titanium dioxide (TiO₂) in sunscreens, silica NPs in coatings for scratch resistance, and carbon nanotubes in lightweight composites. Additionally, environmental applications include water purification (graphene oxide filters) and air pollution control (catalytic NPs for CO₂ reduction). Despite their advantages, challenges like toxicity, scalability, and regulatory hurdles remain critical areas of research.

Table 5. Applications of Silver and Magnesium nanoparticles.

Application Field	AgNPs (Silver Nanoparticles)	MgNPs (Magnesium Nanoparticles)
Pharmaceutical	- Antimicrobial drug delivery - Wound dressings (e.g., silver sulfadiazine) - Anticancer therapy (targeted drug delivery) - Antiviral coatings (e.g., for COVID-19 masks)	- Magnesium-based biodegradable implants - Anti-inflammatory drug carriers - Bone regeneration scaffolds (due to Mg's osteogenic properties)
Agricultural	- Nano-pesticides (fungicidal/bactericidal) - Seed coating for pathogen resistance - Soil remediation (heavy metal detoxification)	- Nano-fertilizers (Mg essential for chlorophyll) - Stress resistance enhancers in crops - Biodegradable mulch films
Medical	- Antibacterial coatings (catheters, implants) - Diagnostic imaging (biosensors) - Dental composites (caries prevention)	- Cardiovascular stents (biodegradable) - Neuroprotective agents (Mg's role in neural health) - Muscle regeneration aids
Industrial	- Food packaging (antimicrobial films) - Textiles (antibacterial fabrics) - Water purification (pathogen removal)	- Lightweight biodegradable materials - Corrosion-resistant coatings - Energy storage (Mg-ion batteries)

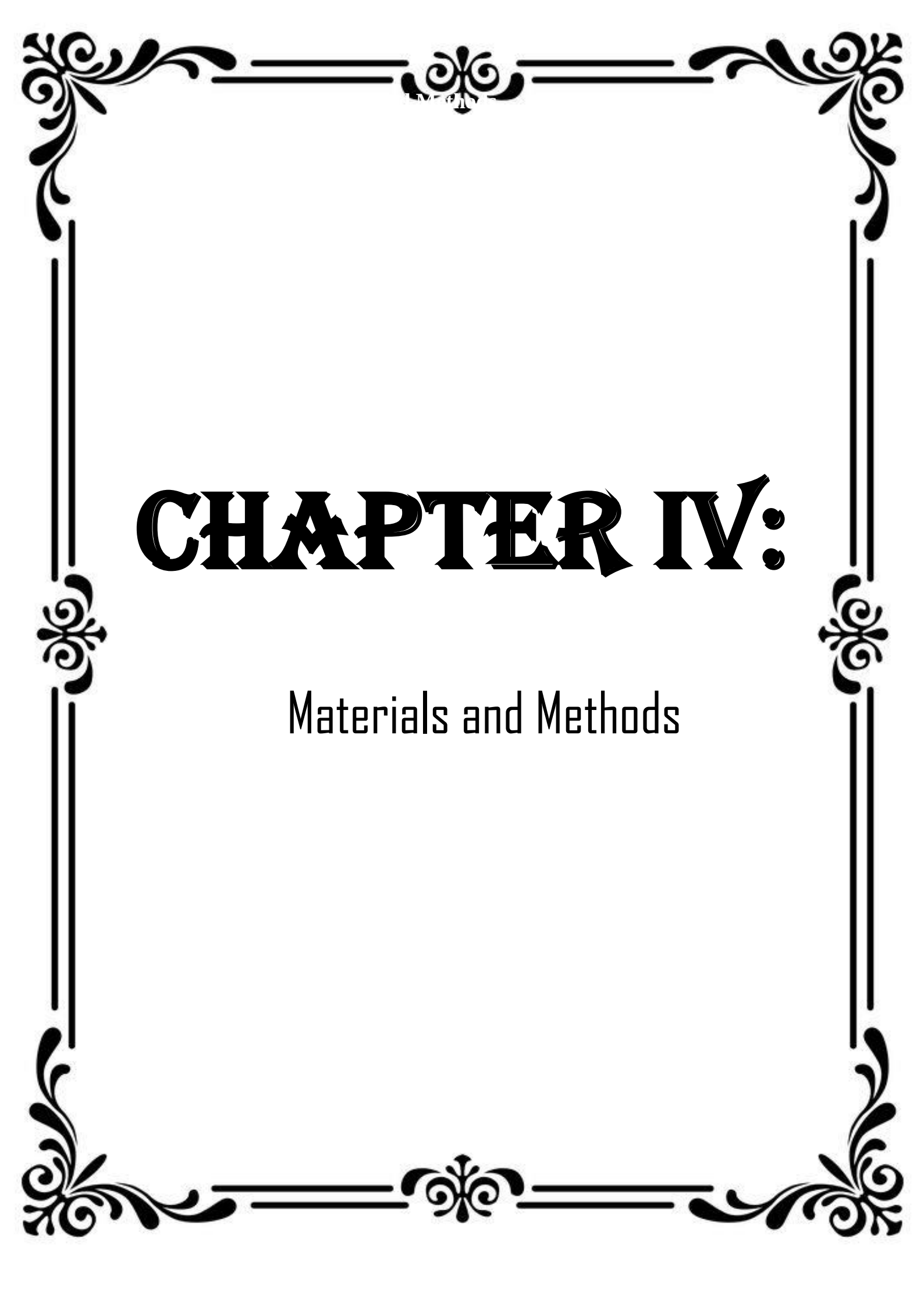
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Second part
Experimental part



CHAPTER IV:

Materials and Methods

This chapter is devoted to describing the methods and techniques used throughout the work, including different materials and chemicals.

IV.1. Objectives of this work

The primary objectives of this doctoral research are to investigate the influence of seasonal variation on the bioactive compound profile of two *Pituranthos* species collected from distinct geographical regions. The study will further examine how climatic and edaphic factors modulate their secondary metabolism. A comparative evaluation of the plants' biological potential will be conducted by assessing the antioxidant, antimicrobial, and/or anti-inflammatory activities of their extracts, essential oils, and biosynthesized nanoparticles.

The methodological approach for this thesis will include the following steps:

1. Analysis of climate data from the two study areas
2. Physico-chemical characterization of soil samples.
3. Collection of the studied plant species.
4. Extraction of essential oils and preparation of ethanolic extracts.
5. Synthesis of nanoparticle
6. Determination of different secondary metabolites
7. Assessment of various organic osmolytes.
8. Determination of mineral elements.
9. Qualitative and quantitative analysis of the ethanolic extracts using Ultra-performance Liquid Chromatography-Mass Spectrometry (UPLC/MS-MS)
10. GC-MS analysis of Essential Oil.
11. Characterization of the nanoparticle using UV-visible spectroscopy, XRD, FTIR, SEM, and EDX.
12. Evaluation of biological activities, including antioxidant, anti-inflammatory, and antibacterial assays.

This chapter details the experimental framework employed to investigate the influence of environmental factors on the *Pituranthos* genus. The presentation of materials follows a causal logic, beginning with the definition of the environmental domain before specifying the biological units studied within it. Consequently, we first describe the Study Area to establish the broad climatic and soil gradients that form the hypothesized selective pressures. This geographical context is essential for justifying the selection of specific *Pituranthos* populations, which are presented subsequently as the plant material. Following this, the protocols for environmental data collection and data analysis are outlined.

IV.2. Characteristics of the study areas

IV.2.1. Geographical location

IV.2.1.1. Ghardaïa

Ghardaïa is located in the northern-central part of the Algerian Sahara, approximately 550 kilometers south of Algiers, the capital of Algeria. It lies at the geographic coordinates of 32° 29' 39" North latitude and 3° 41' 23" East longitude, with an average elevation of 548 meters above sea level. Ghardaïa is characterized by its arid desert climate, with extremely hot summers, mild winters, and minimal annual rainfall. The region is part of the larger Saharan ecosystem, featuring rocky plateaus, dunes, and sparse vegetation adapted to hyper-arid conditions.

IV.2.1.2. Adrar

Adrar is in the southwestern part of Algeria, within the Sahara Desert. Its geographic coordinates are approximately 27° 52' 60" North latitude and 0° 17' 60" West longitude, with an elevation of around 258 meters above sea level. The province is characterized by its arid desert climate, with extreme temperatures and minimal precipitation, making it a representative area for studies on desert ecosystems, climate, and geomorphology.

IV.2.2. Bioclimatic characterization

Climate plays a crucial role in determining the distribution of vegetation. In our study, we considered rainfall and temperature as climatic parameters, as they are both readily available and the variables that have the greatest influence on vegetation.

IV.2.2.1. Weather Data

Several fundamental factors influencing the climatic characteristics of the region are: geographical location, exposure, and altitude. The climatic data for the study regions were supplied by the meteorological national office (O.N.M.) for a period of 10 years, from 2014 to 2024, providing information on precipitation (P, mm) and temperatures (T, °C) in Monthly and Annual averages.

IV.2.2.1.1. precipitation

Rainfall from a quantitative point of view is generally expressed as the average annual rainfall. It can be used as a characteristic element of the climate (Charalampopoulos et al., 2025; Le houerou, 1959). The variation in climatic parameters, such as evapotranspiration and dry season, is mainly a function of average annual rainfall. Indeed, when rainfall decreases, evapotranspiration and the duration of the dry season increase. This led to considering average yearly rainfall as an important factor in climate. (DJEBAÏLI, 1984) defines rainfall as the primary factor that determines the type of climate. In fact, this condition affects both the

maintenance and distribution of vegetation on the one hand, and the degradation of the natural environment through erosion on the other, especially at the beginning of spring (Li et al., 2023; Rousta et al., 2024).

IV.2.2.1.1.1 Seasonal regime

The rainfall pattern is also considered a characteristic of a climate. For plants, the distribution of rainfall is more important than the total annual quantity. The key factor is the availability of water during their development cycle. If autumn and spring rains are sufficient, plants will flourish; however, if the rainfall during these seasons is limited, their growth will be poor (Bai et al., 2023).

IV.2.2.1.2. Temperature

The development of vegetation is heavily influenced by temperature, a factor second in importance only to other climatic elements. Ecological studies emphasize that extreme temperatures, which define the lethal thresholds for plants, are more impactful than average conditions (Shi et al., 2025). At least four variables should be taken into account in the study of temperature:

- **m**: Mean minimum temperatures (°C).
- **M**: Average maximum temperatures (°C).
- **(M+m) /2**: Average temperatures (°C).
- **(M-m)**: The thermal amplitude.

IV.2.2.2. Climatic synthesis

The two parameters, precipitation and temperature, have been selected for this bioclimatic synthesis. They are of great interest for characterizing the influence of the region's climate and determining the dry period.

IV.2.2.3. Climatic indices

IV.2.2.3.1. The de Martonne Index (DMI)

For the assessment of the bioclimatic conditions of the cross-section area, the DMI was applied and computed on an annual basis according to the following formula (Charalampopoulos et al., 2025) :

$$\text{DMI} = P / (T + 10)$$

- **P**: The annual precipitation (in millimeters, mm)
- **T**: Mean annual temperature (in degrees Celsius, °C)
- The number 10 is the coefficient employed for the acquisition of positive values.

Table 6. Aridity index values.

DMI values	Types of Bioclimates
$0 < \text{DMI} < 5$	Hyper-arid
$5 < \text{DMI} < 10$	Aride
$10 < \text{DMI} < 20$	Semi-arid
$20 < \text{DMI} < 24$	Mediterranean
$24 < \text{DMI} < 28$	Semi-humid
$28 \leq \text{DMI} < 35$	Humid
$35 \leq \text{DMI} < 55$	Very humid
$\text{DMI} \geq 55$	Extremely humid

IV.2.2.3.2. Xerothermic index (Bagnouls et Gaussen, 1953):

The Gaussen umbro-thermal diagram was drawn up based on the interannual monthly averages of precipitation and temperature recorded at two weather stations (Gaussen & Bagnouls, 1952).

The umbrothermal diagram by Bagnouls and Gaussen, (1953) consists of plotting average monthly rainfall and average monthly temperatures on the same graph. The dry season corresponds to the period when the temperature curve rises above the precipitation curve ($P \leq 2T$). Precipitation is expressed in millimeters and Temperature in degrees Celsius. This type of graph is called “umbrothermal”. A month is considered dry when the temperature curve is higher than the precipitation curve. The part of the graph between the two curves reflects the duration and intensity of the drought.

