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THEME

STUDY OF SECONDARY METABOLITES IN MEDICINAL PLANTS OF FORAGE INTEREST

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DEDICATION

This work is dedicated to:

To my dear parents

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ABSTRACT

This study aims to evaluate local medicinal-forage plant species as sustainable resources for improving livestock nutrition and health in semi-arid regions.

Based on an ethnobotanical survey conducted in Bordj Bou Arréridj, Algeria, the research documents traditional knowledge of wild forage plants. From this, six medicinal-forage species: *Juniperus phoenicea, Ziziphus lotus, Artemisia herba-alba, Opuntia ficus-indica, Sinapis arvensis,* and *Atriplex canescens*, were selected for comprehensive laboratory analysis of their phytochemical, antioxidant, and nutritional profiles.

Qualitative phytochemical screening identified diverse secondary metabolites, including phenolics, flavonoids, tannins, terpenoids, alkaloids, and saponins, with distinct variations between 70% ethanol and aqueous extracts. Quantitative analysis revealed that *J. phoenicea* and *Z. lotus* had the highest total phenolic and flavonoid contents using hydroalcoholic extraction (169.31 ± 3.88 mg GAE/g DW and 24.18 ± 0.22 mg QE/g DW, respectively), whereas *O. ficus-indica* showed superior extraction efficiency in aqueous media, yielding 190.12 mg GAE/g DW.

Antioxidant assays (DPPH and FRAP) confirmed strong radical scavenging activity, especially in *J. phoenicea* and *Z. lotus*, suggesting potential for reducing oxidative stress in livestock. Proximate analysis showed moderate crude protein levels (6.8–8.2%), with *O. ficus-indica* and *S. arvensis* meeting maintenance requirements for ruminants. *A. canescens* was identified as a rich source of potassium (47.376 mg/g), crucial for electrolyte balance in ruminants. FT-IR and LC-MS/MS analyses confirmed the presence of bioactive compounds such as chlorogenic acid, β-carotene, and thymol, known for their antioxidant, antimicrobial, and metabolic benefits.

These findings support the integration of these native medicinal-forage plants into livestock diets as functional supplements or feed additives to enhance nutrient uptake, immunity, and feeding efficiency, while reducing reliance on synthetic inputs. Their resilience in arid conditions highlights their value in developing climate-adapted, resource-efficient animal production systems tailored to semi-arid environments.

Keywords: Medicinal-forage plants, secondary metabolites, antioxidant activity, livestock nutrition, semi-arid regions, sustainable agriculture.

RÉSUMÉ

Titre : Eude des métabolites secondaires dans des plantes médicinales d'intérêt fourrager.

Cette étude vise à évaluer le potentiel de plantes fourragères médicinales locales comme ressources durables pour améliorer la nutrition et la santé du bétail dans les régions semi-arides.

À partir d'une enquête ethnobotanique menée à Bordj Bou Arréridj (Algérie), l'étude a permis de documenter les savoirs traditionnels relatifs aux plantes fourragères sauvages. Sur cette base, six espèces à double usage nutritionnel et médicinal : *Juniperus phoenicea, Ziziphus lotus, Artemisia herba-alba, Opuntia ficus-indica, Sinapis arvensis* et *Atriplex canescens*, ont été sélectionnées pour une analyse approfondie de leur composition phytochimique, de leur activité antioxydante et de leur valeur nutritionnelle.

Le criblage qualitatif a révélé la présence de divers métabolites secondaires : phénols, flavonoïdes, tanins, terpénoïdes, alcaloïdes et saponines, avec des différences notables entre extraits aqueux et hydroalcooliques. L'analyse quantitative a montré que *J. phoenicea* et *Z. lotus* présentent les teneurs les plus élevées en phénols totaux (169,31 ± 3,88 mg EAG/g MS) et flavonoïdes (24,18 ± 0,22 mg EQ/g MS). *O. ficus-indica*, quant à lui, a montré une extraction optimale dans les solvants aqueux (190,12 mg EAG/g MS).

Les tests antioxydants (DPPH et FRAP) ont confirmé une forte activité antiradicalaire, surtout chez *J. phoenicea* et *Z. lotus*, indiquant un potentiel pour atténuer le stress oxydatif chez les ruminants. L'analyse proximale a mis en évidence des teneurs modérées en protéines brutes (6,8–8,2 %), suffisantes pour répondre aux besoins d'entretien avec *O. ficus-indica* et *S. arvensis*. *A. canescens* s'est distingué par sa richesse en potassium (47,376 mg/g), essentiel à l'équilibre électrolytique. Les analyses FT-IR et LC-MS/MS ont confirmé la présence de composés bioactifs tels que l'acide chlorogénique, le β-carotène et le thymol, reconnus pour leurs effets bénéfiques sur la santé.