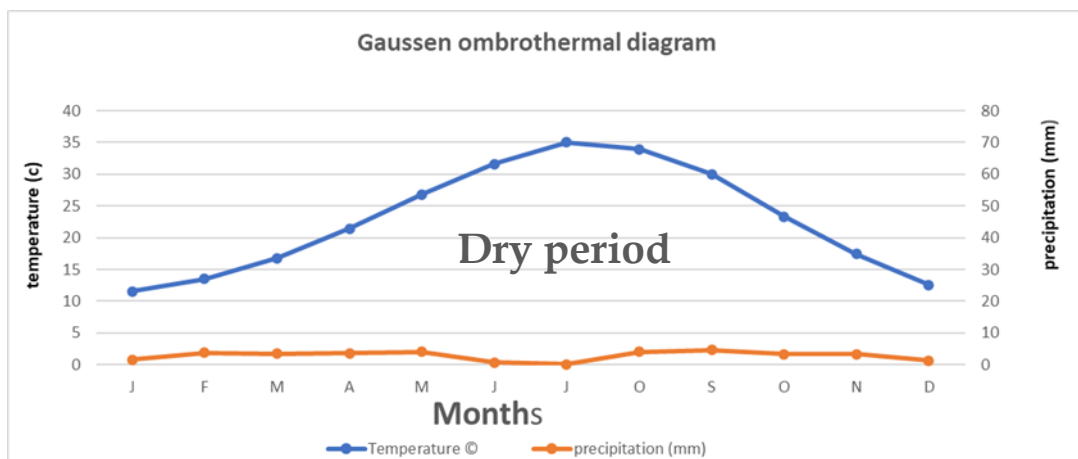


Fig 10. Umbro-thermal diagram of Ghardaïa station (2014-2023)

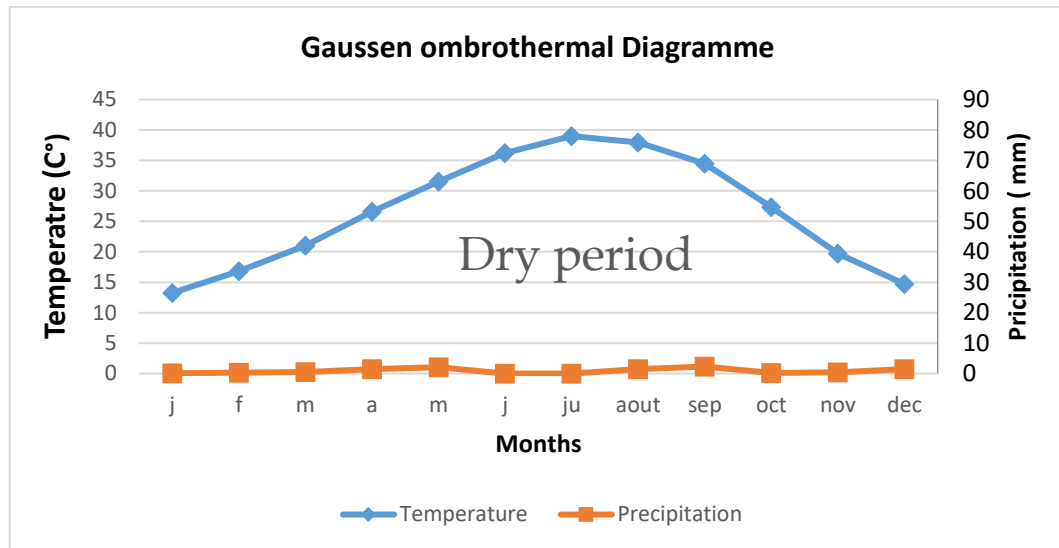


Fig 11. Umbro-thermal diagram of Adrar station (2015-2024)

IV.2.2.3.3. EMBERGER index (1955)

For the assessment of the phytoclimatic conditions of the cross-section area, the Emberger Index (EI), commonly termed the “pluviothermic quotient” (Q or Q1), was applied and computed on an annual basis according to the following formula (Emberger, 1930) :

$$Q = (1000 \times P) / ((M + m) \times (M - m))$$

- **P**: represents the annual average precipitation (mm);
- **M**: represents the average maximum monthly air temperature of the warmest month in absolute degrees Celsius.
- **m**: represents the average minimum monthly air temperature of the coldest month in absolute degrees Celsius.

The following Table 7 presents the relation between the Q values and the types of bioclimates

Table 7. Phytoclimatic classification of Emberger’s pluviothermic quotient (Q).

Q Values	Types of Bioclimates
$Q > 170$	Hyper-humid (or per-humid)
$120 \leq Q \leq 170$	Humid
$65 \leq Q < 120$	Sub-humid
$30 \leq Q < 65$	Semi-arid
$10 \leq Q < 30$	Arid
$Q < 10$	Hyper-arid (or per-arid)

IV.2.2.3.4. Rivas-Martínez Simple Continentality Index

It is a fundamental indicator and widely used tool in modern bioclimatology that quantifies the degree to which a location's climate is influenced by a large land mass, as opposed

to the moderating effect of an ocean. In simple terms, it measures how "continental" a climate is. The index is famously simple to calculate. It requires only the mean temperature of the warmest month (M) and the mean temperature of the coldest month (m), both in degrees Celsius:

$$Ic = M - m$$

Where:

- Ic = Continentality Index
- M = Mean temperature of the warmest month
- m = Mean temperature of the coldest month

This value, Ic, represents the Annual Thermic Range. The resulting value (Ic) is interpreted using a standard scale, which defines different levels of continentality. The higher the value, the more continental the climate (table 8).

Table 8. Typical classification scale based on Rivas-Martínez

Continentality Index (Ic)	Category
< 15	Hyperoceanic
15 – 21	Eu-oceanic / Oceanic
21 – 28	Suboceanic
28 – 38	Continental / Subcontinental
> 38	Hypercontinental

IV.3. Soil analysis

Soil analysis serves as a fundamental diagnostic tool in agricultural and environmental sciences, providing essential data on the physico-chemical and biological parameters that govern edaphic conditions and directly influence floristic composition and vegetative vigor. Through a comprehensive characterization of properties including particle size distribution (texture), hydrogen ion concentration (pH), macro- and micronutrient profiles, and soil organic matter (SOM) content, this practice elucidates the soil's inherent capacity to sustain and nurture plant life. To illustrate, soil pH is a master variable that modulates the bioavailability of crucial nutrients; strongly acidic or alkaline conditions can induce deficiencies or toxicities by locking up elements such as nitrogen (N), phosphorus (P), and potassium (K). Concurrently, soil texture dictates critical hydrological characteristics like water-holding capacity and aeration, which in turn affect root architecture and penetration. Furthermore, SOM is not merely a nutrient reservoir but a key driver of soil structure, microbial diversity, and overall ecosystem resilience, facilitating enhanced nutrient cycling and water infiltration. Consequently, the insights gleaned from a thorough soil analysis are instrumental in formulating targeted soil management strategies such as precision fertilization, strategic liming, or the application of organic composts

to rectify imbalances and optimize conditions for specific plant growth and broader ecological sustainability. For this study, all soil analytical procedures were conducted at the specialized laboratories of the National Institute of Irrigation and Drainage Soils to ensure methodological rigor and accuracy.

IV.4. Plant material

IV.4.1. Description of plants studied

- *Pituranthos chloranthus*

Pituranthos chloranthus is a perennial shrub that exhibits classic adaptations to arid environments. A much-branched, erect or sprawling subshrub, typically reaching 30 to 80 cm in height. The old stems are often woody and rigid. The stems are slender, grooved, and highly branched, giving the plant a bushy appearance. The young branches are often green and photosynthetic. The leaves are finely divided, appearing almost thread-like (filiform). The flowers are arranged in characteristic compound umbels typically yellowish or greenish-white, the fruit is a schizocarp, which splits into two dry, one-seeded mericarps when mature. The mericarps are often ribbed (costate), a key taxonomic feature for identification within the family. The plant has been identified taxonomically according to Quézel et Santa (1963)



Fig 12. *P. chloranthus* from Ghardaïa (original photo)

- *Pituranthos scoparius*:

The Saharan perennial shrub *Pituranthos scoparius* (syn. *Deverra scoparia*), a member of the Apiaceae family, bears compound umbels with white-to-yellowish flowers and schizocarpic fruits. The plant is also known for its signature aromatic scent, which is produced by essential oils in its glandular trichomes (Vilmorin, 1959).

This species is endemic to North African arid zones, where it thrives in rocky desert landscapes and high plateaus like the Tassili n'Ajjer and Saharan Atlas (Hammiche & Maiza, 2006).

This plant was taxonomically identified by Professor Eddoud Ammar of the University of Ouargla, and specimens of it have been placed in the Herbarium of the Faculty of Biology at the University of El Oued Algeria under specimen number L. BIO39PS01.



Fig 13. *Pituranthos scoparius* from Adrar (original photo)

The geographical coordinates (Latitude, Longitude, and Altitude) of the stations sampled are shown in the Table 9.

Table 9. Geographic coordinates

The province	Stations	Latitude	Longitude	Altitude
Ghardaia	Oued drin	32° 21'49.8" N	3° 50'48.4" E	416
Adrar	Ouled ahmed timi	27°51'39.0" N	0°16'51.5" w	258

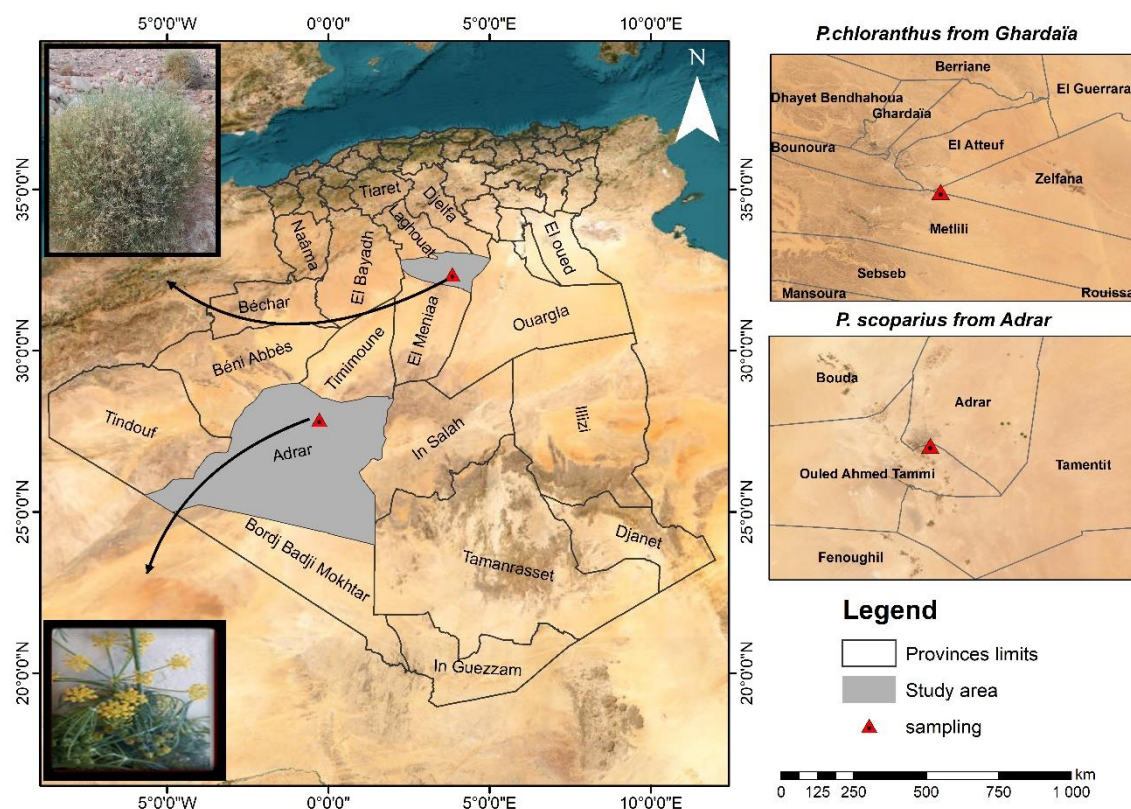


Fig.14. Localization of the plants studied

IV.4.2. Collection of the material plants

The work of the present thesis was carried out on the aerial parts of the fresh and dry plants belonging to the *Piturnthos* genus, which were harvested manually in the early morning and collected in a clean, thick cloth bag. The biomass was then manually rinsed with distilled water to remove impurities and divided into two parts.

The first quantity, used as fresh material for analyzing the osmolytes, was cut into small pieces of 2-3 cm. The second quantity was spread horizontally on a wooden surface covered with a thick, clean cloth, and dried in the laboratory at room temperature (27-29°C) for 07 days. Finally, it was cut into very fine pieces (2-5 mm) for use in the different extraction, then stored hermetically in a dry place.

The aerial parts of both plants, *Pituranthos chloranthus*, *Pituranthos scoparius* were collected from Oued Drin in Metlili province (Ghardaïa), Ouled Ahmed Timi in Adrar, respectively in southern Algeria during two seasons, autumn (November 2023) and spring (May 2024).

IV.4.3. Preparation of Ethanolic Extract

The dry plants were ground using a grinder, and the mixtures were passed through sieve No 14. Before being extracted, the powdered plant was kept in an airtight container. each dried plant material was macerated using ethanol 75% at room temperature for 24h with steady

stirring, this process was repeated three times than the extract was filtered and concentrated using a rotating vacuum evaporator (Mahgoub et al., 2025). The dried extract was stored in an airtight container at a low temperature in the refrigerator for future investigation. The yield based on the dried weight of the samples was determined using the following equation:

$$(\%) = (\text{weight of dry extract}(g)) \div (\text{initial weight of dry sample}(g)) \times 100$$

IV.4.4. Essential Oil Extraction

Hydrodistillation or steam distillation is the reference technique for studying the volatile compounds of a plant species. It is used to extract EOs from fresh or dried plants.

Procedure

Before use, the unit is cleaned with acetone and then rinsed with distilled water to remove all traces of dust and grease, to avoid contamination of the essential oil at during extraction.

The operation consists of introducing 50g of dried plant mass into a large glass flask, and adding a sufficient quantity of distilled water without filling the flask to avoid boiling over.

The mixture is brought to the boil using a balloon heater. The oil-laden vapours pass through the vertical tube and into the cooling coil, where condensation takes place. The oil droplets thus produced accumulate in the tube, previously filled with distilled water. The extraction process takes three hours from the start of boiling.