Ces résultats appuient l'intégration de ces plantes dans les régimes alimentaires du bétail, soit en tant que compléments fonctionnels, soit comme additifs naturels, afin d'améliorer l'efficacité alimentaire, renforcer l'immunité et réduire l'usage des produits synthétiques. Leur résilience climatique souligne leur rôle dans le développement de systèmes d'élevage durables et adaptés aux conditions semi-arides.

Mots-clés : Plantes médicinales-fourragères, métabolites secondaires, activité antioxydante, nutrition animale, régions semi-arides, agriculture durable.

الملخص

العنوان: دراسة المركبات الثانوية في النباتات الطبية ذات الأهمية العلفية

تُقيّم هذه الدراسة الإمكانات التغذوية والعلاجية للنباتات الطبية العلفية المحلية باعتبارها موارد مستدامة لتحسين تغذية الماشية وصحتها في المناطق شبه الجافة.

وبالاعتماد على نتائج المسح الإثنوبوتاني الذي أُجري في ولاية برج بوعريريج (الجزائر)، تم توثيق المعارف التقليدية المتعلقة باستخدام النباتات العلفية البرية، وخاصة الأنواع ذات الخصائص الغذائية والعلاجية المزدوجة. وبناءً على ذلك، تم اختيار ستة أنواع نباتية لإجراء تحليل مخبري شامل لتركيبها الكيميائي النباتي، ونشاطها المضاد للأكسدة، وقيمتها الغذائية، وهي :العرعار الفينيقي(*Juniperus phoenicea*) ، السدر(*Ziziphus lotus*) ، الشيح *Artemisia*) (*herba-alba*، التين الشوكي(*Opuntia ficus-indica*) ، الخردل البري(*Sinapis arvensis*) ، والقطف (*Atriplex canescens*).

أظهر التحليل الكيميائي النوعي وجود تنوع في المواد الثانوية مثل الفينولات، الفلافونويدات، التانينات، التربينويدات، القلويدات والسابونينات، مع اختلافات واضحة بين المستخلصات المائية والإيثانولية (70%). كما أوضحت النتائج الكمية أن نوعي *J. phoenicea* و*Z. lotus* يحتويان على أعلى مستويات من الفينولات والفلوفونويدات الكلية عند استخدام الاستخلاص الهيدروكحولي 3,88 ± 169,31) ملغرام/GAE غ م ج و24,18 ± 0,22 ملغرام/QE غ م ج على التوالي(، بينما أظهر *O. ficus-indica* كفاءة استخراج تعتمد على نوع المذيب، إذ بلغت تركيز الفينولات الكلية فيه 190,12 ملغرام/GAE غ م ج في المستخلص المائي .

أكدت التجارب المضادة للأكسدة DPPH) و (FRAPوجود نشاط قوي في التقاط الجذور الحرة، خاصة لدى النوعين *J. phoenicea* و*Z. lotus*، مما يشير إلى إمكانية استخدامهما لتقليل الإجهاد التأكسدي لدى الماشية. وأشارت التحليلات الغذائية إلى مستويات معتدلة من البروتين الخام (6,8–8,2%)، مع تلبية *O. ficus-indica* و*S. arvensis* لاحتياجات الصيانة الأساسية للماشية العاشبة. كما تميز *A. canescens* بغناه بالبوتاسيوم (47,376 ملغ/غ)، وهو ضروري للتوازن الكهربائي لدى الحيوانات المجترة. وأكدت تحليلات FT-IR و LC-MS/MSوجود مركبات فعالة بيولوجيًا مثل حمض الكلوروجينيك، البيتاكاروتين والثيمول، المعروفة بخصائصها المضادة للأكسدة والجراثيم وتحفيز الأيض .

تدعم هذه النتائج دمج هذه النباتات المحلية في أنظمة تغذية الماشية، سواء كمكملات وظيفية أو كإضافات غذائية طبيعية، بهدف تحسين امتصاص العناصر الغذائية والاستجابة المناعية وكفاءة التغذية العامة، مع تقليل الاعتماد على الإضافات الاصطناعية. وتبرز مقاومة هذه النباتات للظروف المناخية القاسية دورها في تطوير أنظمة تربية ماشية مستدامة وفعالة في استخدام الموارد، والمُلائمة للمناطق شبه الجافة .

الكلمات المفتاحية : النباتات الطبية والعلفية، المواد الثانوية، النشاط المضاد للأكسدة، تغذية الماشية، المناطق شبه الجافة، الزراعة المستدامة .