The essential oil has a low density in relation to water, and floats on the surface of the latter. It is collected and stored in well-sealed opaque bottles at low temperature (4-5°C). The yield based on the dried weight of the samples was determined using the following equation:

$$\% = (W1 \div W0) \times 100$$

?: yield of essential oils as a percentage

W1: mass of essential oils recovered (g)

W0: weight of plant material (g)

IV.4.5. Green synthesis of magnesium-silver oxide nanoparticles

The green synthesis of nanoparticles represents an eco-friendly and sustainable alternative to conventional physicochemical methods. The synthesis of nanocomposites leverages and enhances the intrinsic bioactivity of plant extracts and essential oils by combining them with metallic nanoparticles (Makarov et al., 2014). This synergy creates novel materials with superior and often multifunctional properties such as increased stability, controlled release, and amplified antimicrobial or catalytic activity that the individual components lack (Haleem et al., 2023).

• Procedure

The green synthesis of the silver-magnesium nanocomposite was initiated by combining 200 mL of each diluted plant extract in a 1000-mL Erlenmeyer flask under constant agitation. To this vigorously stirred extract, aqueous solutions of 0.001 M silver nitrate (AgNO_3) and 0.1 M magnesium nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were simultaneously added in a gradual, dropwise manner. The reaction mixture was then heated to 70 °C under continuous stirring. The alkalinity of the solution was critical for nanoparticle formation; therefore, the pH was carefully adjusted and maintained within a range of 9 to 10 using a 1 M sodium hydroxide (NaOH) solution (Nasrollahzadeh et al., 2020).

Over a four-hour reaction period, the formation of the nanocomposite was visually confirmed by the development of a dark green precipitate. This biogenic precipitate was subsequently recovered via centrifugation at 12,000 rpm for 15 minutes.

To ensure high purity, the pelleted nanocomposite was subjected to multiple washing cycles with distilled water and absolute ethanol to remove any residual phytochemicals or unreacted precursors. The purified pellet was then dried in an oven at 45°C to a constant weight. The resulting solid was finely ground into a homogeneous powder using a mortar and pestle, and the dry yield was recorded for each plant extract. Primary confirmation of successful nanoparticle synthesis was achieved using UV-Visible spectroscopy (Shimadzu, Japan), where the absorbance spectra and calculated band gaps were analyzed to verify the formation and optical properties of the nanocomposite.

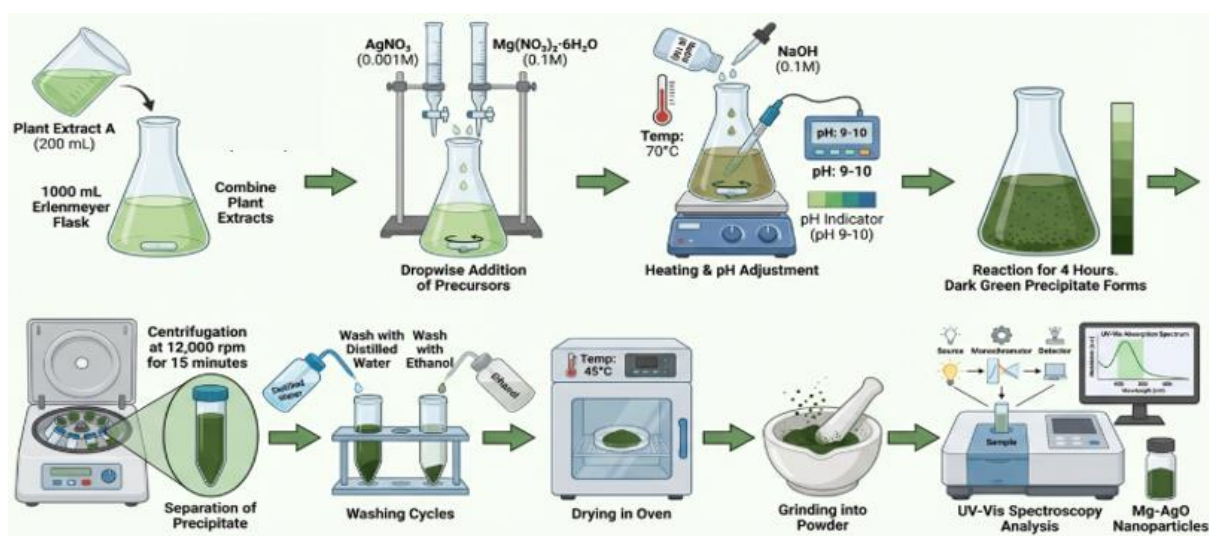


Fig 15. Green synthesis procedure of magnesium-silver oxide nanoparticles

IV.5. Quantification Analysis

IV.5.1. Phytochemical screening

The qualitative phytochemical analysis gives a preliminary idea about the presence of a specific class of phytochemical (Chen et al., 2022). Any change in the colors or composition of the precipitate has been adopted as an indication of a positive response to these tests.

- **Polyphenols**

2 mL of the extract received a few drops of a 2 % (w/v) FeCl₃ solution. FeCl₃ takes on a greenish or blackish-blue coloring when polyphenol derivatives are present.

- **Flavonoids**

2 mL of plant extract was mixed with 2 mL of 1% hydrochloric acid, then 2 mL of the mixture was added to 2 mL of Dragendorff reagent.

- **Alkaloids**

For alkaloid analysis, 1 mL of the extract was divided into two test tubes. The medium was acidified with a few drops of HCl, and Mayer's reagent was added to one tube while Wagner's reagent was added to the other. The appearance of a white or brown precipitate in the respective tubes confirmed the presence of alkaloids.

- **Saponins**

2 mL of extract and 2 mL of distilled water are shaken for 15 seconds and left for 15 minutes. A firm foam height of more than one Cm indicates the presence of saponins.

- **Terpenoids**

In a test tube, 5 mL of plant extract was mixed with 2 mL of chloroform and 3 mL of strong sulfuric acid. The development of a reddish-brown tint indicated the presence of terpenoids.

IV.5.2. Spectrophotometric quantification

IV.5.2.1. Determination of total phenols (TPC)

The principle

The most common and widely accepted method for determining total phenolic content is the Folin-Ciocalteu (F-C) assay (Molole et al., 2022). Its principle is based on a redox (reduction-oxidation) reaction where phenolic compounds act as reducing agents (Bouaziz et al., 2020). The Folin-Ciocalteu reagent itself is a complex mixture containing phosphomolybdic and phosphotungstic acids (H₃PW₁₂O₄₀, H₃PMo₁₂O₄₀). In this reaction, the phenolic compounds (Ar-OH) reduce the metal ions in the reagent. Specifically, they reduce the yellow Mo(VI) and W(VI) to blue Mo(V) and W(V) following the chemical reaction:

Phenolic Compound (Reducing Agent) + Mo(VI)/W(VI) (oxidized, yellow) → Phenolate Oxidized Product + Mo(V)/W(V) (reduced, blue)

This reduction reaction results in the formation of a stable blue-colored complex. The intensity of this blue color is directly proportional to the concentration of phenolic compounds in the sample. This intensity is measured using a UV-Vis spectrophotometer at a wavelength between 725 and 765 nm .

- The reaction occurs optimally under basic conditions. This is why sodium carbonate (Na_2CO_3) is added

The procedure:

0.1 ml of each ethanolic extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water). After standing for 5 minutes, 2 ml of Na_2CO_3 sodium carbonate (20 %) is added to the mixture. After vigorous homogenization and then a 30-minute rest in the dark at room temperature, the absorbance is measured at 760 nm. The gallic acid was used as the standard for the calibration equation, and the total phenolic content was expressed in μg equivalent of gallic acid per mg of extract.

IV.5.2.2. Determination of total flavonoids (TFC)

The principle:

The principle of this method is based on the formation of a stable, acid-stable complex between the aluminum ion (Al^{3+}) from AlCl_3 and the carbonyl and hydroxyl groups of flavonoid molecules. This complexation reaction results in a visible color change, which allows for spectrophotometric quantification (Chandra et al., 2014).

The process can be broken down into three key mechanistic steps:

- The principle relies on the ability of flavonoids to act as ligands by chelating metal ions. Specifically, the Al^{3+} ion from aluminum chloride forms a stable complex by binding simultaneously to the carbonyl oxygen at the C-4 position and a hydroxyl group at either the C-3 or C-5 position on the flavonoid structure.
- The formation of the flavonoid-aluminum complex causes a bathochromic shift, moving the flavonoids' absorption from the UV into the visible spectrum. This results in a measurable yellow color, the intensity of which is directly proportional to the flavonoid concentration.
- The intensity of the yellow color, measured as absorbance at a set wavelength (415 or 510 nm), is directly proportional to the flavonoid concentration and is quantified by comparing it to a standard curve made with a reference flavonoid like quercetin.

The procedure:

1.5 ml of each diluted extract or standard was added to 1.5 ml of AlCl_3 (2 % in ethanol). The absorbance was measured at 415 nm after 30 minutes of incubation. The results are determined using a linear calibration equation with quercetin as the standard. The findings were shown as μg of quercetin for every milligram of extract.

IV.5.2.3. Determination of total condensed tannin (TCT)

The principle

The tannins assay was measured by the vanillin-HCl method. The method quantifies condensed tannins by their acid-catalyzed depolymerization into colored anthocyanidin pigments in a hot butanol/HCl mixture, with the color intensity measured at 550 nm being proportional to their concentration (Broadhurst & Jones, 1978).

The procedure

3 ml of 4 % of a vanillin solution prepared in ethanol and 1.5 ml of concentrated HCl are added to 0.5 ml of extract. After incubation for 15 min, the absorbance is measured at 500 nm by a UV-visible spectrophotometer. The results are expressed in μg equivalents of catechin per milligram of dry vegetable matter ($\mu\text{g CE/mg DM}$)

IV.5.3. Organic Osmolytes

IV.5.3.1. Determination of photosynthetic pigment content

Chlorophyll a and chlorophyll b were determined by mixing 0.1 g of fresh plant material in 10 mL of acetone (80%) at 4°C for 24 hours. After centrifugation for 10 minutes at $5000 \times g$, the absorbance of the supernatant was read at wavelengths 663, 645, and 470 nm using a spectrophotometer (figure 18) (Sumanta Nayek, 2014).

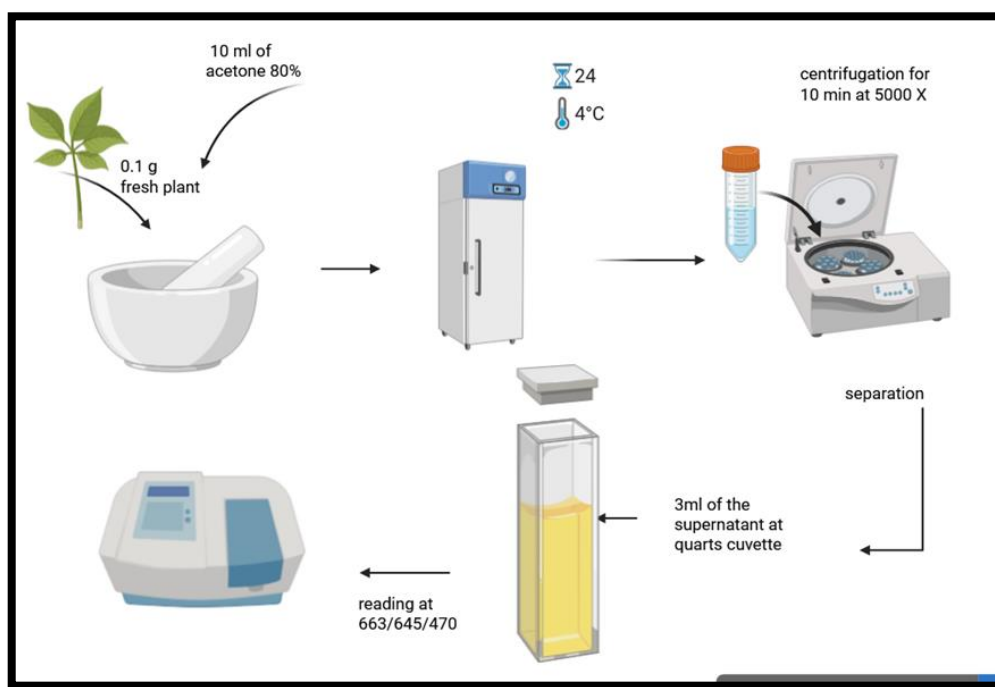


Fig 16. Determination of photosynthetic pigment content

IV.5.3.2. Determination of total soluble sugar

The levels of total soluble sugar in the studied plant samples from the two regions and across different seasons were determined following the protocol that was described in (Doran & Souza, 2021) the analyses were conducted with three biological replicates. Exactly 100mg of fresh plants were homogenized by vortexing in 2 ml of 80% ethanol, followed by incubation for 48 hours in obscurity. The insoluble fraction, including debris, was removed from the ethanol extracted by centrifugation at 3500g for 6 min at room temperature. Half of the ethanol extracted was dried by evaporation, the extract was allowed to cool down, and then was made up to the volume of 20ml with sterile distilled water. Exactly 1 ml of the solution was transferred to another tube, to this 1 ml of 5% phenol was added and vortexed thoroughly, then 5ml of sulfuric acid was added and left on the room temperature for 10 minutes after that in bath room for 15 min to 30min, finally spectrophotometric reading was taken and calculate the quantity of total soluble sugar using the equation:

$$\mu g/gMF = DO490 \times 1.657$$

IV.5.3.3. Determination of proline content

A colometric method was adapted using the Troll and Lindsly (1954) (Troll & Lindsley, 1955) method, which was simplified and developed by Drein and Goring (1974) and updated by Monneveux and Nemmar(1986). From aerial parts samples, 100 mg of each sample was transferred to a tube containing 2ml of methanol 40% and was vortexed thoroughly. These homogenates were incubated in a water bath room 85 °C for 60 minutes. After cooling the extracts, the supernatants were transferred to new tubes for use in the proline assay. Exactly 1 ml of these supernatants was mixed with 1 ml of acetic acid and 25mg of ninhydrin, then we added in each tube 1 ml of the solution (12 ml distilled water +30 ml acetic acid + 8 ml orthophosphate acid), the reaction mixtures in closed tubes were incubated in a boiling water bath for 30 minutes. Tubes were allowed to cool dawn again, then we added 5 ml of toluene and vortexed. The supernatants were separated and transferred to new tubes containing 5 mg of sodium sulfate. We left the mixture for 48 hours, after which readings were taken using a spectrophotometer at 528. and calculate the quantity of proline using the equation:

$$\mu g/gMF = DO528 \times 0.62$$

IV.5.3.4. Determination of glycine betaine

A mixture of 0.5 grams of finely ground dry plant material and 20 milliliters of distilled water was then incubated at 25 degrees Celsius for 48 hours. After that, the sample was filtered, and the subsequent filtrate was stored in the freezer until it was time for analysis. After being defrosted, the extract was diluted with sulphuric acid at a ratio of one to one. Half a milliliter of the solution that was produced was placed in ice water for one hour. The reagent KI-I2 (0.2

mL) was added to the mixture, which was gently agitated with a vortex. The samples were kept at a temperature between 0 and 4°C for 16 h. The samples were then transferred to tubes and centrifuged at 10,000 g for 15 min at 0°C. A micropipette is used to carefully suction the supernatant in. To ensure that the cold complex is successfully isolated from the acid media, it is necessary to keep the tubes in place until the solubility of the complex increases sufficiently with temperature. The reagent, which is 9 milliliters of 1,2-dichloroethane, is used to dissolve the pellet. Prior to that, wash with 0.5 milliliters and wait for five minutes. The tubes should be mixed with a vortex in order to guarantee complete solubility in the solvent. After two hours, the absorbance was determined by using a visible UV spectrophotometer to detect the wavelength at 365 nm (Grieve & Grattan, 1983). The glycine betaine standard (50-200 µg/ml) were produced in 2 N sulphuric acid and sample estimation was performed as previously described.

IV.5.4. mineral elements

The concentrations of essential micronutrients (Zinc (Zn), Copper (Cu), Manganese (Mn), Iron (Fe), Cobalt (Co)), macronutrients (Magnesium (Mg)), and potentially toxic heavy metals (Lead (Pb), Nickel (Ni)) in the plant samples were determined using flame atomic absorption spectroscopy (AAS).

The procedure

The plant materials were first thoroughly washed with distilled water to remove any adhering dust or soil particles. They were then oven-dried at 60°C for 48 hours until a constant weight was achieved. The dried samples were ground into a fine, homogeneous powder using a mechanical grinder and stored in sealed plastic bags at room temperature until analysis.

The digestion of the plant samples was carried out to mineralize the organic matrix and release the metallic elements into solution. The procedure was as follows:

- Approximately 0.5 g of the dried, powdered plant sample was accurately weighed into a clean, pre-ignited porcelain crucible.
- The crucible was placed in a muffle furnace and the temperature was gradually increased to 500°C. The sample was asked at this temperature for 4-6 hours, or until a white or greyish-white ash residue was obtained, indicating complete combustion of organic matter.
- After cooling in a desiccator, the ash was dissolved in 5 mL of a 20% (v/v) hydrochloric acid (HCl) solution. The mixture was gently heated on a hot plate to near dryness to ensure complete extraction of the minerals.
- The residue was then filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask. The crucible and filter paper were rinsed several times with deionized water,

and the filtrate was made up to the final volume (50 mL) with deionized water. A blank solution was prepared following the same procedure without the plant sample.

The concentrations of zinc (Zn), lead (Pb), nickel (Ni), copper (Cu), manganese (Mn), magnesium (Mg), iron (Fe), and cobalt (Co) in the digested solutions were determined using an Atomic Absorption Spectrometer. The instrument was calibrated using a series of standard solutions of known concentrations for each element, prepared by serial dilution from a certified multi-element stock solution. All measurements were performed in triplicate to ensure precision, and results were expressed as mean concentrations in mg/kg of dry weight, along with standard deviations.

IV.6. Ultra-performance Liquid Chromatography-Mass Spectrometry (UPLC/MS-MS)

The comprehensive qualitative profiling of polyphenolic constituents present in our extracts was conducted employing advanced ultra-performance liquid chromatography coupled with single ion monitoring mass spectrometry (UPLC-SI-MS). The analyses were performed on a Shimadzu 8040 system, renowned for its ultra-high sensitivity and equipped with UFMS (Ultra-Fast Mass Spectrometry) technology, which was driven by a Nexera XR LC-20AD binary pump for precise solvent delivery. To establish a reference, polyphenol standards were first optimized via direct infusion, bypassing the chromatographic column. Critical electrospray ionization (ESI) parameters were meticulously optimized to ensure efficient ion generation and detection; these included a Collision Induced Dissociation (CID) gas pressure of 230 kPa, a conversion dynode voltage of -6.00 kV, an interface temperature of 350°C, a desolvation line (DL) temperature of 250°C, a nebulizing gas flow of 3.00 L/min, a heat block temperature of 400°C, and a drying gas flow rate of 15.00 L/min. All analytical standards were prepared at a consistent concentration of 500 µg/L in methanol. The ion trap mass spectrometer was operated with high selectivity in both negative and positive ionization modes, utilizing the Multiple Reaction Monitoring (MRM) mode to enhance detection specificity and sensitivity for target analytes. Chromatographic separation was achieved using a Restek C18 Ultra-force column, 3µm, 150x4.6mm, maintained at a constant temperature of 25°C. For gradient elution, the mobile phase was composed of solvent A (water), solvent B (methanol), and 0.1% formic acid at a rate of 0.4 ml/min with a precise injection volume of 5 µL for all samples and standards. (El-Mesallamy et al., 2023).

IV.7. GC-MS analysis of Essential Oil

The principle consists in transferring compounds separated by gas chromatography via the mobile phase (the carrier gas) into the mass spectrometer, where they are fragmented into ions of varying mass, separated according to their mass. Identification is then carried out by

comparing the retention indices (Ir) and spectral data (mass spectra) of the individualized constituents with the characteristics of reference products contained in spectral libraries.

Identification of volatile constituents of essential oils (EO) was carried out at using GC/MS, while quantitative determination was carried out on a device equipped with a flame ionization detector (GC/FID). Quantification of essential oil constituents is determined by the universal internal standardization method without response coefficient, given that not all products present are isolated, known and clarified. Gas chromatography coupled to GC/MS mass spectrometry. GC-MS-TQ8040 NX gas chromatograph with an Rxi-5Sil MS

Operating conditions
Apolar column: 30 m, 0.25 mm, 0.25 μ m
Temperature programming: 40 °C for 3 min, then increased 25 °C per minute to 125 °C, then 10 °C per minute to 300 °C for 15 min.
Vector gas: Helium was the carrier gas at 1.2 mL/min
Sample: 1:60 split ratio
Injection volume: 5 μ L
Injector: 250 °C
Mass range: 30–500 m/z.

Compounds were identified by comparing their mass spectra and their KI (Kovats Index) with those of the databases and that established by the host laboratory. For proper identification, the following principles are derived from the properties of the columns. The linear retention index is defined by the following formula:

$$KI = 100n + (TR(C_n)) / (TR(C_{n+1}) - TR(C_n)) \times 100$$

KI: Retention index

A: unknown compound,

TR(A): retention time of the unknown compound

TR(C_n): retention time of the hydrocarbon with n carbon atoms immediately preceding the compound

TR (C_{n+1}): retention time of the hydrocarbon with (n + 1) carbon atoms immediately following the compound; The experimental indices are compared with those in the literature and with those in the laboratory databases for confirmation.

IV.8. Characterization of green-synthesized nanocomposite

Comprehensive characterization is essential to confirm the successful green synthesis of the nanocomposite and to determine its fundamental physicochemical properties, which directly influence its application performance.

The methods UV-Vis, FT-IR, XRD, SEM, and EDX were used to characterize the nanocomposite

IV.8.1. UV-visible spectroscopy

UV/Visible spectroscopy (Shimadzu-00463) was used to assess the bio-reduction and optical properties of nanocomposite samples. The optical properties of the nanocomposite samples in the UV and visible ranges were studied by UV-visible absorption spectroscopy. Electron spectroscopy is an absorption spectroscopy concerning transitions from the ground to an excited state. It consists of measuring the attenuation of an incident light ray of intensity (I_0) as a function of the wavelength when it passes through a homogeneous medium of thickness (l) containing an absorbing species. The technique consists of detecting and quantifying the intensity of radiation whose wavelength varies between 200 and 1400 nm; the sample can absorb, transmit, or reflect the photons emitted. The Beer-Lambert law governs Absorbance (Jagadeesh et al., 2024)

IV.8.2. Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) in diffuse reflection mode, performed with a Thermo Scientific-Nicolet iS5 Attenuated Total Reflection, was employed to detect the functional groups in the nanocomposite. The recorded spectra spanned from 4000 to 400 cm^{-1} , exploiting a KBr pellet approach (Jose Chirayil et al., 2017)

IV.8.3. X-ray diffraction (XRD)

The XRD method is most appropriate for powdered samples, which are freshly prepared after drying the samples' respective colloidal solutions. Using XRD equipment (Bruker D8 Advance, Texas, US), the position and intensity of peaks in a sample are compared to patterns in various diffraction databases, allowing the composition of nanoparticles to be quantified (Kumar et al., 2022) The principle of operation of the XRD method includes the dispersing of X-rays due to the revolution of electrons in the nucleus of the atom when the rays hit the nanoparticles. Dispersed X-rays are reflected in different directions, causing interference patterns. These patterns are destructive or constructive, but only dispersed X-rays that go through constructive interaction give rise to diffraction. Characterization of nanoparticles by XRD begins with identifying the phase of the sample material. The type of crystal in the sample is determined by a search-and-match process, which is carried out in regions where high peak intensities are observed. The different XRD peak configurations are the result of different atom arrangements. In an XRD approach, interference occurs when the light of a given wavelength highlights a periodic structure having a predetermined spacing. The principle of XRD follows Bragg's law ($n\lambda = 2d\sin\theta$), where λ , n , d , and θ respectively denote the X-ray wavelength,

integer, plane spacing, atomic, and the half-angle of diffraction. Therefore, information can be obtained regarding sample crystal defects, crystal size, crystal phase, shape anisotropy, deformation, and texture by evaluating the diffraction peaks' width, shape, and position.

IV.8.4. Scanning electron microscopy (SEM)

The morphology of the surface and magnesium, silver, and oxygen distribution in the sample were verified by SEM-EDAX experiments (Carl Zeiss, Germany, Gemini 300). SEM is an electron microscope that generates images of the sample's top surface by scanning it with a stream of high-energy electrons. The external surface structure of the materials was studied using the scanning electron microscopy (SEM) approach. SEM provides information on surface morphology and material composition (Firdous et al., 2025). The SEM technique has several advantages in dimensional morphological analysis, but the information regarding the distribution size and average population is limited. Studying a nanoparticle solution using SEM requires drying it to a powder before mounting it on a sample holder and coating the sample surface with a conductive metal, such as using a cathode sputtering device. The external surface sample is examined when a high-spirited flow of electrons is incident on it. The high-resolution enlarged images produced when the narrow electron beam reveals details smaller than 1-5 nm produce a characteristic three-dimensional image to understand the structure of the surface sample (Jagadeesh et al., 2024).

IV.8.5. Energy dispersive spectroscopy (EDS)

EDS is utilized to gain insight into the elemental composition of metal nanoparticles, offering a foundational comprehension of the sample (Jagadeesh et al., 2024)

IV.9. In vitro biological activity tests

IV.9.1. Antioxidant activity

IV.9.1.1. DPPH radical-scavenging activity

The principle

The assay is based on the scavenging ability of antioxidants towards the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Ozsoy et al., 2008).

DPPH is a stable, organic nitrogen-centered free radical. Its stability comes from the delocalization of the unpaired electron over the entire molecule, which is shown by its deep purple color with a characteristic absorption maximum at around 515-517 nm.

When an antioxidant molecule is introduced, it donates a hydrogen atom ($H\bullet$) to the DPPH radical, neutralizing it. This reduction reaction converts the purple DPPH $\bullet$ into a stable, yellow-colored diamagnetic molecule, 1,1-diphenyl-2-picrylhydrazine (DPPH-H) The reaction can be summarized as:



The antioxidant (AH) quenches the DPPH radical by transferring a hydrogen atom, resulting in the reduced, yellow form. The more potent the antioxidant, the more DPPH radicals it can reduce, and the stronger the observed color change (Ahn et al., 2007).