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LIST OF ABBREVIATIONS

MAPs: Medicinal and Aromatic Plants

SMs: Secondary Metabolites

CP: Crude Protein

CT: Condensed Tannins

DM: Dry Matter

VFA: Volatile Fatty Acids

SOD: Superoxide Dismutase

CAT: Catalase

GSH-Px: Glutathione Peroxidase

ROS: Reactive Oxygen Species

FT-IR: Fourier-Transform Infrared Spectroscopy

LC-MS/MS: Liquid Chromatography–Mass Spectrometry/Mass Spectrometry

RFC: Relative Frequency of Citation

DPPH: 2,2-Diphenyl-1-picrylhydrazyl (antioxidant assay)

FRAP: Ferric Reducing Antioxidant Power

NRC: National Research Council

ARC: Agricultural Research Council

ANFs: Anti-Nutritional Factors

IC50: The concentration of extract required to scavenge 50% of DPPH radicals

INTRODUCTION

Introduction

Livestock production in semi-arid regions faces significant challenges due to harsh environmental conditions, which directly affect animal health and indirectly limit forage availability. In Algeria, where more than 95% of the land is classified as semi-arid or arid (~80% arid and ~15% semi-arid) (Benhanifia et al., 2004), these challenges are particularly acute, threatening the socio-economic stability of rural communities reliant on livestock farming (Kanoun et al., 2007; Bengouga et al., 2019). Globally, climate change exacerbates these issues by intensifying feed scarcity and environmental degradation (Nardone et al., 2010). Traditional feeding practices, heavily dependent on commercial feeds and synthetic additives, are increasingly recognized as economically unsustainable and raise concerns about their environmental impact and implications for animal health (Miara et al., 2019). These factors underscore the urgency for innovative, sustainable solutions in livestock nutrition.

Medicinal forage, defined as plants that provide both nutritional and medicinal benefits to animals, represents a promising alternative. These plants are rich in bioactive compounds, or secondary metabolites (SMs), which can enhance animal health and productivity (Salmasi, 2024). Algeria's rich diversity of medicinal and aromatic plants, shaped by its climatic and topographic variability (Benarba, 2015), provides a unique opportunity to explore their potential as forage resources. However, the nutritional profiles of many species remain understudied, representing a critical gap in the literature. Addressing this gap could unlock the potential of medicinal plants to support livestock maintenance requirements, improving feed efficiency while reducing economic costs (Ragab Elwa et al., 2022).

Historically, secondary metabolites such as phenolics, flavonoids, tannins, and terpenoids were considered anti-nutritional factors (ANFs) due to their potential to inhibit protein digestion and disrupt nutrient bioavailability (Boufennara et al., 2013). Recent studies, however, highlight that these compounds, at optimal concentrations, offer significant functional benefits. Phenolics and tannins, for instance, exhibit antioxidant properties by scavenging free radicals, thereby mitigating oxidative stress and enhancing animal immunity (Adli et al., 2024). Certain SMs also regulate gut microbial populations, reducing pathogenic infections and improving nutrient absorption (Kazemi and

Mokhtarpour, 2022). Despite these advances, the mechanisms underlying these effects, particularly in ruminants, remain poorly understood, marking an important research frontier.

The incorporation of medicinal plants into animal diets has gained traction as a novel approach to improving livestock health and productivity. These plants, through their bioactive compounds, offer numerous benefits beyond traditional feed ingredients (Bhatt, 2015). Medicinal plants and herbs are increasingly recognized as eco-friendly alternatives to synthetic additives, enhancing feed intake, digestion, and overall animal performance (Paskudska et al., 2018). Advancing this field requires a comprehensive understanding of the chemical composition, physiological effects, and practical applications of these plants, as well as consumer preferences and expectations. This study seeks to address these knowledge gaps by investigating the phytochemical profiles and bioactive properties of selected medicinal plants, providing a scientific basis for their use in livestock nutrition.

Secondary metabolites have been extensively studied for their adverse effects when ingested in excess (Colegate et al., 1994; D'Mello, 1997; Cheeke, 1998; Acamovic and Brooker, 2005). However, recent research highlights their beneficial effects, including antioxidant, antimicrobial, and immune-boosting properties (Jeon et al., 2003; Nash, 2004; Bento et al., 2004; Cushnie and Lamb, 2005). These findings suggest that SMs can improve ruminal fermentation, enhance protein utilization, and reduce methane emissions, thereby contributing to more sustainable livestock production systems (Olagaray and Bradford, 2019; Orzuna-Orzuna et al., 2023).