This method is based on measuring the ability of antioxidants to trap the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH-purple) radical, which is reduced to the nonradical form DPPH-H (diphenylpicrilhydrazine). yellow color by accepting a hydrogen atom. The effectiveness of an antioxidant is determined by measuring the decrease in purple coloration due to a recombination of the DPPH• radicals, measurable by spectrophotometry at 517 nm (Prieto et al., 1999).

The procedure

A volume of 0.2 mL for each samples (1-500 µg/mL) was added to 0.8 mL 0.004% DPPH solution in methanol. The absorbance for the reaction mixture was measured at 517 nm after 30 min of incubation at room temperature. In three independent trials (n = 3), using ascorbic acid as standard.

The radical scavenging activity is calculated as a percentage of inhibition using the following formula:

$$\% \text{ DPPH Scavenging Activity} = [(A_0 \text{ control} - A_1 \text{ sample}) / A_0 \text{ control}] \times 100$$

Where:

A₀ control: is the absorbance of the control reaction (containing all reagents except the test sample).

A₁ sample: is the absorbance of the test sample.

- The results are often expressed further as IC₅₀ (value the most common way to report the potency of an antioxidant). The IC₅₀ (Half Maximal Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of the DPPH radicals. A lower IC₅₀ value indicates a higher antioxidant potency.

IV.9.1.2. Reducing power (FRAP) assay

The FRAP (Ferric Reducing Antioxidant Power) assay is a widely used, direct, and colorimetric method to measure the reducing capacity of an antioxidant (Zemmouli et al., 2025)

The principle

The fundamental principle of the FRAP assay is that antioxidants can be considered as reductants, and their potency can be measured by their ability to reduce the Ferric ion (Fe³⁺) to the Ferrous ion (Fe²⁺) in a stoichiometric, redox reaction (Belyagoubi-Benhammou et al., 2017).

The assay uses a colored, oxidized complex that, upon reduction by an antioxidant, changes to an intensely colored blue complex. The increase in blue color intensity is directly proportional to the combined reducing power of the antioxidants present in the sample.

The procedure

The reductive ability of test extracts, essential oils, and the nanocomposite was tested with very slight modifications. Prepare the reaction solution by adding 1 mL with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide. Subsequently, they were incubated for 25 minutes at 50°C. 2.5 mL of 10% trichloroacetic acid (TCA) was added to halt the reaction and then the mixture was centrifuged for 10 minutes at 3000 rpm. Mix 2.5 mL of supernatant with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl_3 . All experiments performed in triplicate. Absorbance was measured at 700 nm and compared to the synthetic standard ascorbic acid. That decreasing power was EC_{50} .

IV.9.1.3. Total antioxidant capacity (TAC) assay

The total antioxidant capacity of extracts, essential oils and the nanocomposite are determined by the method of phosphomolybdenum, this technique is based on the reduction of molybdenum Mo (VI) present in the form of molybdate ion MoO_4^{2-} a molybdenum Mo (V) as MoO_2^+ in the presence of the samples to form a phosphate / Mo (V) green complex at acidic pH (Prieto et al., 1999)

The protocol

A volume of 0.3 ml of each sample is mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and the tubes are incubated at 95 °C for 90 min. The mixture is allowed to cool, and the absorbance is measured at 695 nm. Total antioxidant activity (TAC) was measured in micrograms of ascorbic acid per milligram of samples.

IV.9.2. Anti-inflammatory activity

To study the potential anti-inflammatory effects of both plants studied *Pituranthos chloranthus*, *Pituranthos scoparius* used in vitro inhibition of protein denaturation method using bovine serum albumin (BSA), and the inhibition of protein denaturation method using egg albumin (Abid et al., 2024).

IV.9.2.1. Denaturation of bovine serum albumin BSA

Protein denaturation is a well-recognized cause of inflammatory conditions; the ability of a compound or an extract to reduce or inhibit heat-induced protein denaturation is an indication of possible anti-inflammatory activity. To evaluate the anti-denaturation potential of the

ethanolic extracts, essential oils, and the Mg/AgO nanocomposite, we followed the protocol below.

The procedure

The Test solutions were prepared at four concentrations (1.75, 2.5, 5, and 10 mg/mL) in Tris-HCl buffer (0.02 M, pH 6.6). Each sample was mixed with an equal volume of 2% (w/v) bovine serum albumin solution. The mixtures were first incubated at 37°C for 15 min, and then heated to 72°C for 5 min. Following cooling, absorbance was measured spectrophotometrically at 650 nm. Diclofenac was used as the positive control, with all experiments performed in triplicate. The percentage inhibition of albumin denaturation was calculated using the following formula:

$$\% \text{ inhibition of albumin denaturation} = [(A_0 \text{ control} - A_1 \text{ sample}) / A_0 \text{ control}] \times 100$$

Where:

A0 control: is the absorbance of the control reaction (containing all reagents except the test sample).

A1 sample: is the absorbance of the test sample.

IV.9.2.2. Denaturation of egg albumin

The anti-inflammatory activity of *P. chloranthus* and *P. scoparius* Desf was performed using egg albumin with some modifications.

The protocol

Egg albumin was prepared by drawing out the egg white from the yolks. The volume of the egg white was measured, and then they are added to a Tris-HCl solution that had already been prepared (20 mM, pH = 6.8) to produce a solution that was diluted (1/100, volume/volume). After a prolonged period of gentle agitation lasting ten minutes, the solution was then filtered. 2 ml of this solution was added to test tubes that contained 1ml of varied concentrations of the extract or Diclofenac (1.75, 2.5, 5, 10 mg/mL). The mixture was incubated at 74°C for 30 min. The absorbance was measured at 650 nm. The percentage inhibition of denaturation, which is an index of anti-inflammatory activity, was calculated using the following equation:

$$\text{Inhibition of protein denaturation \%} = [(A_0 - A_1) / A_0] \times 100$$

A0: the absorbance of the control. A1: the absorbance of the sample

IV.9.3. Antibacterial activity assay

To assess antimicrobial activity of the ethanolic extracts, essential oils and the nanocomposite Mg/AgO, we used the disc diffusion method.

Antimicrobial tests were carried out on reference strains from the American type culture collection (ATCC), kindly provided by the Institut Pasteur d'Algérie, these strains were chosen for their high pathogenicity and multi-resistance. Bacteria are Gram-negative and/or Gram-positive pathogenic species. They include the following strains:

Gram+: *Staphylococcus aureus* ATCC2592 (1), *S. aureus* ATCC 29213 (2), *Enterococcus faecalis* ATCC 49532 and *Bacillus subtilis* ATCC 6633

Gram-: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 70603

- Bacterial strains were stored at room temperature in tubes containing 3 mL of preservation agar.

IV.9.3.1. Solid-state diffusion method

The principle

This technique is similar to the standard antibiogram. The principle of this method is based on the migration properties of extracts on solid media. blotting paper or Watman 3 paper disks impregnated with the antibiotics or extracts to be tested are deposited on the surface of an agar medium previously inoculated with a pure culture. After incubation (18h to 24h at 37°C), a circular zone free of colonies, known as the zone of inhibition, is observed around the discs. The larger the diameter of this zone, the more sensitive the strain is to the antibiotic. The smaller the diameter, the more resistant the bacteria (Avril & Fauchère, 2002)

The procedure

- **Preparation of bacterial inoculum**

The bacteria to be tested were seeded onto Petri dishes containing selective media appropriate to the bacterial strains, then incubated at 37°C for 24 hours, in order to obtain young, well-isolated colonies. After incubation, 1 to 2 well-isolated and perfectly identical bacterial colonies are picked with a platinum loop, then emulsified in a tube containing 10 ml physiological water, while vortexing. The inoculum density was adjusted to 0.5 McFarland (10^8 CFU ml⁻¹)

- **Seeding**

Twenty milliliters (20ml) of Muller Hinton agar are poured into petri dishes. After the culture medium has solidified, 100 µl of the microbial suspension to be tested (10^8 CFU/ml) is spread on the surface.

- **Disc deposit**

Antibiotic discs or extracts are placed on the surface of the previously inked Mueller-Hinton agar plate using forceps flamed with a Benzen beaker. The discs are impregnated in 100

μL of extract and essential oil at concentrations of 50 mg/mL, 75mg/mL and 100 mg/mL in 10% DMSO and 100 μL of nanocomposite at concentrations of 6.26 μg/mL, 3.125 μg/mL and 1.612 μg/mL in 10% DMSO (15 μg/disk gentamicin as positive control; DMSO as negative control). The disks are deposited at equal distances on the MH agar surface. The agar plate prepared in this way is kept at laboratory temperature for 30 minutes to allow pre-diffusion. It is then incubated at 37°C for 24 hours. Trials are carried out in three replicates. After incubation, the diameters of the inhibition zones were measured and classified as insensitive (–) (<8 mm), sensitive (+) (9–14 mm), very sensitive (++) (15–19 mm), or extremely sensitive (+++) (≥20 mm) (Elhouda-Mekhadmi et al., 2024)

IV.10. Statistical analysis

The experimental data, structured by a factorial design encompassing the factors Plant, Season, and Bioactivity, were subjected to statistical analysis using R software (version 4.5.1).

First of all, we calculate the average(mean) and variation (SD) for each variable. Secondaly we check the normality using the Shapiro-Wilk test for choosing between parametric and non-parametric testes.

Comparative tests between parameters were part of the analysis, while the Kruskal–Wallis test was used for abnormal data, and the Dunn's test with Bonferroni correction was used to determine which specific extract pairs differ significantly ($P_{adj} < 0.05$). In addition, This approach allowed for the simultaneous assessment of whether the bioactivity of the plants was significantly affected by species, seasonal variation, and the type of assay, as well as their interactions. Subsequent post-hoc analyses were performed to deconstruct significant interactions. The Principal Component Analysis (PCA) was employed to condense the complexity inherent in the measured variables (climate, soil, osmolytes, and phytochemicals) and to identify the primary sources of variation across the two plant species and two seasons. All graphical representations of the data were also generated within the R environment.

To clarify the sample nomenclature used throughout this study, the codes represent the origin and harvest season of the two plant species from the genus *Pituranthos*. The letter A denotes the specimen of *Pituranthos scoparius* collected from the Adrar region, while G denotes the specimen from Ghardaia. The numerical suffix indicates the harvest season: 1 for autumn and 2 for spring. Therefore, the four experimental groups are **A1** (*Pituranthos chloranthus* from Adrar, autumn), **A2** (*Pituranthos scoparius* from Adrar, spring), **G1** (*Pituranthos chloranthus* from Ghardaia, autumn), and **G2** (*Pituranthos chloranthus* from Ghardaia, spring).

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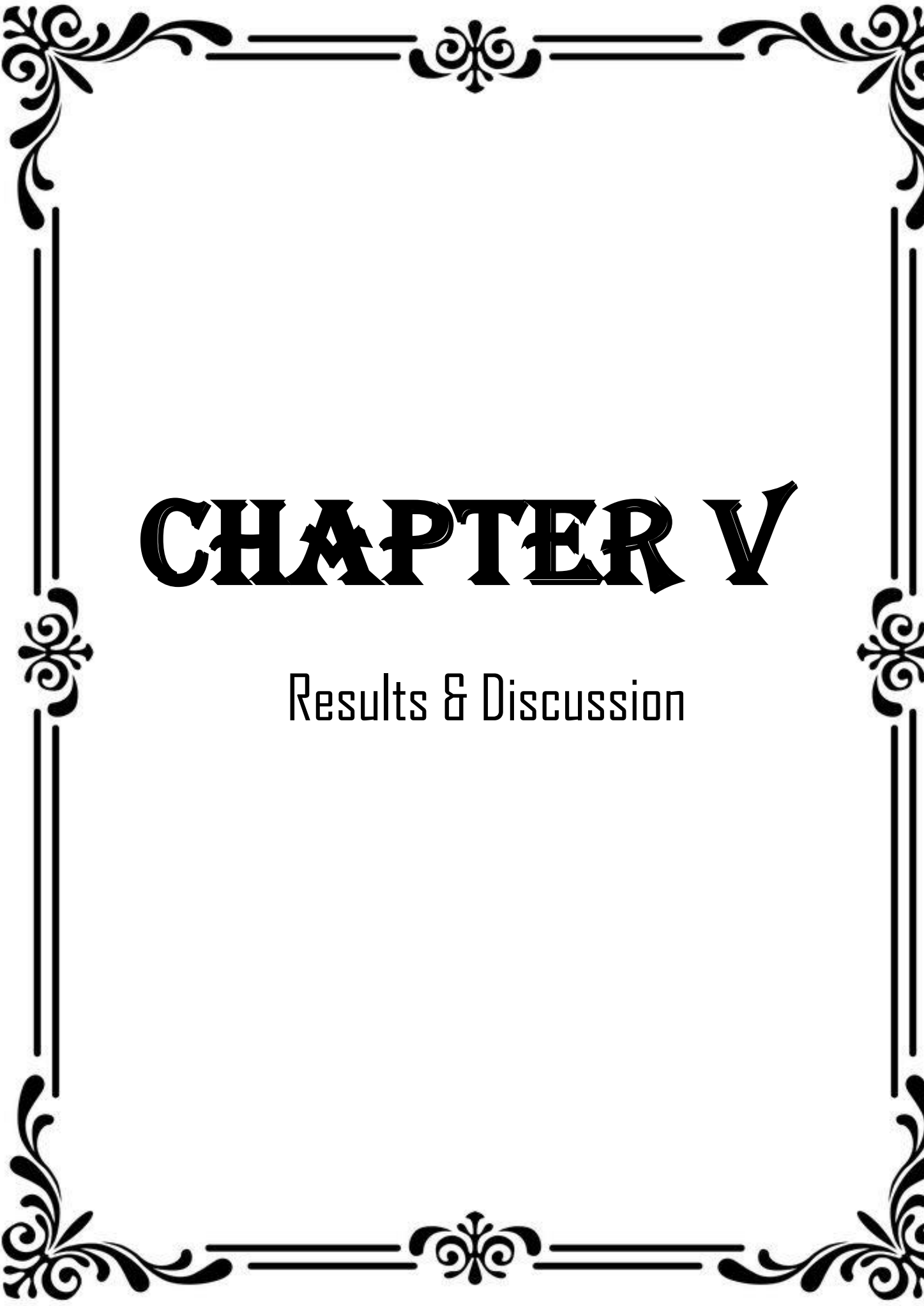
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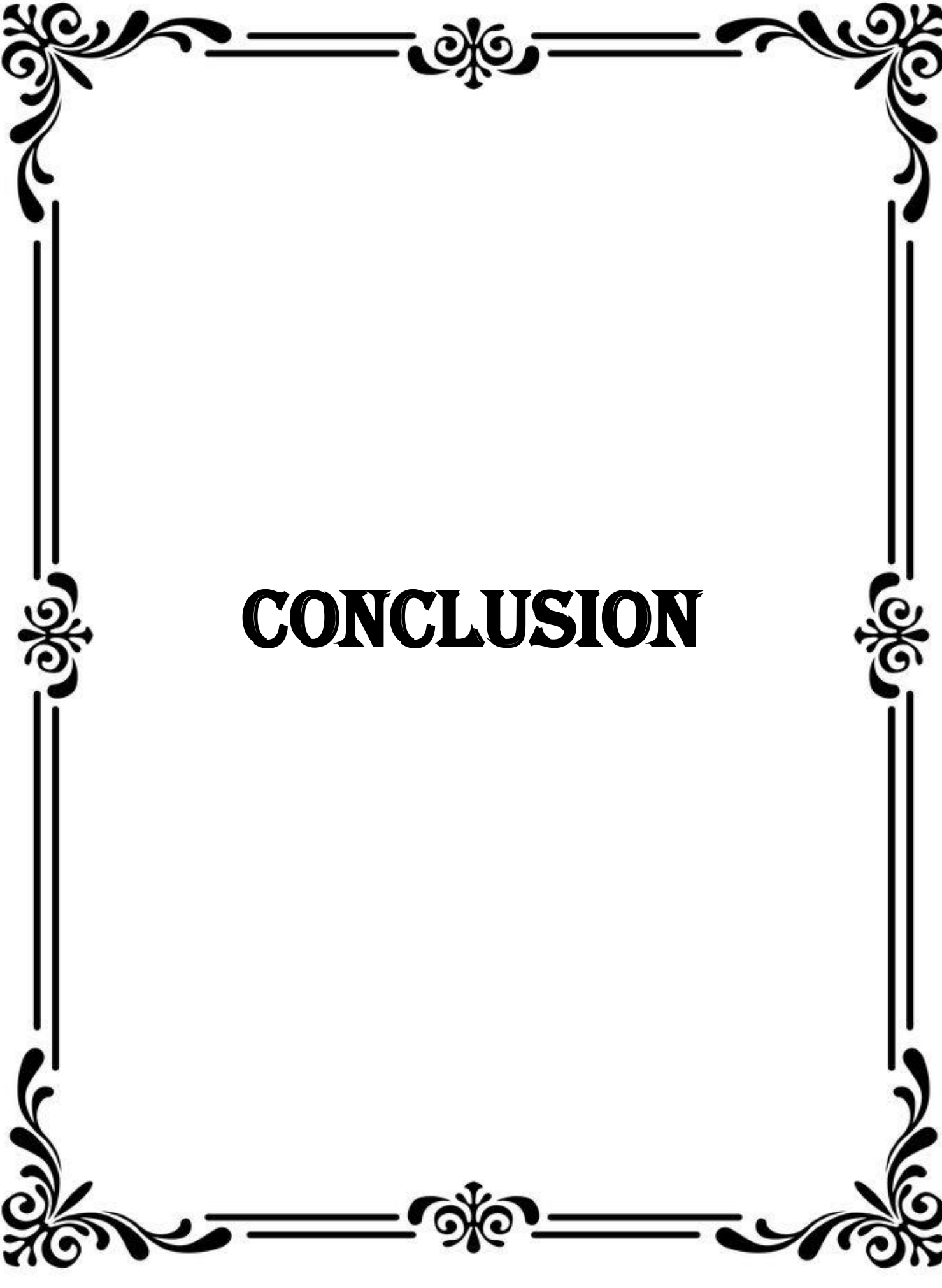
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CHAPTER V

Results & Discussion





CONCLUSION

This thesis set out to investigate the adaptation mechanisms of the *Pituranthos* genus to environmental stress. The research was guided by several key objectives: to analyse the climate and soil properties of the two regions from which the plants were studied, to study the osmolytes, to quantify different secondary metabolites including their essential oils, and to evaluate the capacity of this genus to synthesize nanoparticles and assess the in vitro biological activities of all derived extracts (ethanolic, essential oil, and nanoparticles). The journey through this research has not only successfully addressed these objectives but has also yielded significant insights with broader implications for the fields of plant ecology and phytochemistry.

Climatic analysis established that both study regions exist in a hyper-arid environment, as confirmed by bioclimatic data and standardized aridity indices. This finding provided a crucial foundation for the study, confirming that the sampled *Pituranthos* populations are subject to significant environmental stress.

The analysis of soil properties revealed a clear correlation between the distinct ecosystems and the adaptive strategies of the plants. The data indicate that each species possesses unique mechanisms, a finding that sheds new light on the theoretical model of highly species-specific adaptation to edaphic factors.

Analysis of osmotic adjustment mechanisms revealed distinct, species-specific strategies for environmental stress tolerance in the two *Pituranthos* species. Contrary to initial hypotheses, osmolyte accumulation was highly dependent on both species and geographic origin. A striking example was the substantial accumulation of glycine betaine in *Pituranthos chloranthus* from Ghardaïa, which reached a concentration of 230.66 ± 9.62 mg/l. These findings illuminate the sophisticated nature of plant stress adaptation, demonstrating a direct link between a plant's resilience and its capacity to mobilize specific osmolytes, such as glycine betaine, under abiotic duress.

This study conclusively demonstrates the significant bioactive potential of two Algerian medicinal plants: *Pituranthos scoparius* and *Pituranthos chloranthus* from the Apiaceae family. Their complex phytochemical profiles are exceptionally rich in polyphenols and flavonoids, while their essential oils are dominated by potent monoterpenes and phenylpropanoids, notably myristicin, D-limonene, and α -phellandrene. These distinct chemical compositions directly correlated with the robust biological activities observed. The essential oils exhibited superior antioxidant capacity in both DPPH and FRAP assays. Furthermore, the ethanolic extract possessed the broadest antibacterial spectrum, even surpassing the efficacy of the standard antibiotic gentamicin against several resistant strains. The Ag/MgO nanocomposite also

revealed a selective, yet remarkable, anti-inflammatory potential, particularly in the BSA assay. When contextualized with prior research on the *Pituranthos* genus, the enhanced antioxidant and antibacterial activities reported here underscore the profound influence of Algeria's unique environmental conditions and optimized extraction methodologies on the plants' bioactivity.

Finally, by successfully developing a predictive model, this thesis provides a practical tool for agricultural botanists, conservationists, and phytochemical researchers. The model for the *Pituranthos* genus directly integrates the critical relationships between environmental stressors like temperature and precipitation, soil properties, and secondary metabolite production identified in this study. Consequently, it offers a viable path toward two key applications: optimizing the cultivation of medicinal plants to maximize yields of desired bioactive compounds, and informing targeted conservation strategies for at-risk species in a changing climate.

The most significant contribution of this work lies in demonstrating the deterministic relationship between a plant's phytochemical profile and its resulting pharmacological activities. These findings prove that the value of plant compounds to the pharmaceutical industry is not serendipitous but is critically shaped by environmental pressures, offering a predictive framework for targeted drug discovery.

This study, while providing significant insights, has certain limitations. The phytochemical and biological data represent a snapshot from specific locations and a single harvest period, and may not capture the full natural variability. Furthermore, the robust *in vitro* bioactivity reported requires validation in *in vivo* models to assess its pharmacological relevance. The specific molecular mechanisms behind the observed antibacterial and anti-inflammatory effects also remain to be elucidated. Finally, the predictive model, built on Algerian ecological data, would benefit from validation across a wider geographic range. These limitations, however, clearly define a path for future research, including seasonal monitoring, mechanistic studies, and the expansion of the predictive model to other regions and related species.

In conclusion, this thesis has successfully elucidated the core mechanisms by which the *Pituranthos* genus adapts to environmental stress. By providing a coherent, evidence-based narrative, it moves the field beyond merely documenting bioactivity towards a predictive understanding of how to conserve and enhance the pharmaceutical potential of medicinal plants. The findings affirm the critical role of abiotic factors and offer both a theoretical advance and a practical framework, establishing a solid foundation for future research and conservation strategies.



ANNEXES

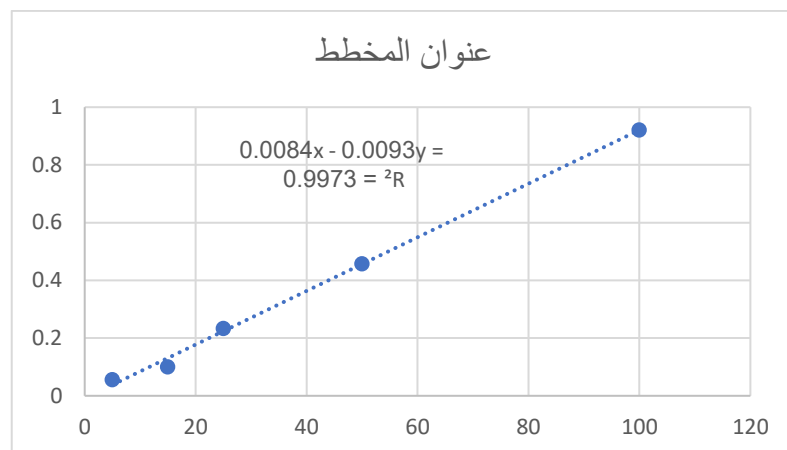


Figure 1. Glycine betaine calibration curve.

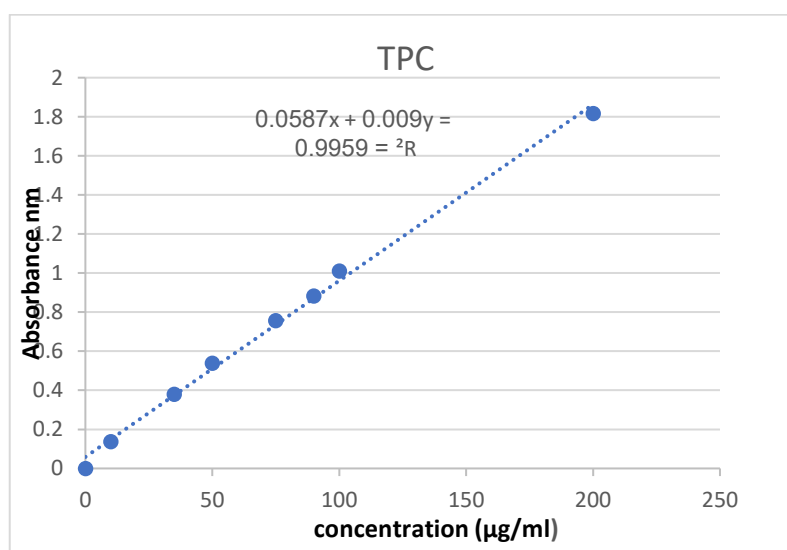


Figure 2. Gallic acid calibration curve.