This dissertation focuses on six medicinal plants—*Atriplex canescens*, *Artemisia herba-alba*, *Juniperus phoenicea*, *Ziziphus lotus*, *Sinapis arvensis*, and *Opuntia ficus-indica*—selected for their ecological adaptability, traditional use in livestock nutrition, and high content of bioactive compounds (Makkar et al., 2007). These species offer dual benefits, contributing to both nutritional value and therapeutic potential in livestock diets.

The primary objectives of this research are to:

1. Document knowledge on wild forage plants through an ethnobotanical survey in Bordj Bou Arreridj, Algeria, to identify species that serve dual purposes as both forage and medicinal resources.
2. Identify and quantify key secondary metabolites in the selected plants and evaluate their antioxidant properties.

3. Develop evidence-based recommendations for the effective inclusion of these plants in livestock diets.

This dissertation is structured as follows:

- Chapter I: Literature Review—Explores the secondary metabolites, their historical classification as anti-nutritional factors, and current research on their health-promoting functions.
- Chapter II: Materials and Methods—Details the study area, plant selection criteria, phytochemical analysis techniques, and laboratory experiments to evaluate nutritional and bioactive properties.
- Chapter III: Results and Discussion—Presents findings on the phytochemical composition of selected plants, their functional bioactivity, and implications for animal nutrition.
- Conclusion and Perspectives—Summarizes key findings, discusses their relevance for sustainable livestock systems, and outlines recommendations for future research.

CHAPTER I: LITERATURE REVIEW

1 Importance of the livestock sector

1.1 Socio-economic contribution

Livestock production is a cornerstone of the socio-economic fabric in rural communities, particularly in developing countries where it plays a significant role in poverty-decreasing tactics (FAO, 2018). It sustains the livelihoods of over 1 billion smallholder livestock producers, contributing approximately 40% of the agricultural Gross Domestic Product (GDP) and accounting for 2% to over 33% of household incomes (Alders et al., 2021). In Algeria, where more than 95% of the land is classified as semi-arid or arid (~80% arid and ~15% semi-arid) (Benhanifia et al., 2004), livestock farming is a cornerstone of rural livelihoods, providing income, food security, and employment for millions of people (Bengouga et al., 2019). Livestock, including sheep, goats, and cattle, are not only a source of meat, milk, and wool but also serve as a form of capital that can be traded or sold during times of economic need (Kanoun et al., 2007). In addition to their direct economic contributions, livestock are integral to cultural practices and social structures (Dabasso et al., 2022), often symbolizing wealth and status within communities. Additionally, manure from livestock is a key component of soil fertility management in crop production, promoting circular agricultural systems (Doyeni et al., 2023). Given the anticipated need for a 50% to 70% increase in animal products to feed 9 billion people by 2050 (Alexandratos and Bruinsma, 2012), the role of livestock in enhancing food security and agricultural sustainability cannot be overstated.

1.2 Challenges in livestock production

Livestock production faces significant challenges that threaten its sustainability and productivity, particularly in the context of climate change. Previous studies have highlighted inappropriate animal husbandry practices, with approximately 30–35% losses reported in the animal breeding sectors of emerging countries (FAO, 2002). Climate change affects livestock systems both directly and indirectly: directly through its influence on animal physiology, behavior, production, and welfare, and indirectly by altering feed availability, composition, and quality (Henry et al., 2018).

One of the most pressing issues is forage scarcity, which is exacerbated by prolonged droughts, overgrazing, and land degradation (Ахметова et al., 2024). Droughts, in particular, have become more frequent and severe due to climate change, leading to reduced availability of natural pastures and water resources (Koutroulis et al., 2024). This scarcity of forage not only compromises animal health and productivity but also forces farmers to rely on

expensive commercial feeds, which are often unaffordable for small-scale producers (Siad et al., 2022). Furthermore, environmental degradation, such as soil erosion and desertification, further limits the availability of grazing land, creating a vicious cycle of resource depletion and declining livestock productivity. Compounding these challenges are the rising costs of commercial feeds and veterinary care, which place additional financial strain on smallholder farmers and limit their ability to invest in improved livestock management practices (Jensen et al., 2009).

Oxidative stress, a state of redox imbalance characterized by excessive reactive oxygen species (ROS) production overwhelming endogenous antioxidant defenses (Hassan et al., 2024), poses significant challenges to animal health, productivity, and product quality. This disequilibrium arises from both metabolic processes and exogenous stressors, including prolonged transport, poor handling practices, nutritional deficiencies, and environmental extremes such as erratic airflow, high temperature, and noise pollution (Minka and Ayo, 2009; Fayem and Muchenje, 2012). These factors disrupt homeostasis, triggering systemic stress responses that compromise physiological and metabolic functions. In livestock, oxidative stress manifests through diverse pathways, it exerts profound effects on animal production efficiency and reproductive performance (Tang et al., 2024). Physiologically, it can induce cardiac arrhythmias, hypertension, and impaired thermoregulation, undermining animal welfare (Cataldi, 2010). Productively, it reduces feed intake, lowers milk yield, diminishes milk quality, and shortens productive lifespans (Guo et al., 2023). Its most profound impacts, however, are observed in meat quality. Oxidative stress promotes lipid peroxidation in muscle tissues, degrading polyunsaturated fatty acids and generating harmful compounds such as malondialdehyde (Costantini and Bonadonna, 2010). This process accelerates meat discoloration, flavor deterioration, and nutrient loss while reducing shelf stability (Falowo et al., 2014). Additionally, oxidative damage to proteins increases meat toughness, directly affecting tenderness and consumer acceptability. The interplay between oxidative stress and meat quality is further modulated by genetic, metabolic, and pre-slaughter handling factors. For instance, interspecies confinement and aggressive handling during transport exacerbate ROS production, amplifying postmortem oxidative damage (Warriss, 2010).

1.3 Need for sustainable solutions

Addressing these challenges faced by the livestock sector in semi-arid regions requires innovative and sustainable solutions that enhance resilience and productivity while

minimizing environmental impact. One promising approach is the development of sustainable feeding strategies that leverage locally available resources, such as drought-tolerant forage plants and agricultural by-products (Craine et al., 2013; Henry et al., 2018). By reducing dependence on commercial feeds and improving the nutritional value of locally sourced forage, these strategies can help mitigate the effects of forage scarcity and enhance animal health and productivity. Integrating traditional knowledge with modern scientific research can provide valuable insights into the use of locally adapted plants as complementary forage resources, offering both nutritional and health benefits to livestock (Lobo et al., 2024). Such efforts not only improve the resilience of livestock systems but also contribute to the broader goals of climate adaptation and sustainable development in semi-arid regions.

2 Medicinal plants in livestock systems

2.1 Definition of medicinal plants

Medicinal plants, as defined by the World Health Organization (WHO), are plants that contain substances with therapeutic properties, either in their entirety or in specific organs, which can be used directly for therapeutic purposes or as precursors for drug synthesis (Farnsworth and Soejarto, 1991). These bioactive substances, often referred to as phytochemicals or active principles, are responsible for the medicinal properties of plants and can be found in various parts of the plant, including roots, stems, leaves, bark, fruits, and seeds (Liu, 2004; Hamuel et al., 2009). Certain plants are used for both humans and animals, serving as food and/or for therapeutic purposes (Sghaier, 2014; Hamzah et al., 2017), in the context of livestock systems, medicinal plants serve as nutri-medicinal resources, providing both health benefits and nutritional value. For example, plants like *Lavandula officialis* and *Artemisia herba-alba* are rich in secondary metabolites, such as flavonoids and phenolics, which enhance animal health and productivity (Al-Masri, 2013).

2.2 History of medicinal plants in animal approach

Stress factors and inadequate nutrition significantly impact animal productivity, health, behavior, and overall well-being, increasing susceptibility to diseases. This not only raises the need for veterinary interventions but also poses risks to food consumers, reduces productivity, and threatens the environmental sustainability of livestock rearing systems and associated food chains. Good nutrition is a fundamental requirement for all farm animals, serving as a key contributor to maintaining animal welfare and health.

The incorporation of medicinal forage supplements into animal nutrition is gaining attention as a novel approach to enhance livestock health and productivity. This method leverages the bioactive compounds found in medicinal plants, which offer benefits beyond traditional feed ingredients. The use of medicinal plants in livestock systems has a long history, particularly in semi-arid regions and remote areas where access to conventional veterinary services is limited or too costly for local farmers (Tabuti et al., 2003; Nyamanga et al., 2008). These plants, often referred to as "medicinal forage," provide both nutritional and therapeutic benefits, containing active compounds or secondary metabolites that support animal health (Salmasi, 2024). This dual role of medicinal plants as both feed and medicine has made them an integral part of traditional livestock management practices, offering sustainable and cost-effective solutions to modern challenges in animal husbandry.

Health wise, medicinal plants are increasingly recognized for their ability to improve livestock health and productivity through their bioactive compounds by boosting immunity, reducing oxidative stress, and managing diseases (Huyghebaert et al., 2011). The World Health Organization has emphasized the importance of investigating and mobilizing traditional systems of medicine, including the use of medicinal plants, to address global health challenges (Chan, 2008). In veterinary medicine, medicinal plants are widely used to treat a variety of livestock species, including cattle, horses, sheep, goats, and pigs, which collectively represent approximately 70% of animals treated with herbal remedies (Viegi et al., 2003). This growing interest in herbal remedies is driven by both the trend toward natural products and the increasing evidence of their efficacy (Franz et al., 2010; Laudato and Capasso, 2013).