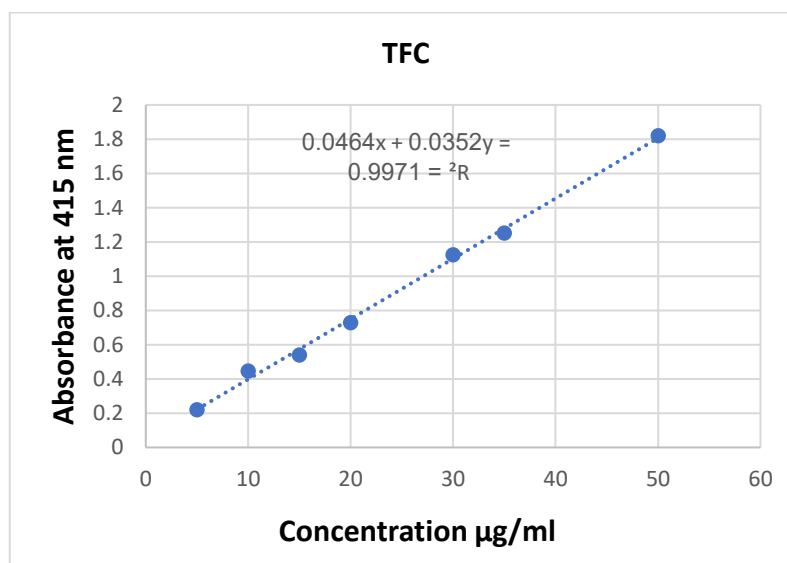


Figure 3. Quercetin calibration curve

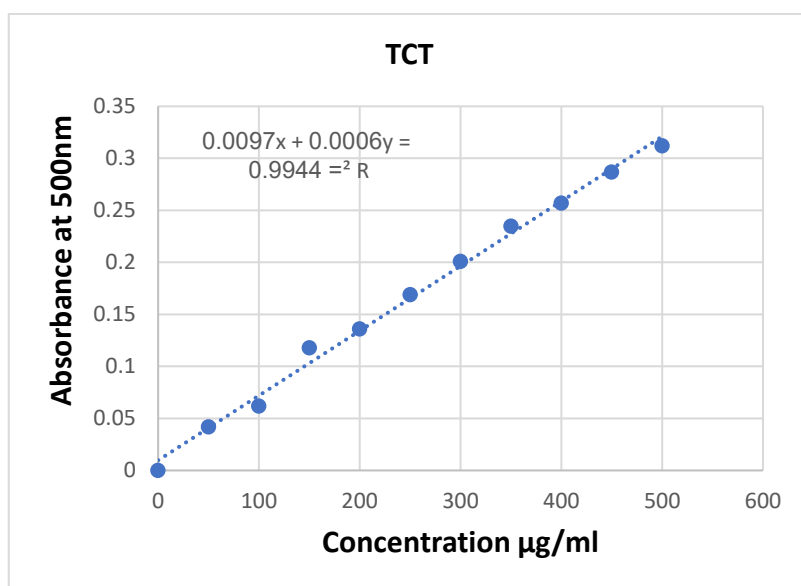


Figure 4. Catechin calibration curve.

Table 1. Chemical constituents detected in the *Pituranthos scoparius* (Autumn) ethanolic extract by UPLC-ESI-MS-MS analysis

<i>Compound</i>	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Ret. Time	Height	Area
<i>Ascorbic Acid</i>	C ₆ H ₈ O ₆	176.12	(+)	177.1500> 130.9500	9.097	27109	408575
<i>8-hydroxyquinoline</i>	C ₉ H ₇ NO	145.16	(+)	146.2000> 101.0000	7.353	2760	21919
<i>Beta caroten</i>	C ₄₀ H ₅₆	536.87	(+)	536.9000> 282.1000	15.898	12023	128980
<i>Curcumin</i>	C ₂₁ H ₂₀ O ₆	368.4	(+)	368.9000> 177.0500	10.977	2600	21465
<i>Rutin</i>	C ₂₇ H ₃₀ O ₁₆	610.5	(+)	611.2000> 356.2000	16.982	7742	80419
<i>Oleanolic Acid</i>	C ₃₀ H ₄₈ O ₃	456.7	(+)	457.1000> 411.2500	14.321	11223	114761
<i>kojik Acid</i>	C ₆ H ₆ O ₄	142.11	(+)	142.8000> 41.0000	11.967	816	5317
<i>Chrysin</i>	C ₁₅ H ₁₀ O ₄	254.24	(+)	254.8000> 153.0500	7.813	1780	9153

Salicin	$C_{13}H_{18}O_7$	286.28	(+)	287.0000> 56.9000	11.122	11776	131818
Riboflavin	$C_{17}H_{20}N_4O_6$	376.4	(+)	368.9000> 145.0000	7.526	3301	34319
Quercetine	$C_{15}H_{10}O_7$	302.23	(+)	302.4000> 285.1000	11.415	41377	440944
Vitexin	$C_{21}H_{20}O_{10}$	432.4	(+)	432.9000> 313.1500	13.212	300	891
Vanillin	$C_8H_8O_3$	152.15	(+)	153.2000> 93.0000	7.696	4546	40103
Catechin	$C_{15}H_{14}O_6$	290.27	(+)	291.1000> 179.1000	13.765	92713	798945
Thymol	$C_{10}H_{14}O$	150.22	(+)	151.1000> 91.1500	8.989	11339	245383
Caffeic Acid	$C_9H_8O_4$	180.16	(-)	179.2000> 135.0000	7.225	34903	460126
Chlorogenic Acid	$C_{16}H_{18}O_9$	354.31	(-)	353.1000> 191.1000	6.669	5901	143015
Vanillic Acid	$C_8H_8O_4$	168.15	(-)	167.2000> 152.0000	7.723	1880	21676
Gallic acid	$C_4H_4O_4$	170.12	(-)	169.2000> 125.0500	9.428	400	2057
Salycilic acid	$C_7H_6O_3$	138.12	(-)	137.0000> 93.0000	6.861	17127	220701

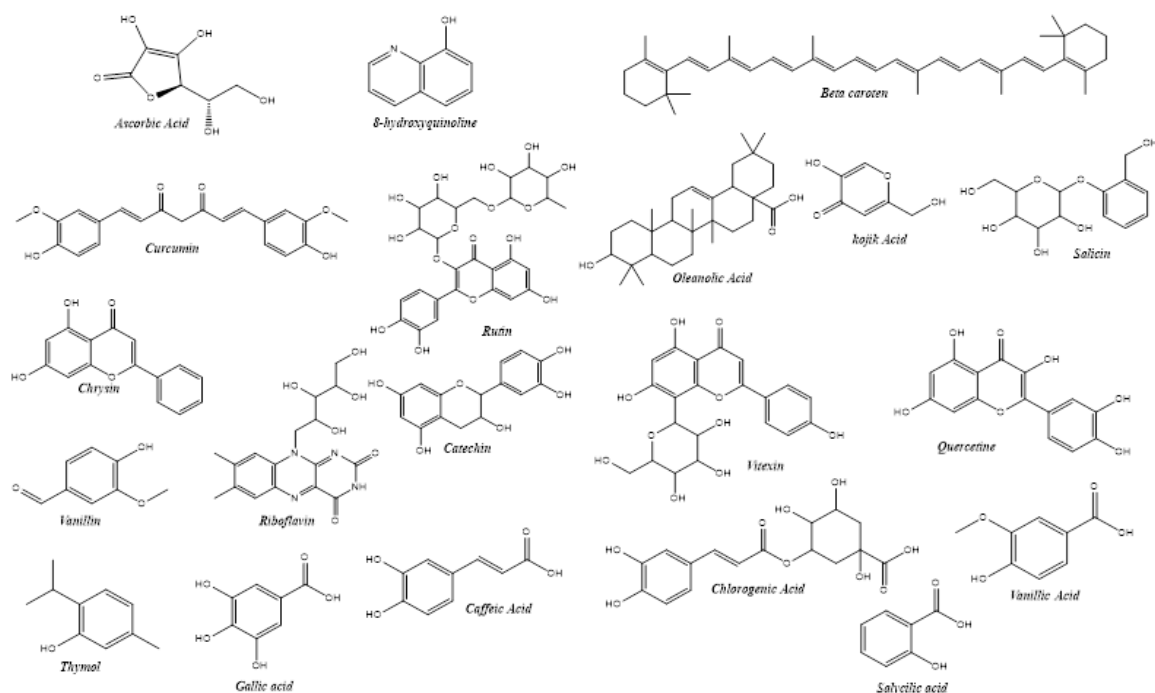


Figure 5. Chemical structure of the compounds detected in the ethanolic extract of *Pituranthos scoparius* (Autumn) by UPLC-ESI-MS-MS analysis

Table 5. GC-MS analysis of essential oils of *Pituranthos scoparius* (Spring)

	R T	Area	Area %	height	Height %	similarity	R I	Name	cas
1	2.525	345552	0.03	134189	0.04	93	667	hexane	110-54-3
2	2.578	332775	0.02	155151	0.04	95	670	Butanal, 2-methyl-	96-17-3
3	3.023	792857	0.06	356643	0.09	96	699	Furan, 2-ethyl-	3208-16-0
4	4.056	27461697	2.01	13650765	3.56	98	766	Benzene, methyl-	108-88-3
5	4.550	554077	0.04	256235	0.07	89	798	Hexane, 2,4-dimethyl-	589-43-5
6	5.160	343237	0.03	236427	0.06	93	844	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	0-00-0

7	5.505	134280	0.01	46531	0.01	93	871	Benzene, 1,2-dimethyl-	95-47-6
8	5.626	198159	0.01	97853	0.03	88	880	Cyclopentane, 1,1,3-trimethyl-	4516-69-2
9	5.756	1042379	0.08	586199	0.15	94	890	1-Nonene	124-11-8
10	5.858	2729033	0.20	1302209	0.34	94	898	4,4-Dimethyl octane	15869-95-1
11	5.935	151868	0.01	73123	0.02	80	904	2-Bornene	464-17-5
12	6.173	26288472	1.92	13498936	3.52	93	927	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyl-1,2,8,6,7,1)--	0 5-2
13	6.272	59959090	4.38	29515339	7.70	94	936	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	2-ene 7785-70-8
14	6.451	5710266	0.42	3006414	0.78	93	953	Camphene	79-92-5
16	6.610	412917	0.03	86951	0.02	80	968	Benzaldehyde	100-52-7
17	6.681	13315483	0.97	6287516	1.64	94	975	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methyl-3,1,3,8,7,1-)	4-1 -5
18	6.750	15049902	1.10	7214559	1.88	93	981	2(10)-Pinene	127-91-3
19	6.826	23446501	1.71	10642097	2.78	93	989	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-1,8-	7(21-S6)7- -3
20	6.853	33695294	2.46	10667056	2.78	89	991	2,3-Dehydro-1,8-cineole	92760-25-3

21	7.130	347328 148	25.3 9	431353 35	11.2 5	93	1019	alpha.- phellandrene (only name in Wiley6)	1529-99- 3
22	7.173	581331 6	0.42	464959 6	1.21	90	1023	2,4,6- Octatriene, 2,6- dimethyl-	673-84-7
23	7.259	120716 500	8.82	299626 44	7.82	87	1032	Benzene, 1,2,3,4- tetramethyl-	488-23-3
24	7.337	208806 915	15.2 6	418368 73	10.9 2	91	1040	(+)-m-Mentha- 1(6),8-diene	1461-27- 4
25	7.544	112826 42	0.82	466621 6	1.22	95	1061	.gamma.- Terpinene	99-85-4
26	7.670	224044 9	0.16	615285	0.16	87	1074	5-Isopropyl-2- methylbicyclo[3 .1.0]hexan-2-ol	2-ol 17699- 16-0
27	7.820	162694 51	1.19	863485 2	2.25	95	1089	4- Isopropylidene- 1-cyclohexene	586-62-9
28	7.892	318267 08	2.33	127181 49	3.32	94	1096	Tetradecane	629-59-4
30	8.096	991729	0.07	554665	0.14	82	1117	2-Caren-4-ol	6617-35- 2
31	8.228	134883 97	0.99	554520 6	1.45	95	1130	2-Cyclohexen- 1-ol, 1-methyl- 4-(1- methylethyl) 2- ,9 c8i0s-	3 -82-5
32	8.342	209543 7	0.15	943923	0.25	81	1141	Cyclohexane, 1,3- butadienyldiene -	53864- 08-7

33	8.382	110498 9	0.08	730667	0.19	80	1145	Ethyl - cyclohexene - aldehyde	0-00-0
34	8.422	898564 3	0.66	371962 7	0.97	92	1149	2-Cyclohexen- 1-ol, 1-methyl- 4-(1- methylethyl) 2- ,9 c8i0s	3 -82-5
35	8.467	652135 3	0.48	245354 6	0.64	82	1153	3,6-Octadienal, 3,7-dimethyl-	55722- 59-3
36	8.594	643669	0.05	279716	0.07	70	1166	4,4- Dipropylheptan e	17312- 72-0
37	8.640	741914	0.05	351459	0.09	81	1171	Santalol, E- cis, epi-.beta.-	14490- 17-6
38	8.659	501271 6	0.37	273095 8	0.71	85	1176	1-Adamantanol	768-95-6
39	8.731	717776 3	0.52	335710 3	0.88	78	1180	1-Adamantanol	768-95-6
40	8.913	140033 272	10.2 4	331400 91	8.65	96	1198	3,6-Dimethyl- 2,3,3a,4,5,7a- hexahydro-1- benzo 7fu0r7a8n6 -	44-6
41	8.982	190578 91	1.39	821534 5	2.14	76	1204	Bicyclo[3.3.0]o ctan-3-one, 7- ethylidene-	0-00-0
42	9.053	279885 45	2.05	956328 4	2.50	92	1211	3-isopropyl-6- methyl-7- oxabicyclo[4.1. 0]hept- 47- 2e1n3e8	69-3