Nutrition wise, medicinal plants enhance nutrient digestibility, appetite, and digestion while exhibiting antimicrobial and antioxidant properties, making them a valuable alternative to synthetic chemical products, which often leave toxic residues and contribute to antimicrobial resistance (Lavinia et al., 2009; Savoia, 2012; Singh, 2019) Functionally, medicinal plants act as sialogogues, stimulating saliva secretion to facilitate swallowing (Hernández et al., 2004; Paskudska et al., 2018), and enhance bile acid synthesis in the liver, which is essential for lipid digestion and absorption. They also accelerate digestion and reduce feed passage time through the digestive tract (Platel and Srinivasan, 2001; Suresh and Srinivasan, 2007; Korošec et al., 2009). Additionally, these plants stimulate the secretion of pancreatic enzymes, such as lipases, amylases, and proteases, and enhance the activity of digestive enzymes in the gastric mucosa (Srinivasan, 2005).

These mechanisms collectively improve rumen fermentation, regulate nutrient intake and digestion, and enhance production outcomes, including growth, lactation, and reproduction (Vondruskova et al., 2009; Hashemi and Davoodi, 2011). The benefits of medicinal plants are reflected in improved production performance in dairy and feedlot cattle (Yang et al., 2008), significant impacts on the quality, composition and healthiness of animal products (Priolo et al., 2002; Karami, 2010; De Brito et al., 2017) and enhanced feed intake and wool growth in lambs (Celi and Raadsma, 2010).

In Algeria, traditional knowledge systems have long recognized the dual-purpose benefits of certain plants, such as *Artemisia herba-alba* and *Opuntia ficus-indica*, which are used to treat livestock diseases and improve nutrition (Zirmi-Zembri and Kadi, 2016; Miara et al., 2019). Similarly, in other regions of the world, medicinal plants have been integral to livestock management practices, offering a sustainable and cost-effective alternative to synthetic drugs and feeds (Ramírez et al., 2021).

2.3 Phytochemistry of medicinal plants

The term phytochemical is derived from two parts: “phyto”, meaning plant, and “chemical”, referring to the diverse array of natural compounds found in plants (Swamy and Kumar, 2022). These compounds can be broadly classified into two major categories based on their functional roles in plants: primary metabolites and secondary metabolites. While primary metabolites are essential for basic physiological processes such as growth, development, and reproduction, secondary metabolites play specialized roles in plant adaptation and defense. These two classes of metabolites differ significantly in their distribution, chemical structure, and functional roles within plants.

2.3.1 Primary metabolites

Primary metabolites are essential organic compounds involved in the basic physiological processes of plants, including growth, development, and reproduction. These metabolites, such as proteins, carbohydrates, and lipids, play a critical role in livestock nutrition. Proteins provide amino acids necessary for muscle development, immune function, and enzyme production, while carbohydrates and lipids serve as primary energy sources, supporting metabolic processes and overall animal health (Makkar et al., 2007). These nutritive components are important determinants of livestock growth rates, reproductive success, and behavior. The composition of primary metabolites in forage plants varies significantly, with fiber content ranging from 23% to 90%, protein from 2% to 36%,

and minerals from 2% to 22% (Lee, 2018). Furthermore, the composition of these primary metabolites is influenced by environmental conditions (Lee, 2018). Forage plants grown in hotter and drier regions tend to be of lower nutritive quality compared to those grown in cooler and wetter regions, often containing higher levels of fiber and lignin and lower levels of protein, which are associated with reduced digestibility. This variability underscores the importance of selecting plant species with optimal nutrient profiles to enhance livestock nutrition and productivity, particularly in resource-constrained environments.

2.3.2 Secondary metabolites

Unlike animals, plants cannot adapt to their environment through behavior; instead, they rely on the biosynthesis of a diverse array of compounds known as secondary metabolites to survive and thrive in diverse environments (Tissier et al., 2014). These metabolites play a crucial role in mediating interactions between plants and their environment, including defense against herbivores, pathogens, and environmental stressors, adaptation, and signaling. Secondary metabolites are often defined in contrast to primary metabolites, by definition, secondary metabolites encompass all the other metabolites synthesized by the organism other than primary metabolites. However, the term "secondary metabolites" can be misleading, as it implies that these compounds are of secondary importance or even waste products of primary metabolism (Bennett and Wallsgrove, 1994). To address this misconception, the term "specialized metabolites" has been proposed, reflecting their critical ecological and functional roles (Davies, 2013; Tissier et al., 2014).

Secondary metabolites exhibit remarkable chemical diversity, with over 50,000 different compounds identified to date, though the exact number remains unknown (Verpoorte et al., 2000; Pichersky and Lewinsohn, 2011; Tissier et al., 2014). Some estimates suggest that the total number of secondary metabolites could exceed 200,000, highlighting the vast biochemical potential of plants (Pichersky and Gang, 2000; Kampranis et al., 2007; Tissier et al., 2014;).

2.3.2.1 Biosynthesis of secondary metabolites in medicinal plants

Secondary metabolites play a crucial role in helping plants adapt to environmental stresses, both biotic (e.g., pathogenesis) and abiotic (e.g., temperature, drought, salinity, heavy metals, and herbicide exposure). These stresses often promote the accumulation of secondary metabolites, as plants upregulate their synthesis to develop adaptive strategies for survival under harsh conditions (Ashraf et al., 2018). The biosynthesis of secondary

metabolites is closely interlinked with primary metabolism, as primary metabolites serve as critical precursors for the production of secondary metabolites (Figure 1).

Terpenes, one of the largest classes of secondary metabolites, are synthesized through two major pathways: the mevalonic acid (MVA) pathway and the 2-C-methylerythritol 4-phosphate (MEP) pathway. The MEP pathway occurs in the plastids and utilizes products of glycolysis, such as pyruvate or acetyl-CoA, to synthesize isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These compounds act as universal precursors for all terpenoids, which are localized in various cellular compartments (Nagegowda, 2010).

Phenolic compounds, another major class of secondary metabolites, are produced via the shikimic acid and malonic acid pathways (Ghasemzadeh and Jaafar, 2011). In response to stress, key enzymes such as phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) regulate the synthesis of phenols (Sharma et al., 2019).

Nitrogen-containing secondary metabolites, such as alkaloids, incorporate nitrogen into their structures and are synthesized from amino acid precursors like lysine, tyrosine, and tryptophan.

The regulation of secondary metabolite production is a complex process that occurs at multiple levels, including transcriptional, post-transcriptional, translational, and post-translational regulation. This multi-layered regulatory mechanism ensures that plants can

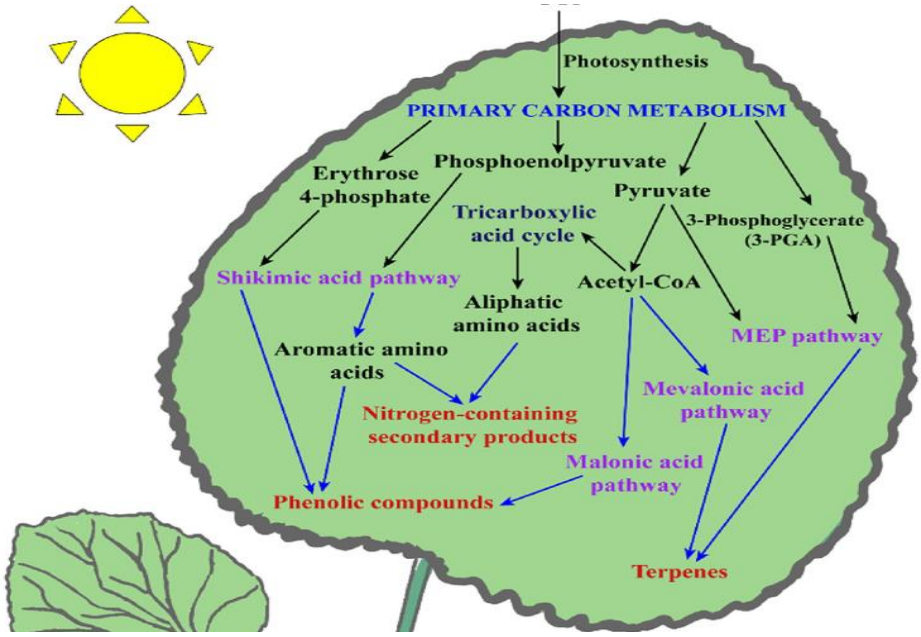

Figure 1: The major pathways of secondary metabolite biosynthesis (Zhao et al., 2023)

fine-tune the production of secondary metabolites in response to environmental cues and physiological needs.