43	9.204	117259 25	0.86	321813 5	0.84	82	1225	Benzaldehyde, 4-(1-methylethyl)-	122-03-2
44	9.270	151751 2	0.11	758303	0.20	78	1231	1-Cyclohexen- 1-ol, 2,6- dimethyl-, acetate	6203-89- 0
45	9.320	180730 9	0.13	813649	0.21	85	1236	2-Cyclohexen- 1-ol, 2-methyl- 5-(1-methylethyl	536-30-1
46	9.360	107701 1	0.08	538028	0.14	88	1239	Dill ether	74410- 10-9
47	9.463	227358 66	1.66	840540 3	2.19	94	1249	D-Carvone	2244-16- 8
48	9.524	443615 7	0.32	200256 6	0.52	93	1255	Cyclohexen-1- one, 2-methyl- 5-(1-methylethy 4l)9-9	7S1)-- 8
49	9.620	249729 2	0.18	580360	0.15	74	1263	1,2,3,4- tetrahydro- 1,2,3-trimethyl- naphthalene	0-00-0
50	9.685	239045 0	0.17	879310	0.23	90	1269	(1R,2S,4S,5R,7 R)-5-isopropyl- 1-methyl-3,8-d	0-00-0
51	9.771	222602 9	0.16	903677	0.24	78	1277	8- Hydroxymethyl -trans- bicyclo[4.3.0]no n-3-en 0e-	0-00-0
52	9.876	438358 0	0.32	149177 5	0.39	81	1287	(4S)-4- isopropylcycloh	23963- 70-4

								exene-1-carbaldehyde	
53	9.987	102603 93	0.75	372587 4	0.97	93	1297	Phenol, 2-methyl-5-(1-methylethyl)-	499-75-2
54	10.05 2	461295 4	0.34	155427 2	0.41	89	1303	3-Cyclohexene-1-ethanol, .beta.,4-dimethyl-	[S 1-3(R83*,5S-*7)5]--1
55	10.13 5	875037	0.06	334417	0.09	82	1310	Pentanoic acid, cyclobutyl ester	0-00-0
56	10.19 9	200844 7	0.15	676203	0.18	78	1316	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl	-5 3404-49-2
57	10.29 8	101010 99	0.74	491596 5	1.28	83	1325	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl -	-1 8679-48-6
58	10.36 1	161655 0	0.12	590946	0.15	74	1330	.alpha.-Cuprenene	29621-78-1
59	10.44 2	629948	0.05	259334	0.07	79	1337	Eucalyptol	470-82-6
60	10.60 4	416770	0.03	113074	0.03	78	1351	1,1,5,6-Tetramethylindane	942-43-8
61	10.76 2	294999	0.02	82300	0.02	79	1365	(E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene	T00-00
62	10.89 0	237444	0.02	68982	0.02	77	1376	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl) 1-,6 t7ra2n1s-	3 9-4

63	10.96 5	575240	0.04	315626	0.08	84	1383	2-Buten-1-one, 1-(2,6,6- trimethyl-1,3- cyclohex 2a3d7ie2n6-- 19-3yl)-4- , (E)-	2a3d7ie2 n6--19- 3yl)-4- ,
64	11.02 6	388934 0	0.28	178737 1	0.47	79	1388	Ethanone, 1- (1,3a,4,5,6,7- hexahydro-4- hydrox	55683- 15-3
65	11.07 2	132472 0	0.10	676508	0.18	72	1392	3-Methyl-2-(3- methylpentyl)- 3-buten-1-ol	13066- 54-1
66	11.12 0	241660 8	0.18	983410	0.26	77	1397	1,4-Dimethyl-7- (prop-1-en-2- yl)decahydroaz ul e2n1	-649-o8l- 41-9
67	11.22 6	741072	0.05	266064	0.07	63	1406	1,1,2,2- Tetramethyl-5- methylidene- octahydroc	0-00-0
68	11.30 0	152076 1	0.11	732632	0.19	85	1412	2-Butanone, 4- (2,6,6- trimethyl-1,3- cyclohexad	20418- 36-7
69	11.38 5	207444 9	0.15	713373	0.19	77	1419	beta.-Terpineol	138-87-4
70	11.52 5	362314	0.03	159790	0.04	71	1431	(1R,2S,6S,7S,8 S)-8-Isopropyl- 1-methyl-3-met	1852-44- 3
71	11.56 8	827029	0.06	334994	0.09	94	1435	Bicyclo[7.2.0]u ndec-4-ene, 4,11,11- trimethyl	118-65-0

72	11.60 6	133037	0.01	101974	0.03	72	1438	(1S,6R)-3-methyl-6-propan-2-yl-1-cyclohex	00-00-0
73	11.68 4	588548	0.04	179813	0.05	77	1444	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)	1-,6 t7ra2n1s- -3 9-4
74	12.14 0	671757	0.05	281369	0.07	86	1483	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexe	14901- 07-6
75	12.27 4	109087 99	0.80	550599 4	1.44	95	1494	(-)-Germacrene D	23986- 74-5
76	12.38 5	476559	0.03	213321	0.06	92	1504	Naphthalene, decahydro-4a-methyl-1-methylen	17-0667- 0
77	12.46 0	102568 4	0.07	310283	0.08	81	1510	7-epi-trans-sesquisabinene hydrate	0-00-0
78	12.61 1	404074 6	0.30	172357 3	0.45	91	1523	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	)- 607- 91-0
79	12.66 6	264408 6	0.19	885472	0.23	78	1527	Chrysantenyl 2-methuylbutanoate	53820- 13-6
80	12.85 0	242329	0.02	73300	0.02	76	1543	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethen	97-42-7

81	13.24 4	331319 9	0.24	158327 7	0.41	83	1576	1H-Cyclobuta[c]pentalen-5-one,2,2a,4a,5,6,7	e1x5a3hy 2d-2ro2- 21,
82	13.51 6	140245 8	0.10	684416	0.18	86	1599	Menth-1-en-9-yl tiglate, p-	0-00-0
83	13.63 2	497499	0.04	250309	0.07	88	1609	1,3,8-p-Menthatriene	18368- 95-1
84	13.77 5	391251 50	2.86	125374 01	3.27	92	1622	1,3-Benzodioxole, 4,7-dimethoxy-5-(2-propen y5l)	2-3 -80-8
85	13.89 5	72691	0.01	64597	0.02	72	1632	(1S,4R,5R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.	81718- 24-0
86	14.01 5	169933	0.01	78609	0.02	86	1642	)-4-methyl-3-cyclohexen-1-yl]propyl-2-	0-00-0
87	14.15 1	190715	0.01	66680	0.02	83	1654	tau.-Muurolol	19912- 62-0
88	14.37 5	871223	0.06	280806	0.07	92	1673	Neointermedeol	5945-72- 2
89	14.80 6	318489	0.02	175925	0.05	79	1700	(S,E)-2,5-Dimethyl-4-vinylhexa-2,5-dien-1-yl	20384- 05-8
90	15.05 9	247716	0.02	115550	0.03	78	1733	1,3,8-p-Menthatriene	18368- 95-1
91	15.11 2	331305	0.02	133723	0.03	74	1738	2-(4-tert-butylphenoxy)acetic acid ethyl ester	3344- 19-2

92	15.34 5	285309	0.02	87274	0.02	83	1759	Menth-1-en-9-yl angelate	, p- 0-00-0
93	15.52 6	560528 0	0.41	224563 1	0.59	84	1775	1H-Cyclobuta[c]pentalen-5-one,2,2a,4a,5,6,7-	8152-22-124
94	15.64 5	644154	0.05	170942	0.04	78	1785	(S,E)-2,5-Dimethyl-4-vinylhexa-2,5-dien-1-yl	20384-05-8
95	15.77 1	228898 2	0.17	913367	0.24	86	1797	Menth-1-en-9-yl angelate	p- 0-00-0
96	15.91 5	103805 2	0.08	350367	0.09	86	1810	1,3,8-p-Menthatriene	18368-95-1
97	16.13 1	677618	0.05	396768	0.10	94	1829	Neophytadiene	504-96-1
10 0	16.31 6	96022	0.01	45403	0.01	82	1846	1H-Cyclobuta[c]pentalen-5-one,2,2a,4a,5,6,7	81532-22-1
10 1	16.54 8	158820	0.01	55499	0.01	85	1868	Menth-1-en-9-yl tiglate,	p- 0-00-0
10 2	18.99 5	153750	0.01	34587	0.01	81	2105	Phytol	150-86-7

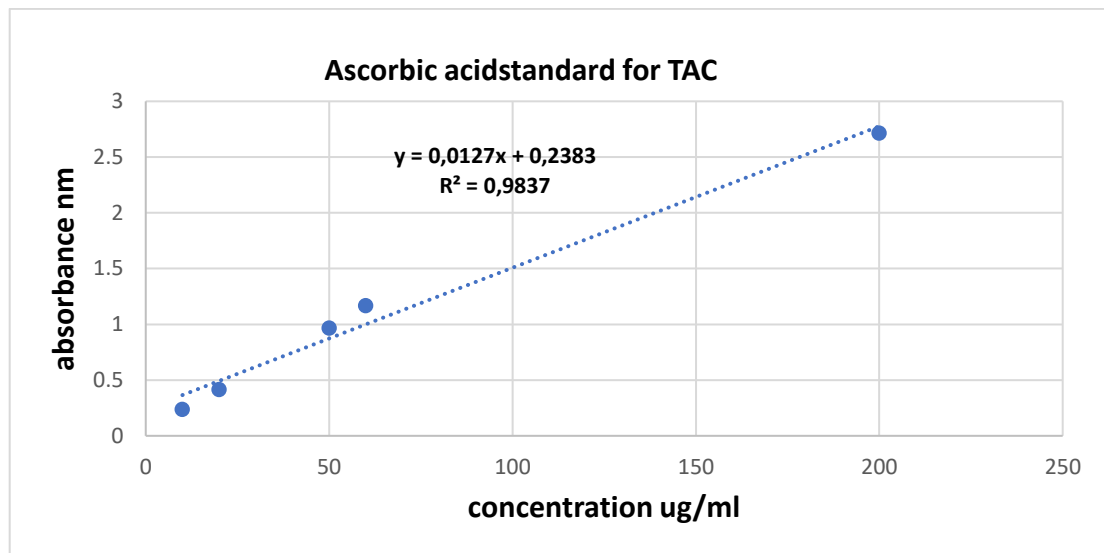


Figure6. Ascorbic acid as standard for TAC assay.

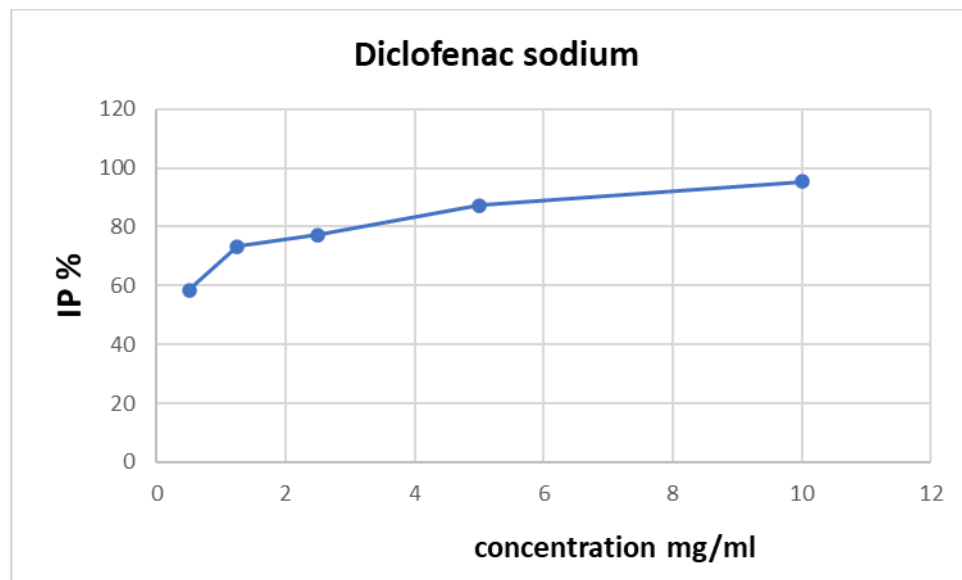


Figure 7. Curve represented the inhibitory effect of denaturation of BSA by Diclofenac Sodium

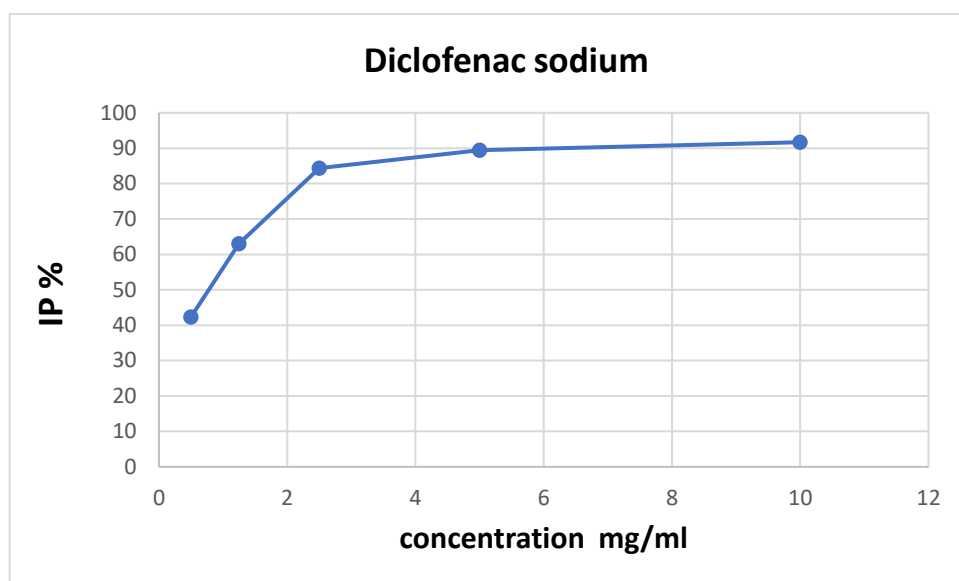


Figure 8. Curve represented the inhibitory effect of denaturation of Egg albumin by Diclofenac Sodium