2.3.2.2 Classification of secondary metabolites

Based on their based on their biosynthetic pathways and chemical structure, secondary metabolites are typically classified into three major groups (Figure 2): terpenoids, nitrogenous compounds (alkaloids) and phenolics (Reshi et al., 2023):

- **Terpenes**

Terpenes represent the largest and most diverse class of natural compounds, with over 50,000 known molecules identified to date (Cox-Georgian et al., 2019; Pasquini et al., 2021). Structurally, most terpenes follow the general formula (C5H8)n, and their thermal decomposition yields C5 isoprene units as a primary product (Mewalal et al., 2017; Hanuš and Hod, 2020). Terpenes are classified based on the number of isoprene units (n) or carbon atoms in their structure. For example, mono- and sesquiterpenes (C10 and C15, respectively) are highly volatile and often constitute the primary components of essential oils (EOs). In contrast, di- and triterpenes (C20 and C30, respectively) are more complex, less volatile, or even non-volatile, and are typically found in plant gums and resins at low concentrations (Bicas et al., 2009; Haberstroh et al., 2018).

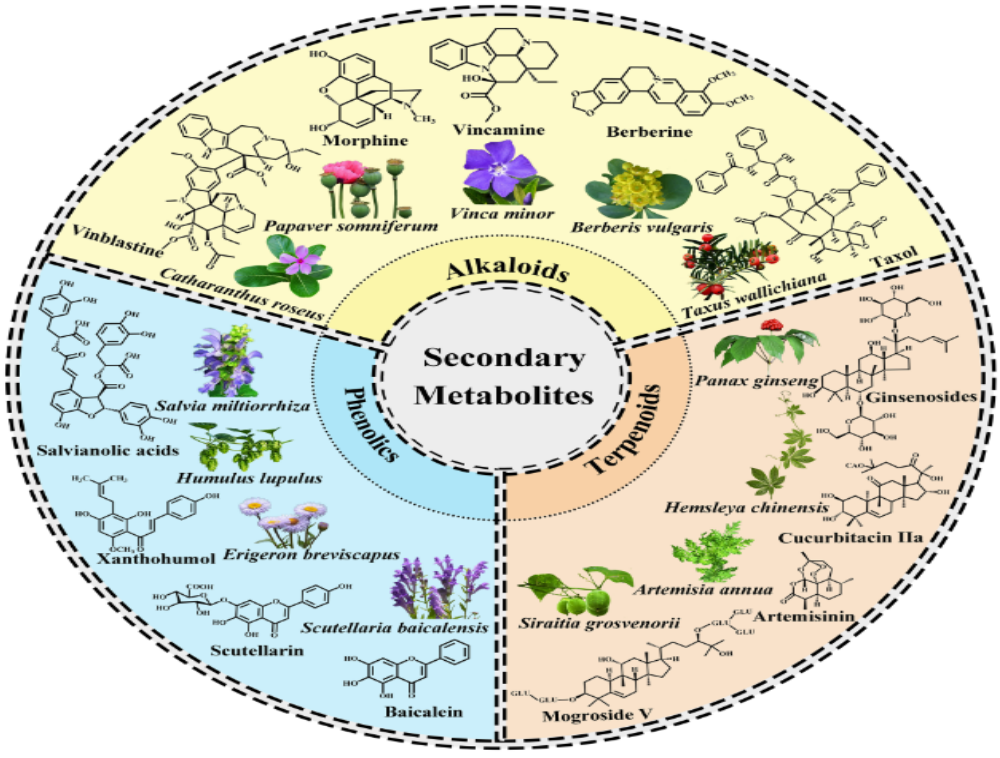

Figure 2: Chemical structure examples of secondary metabolites (Zhao et al., 2023)

Terpenes can also be categorized based on their structural complexity: acyclic (open structure), cyclic (one ring structure), and bi-, tri-, or tetra-cyclic (two, three, or four rings, respectively).

Although terpenes are technically defined as hydrocarbons constructed from five-carbon isoprene units, many authors use the term more broadly to encompass terpenoids. Unlike terpenes, terpenoids contain oxygen in various functional groups, including alcohols, aldehydes, ketones, acids, esters, and ethers, which significantly enhance their chemical and functional diversity (Mosquera et al., 2021; Pasquini et al., 2021).

Terpenes are widely distributed in various plant parts, including leaves, flowers, stems, buds, fruits, seeds, roots, and bark, contributing to the plant's aroma, color, and flavor (Cox-Georgian et al., 2019; Uwineza and Waśkiewicz, 2020). Functionally, terpenes play diverse roles in plants, ranging from structural functions, such as cholesterol's role in maintaining membrane integrity, to more specialized roles, such as carotenoid pigments in photosynthesis and gibberellins in regulating cell growth and defense (Mostofian et al., 2020). Additionally, terpenes exhibit allelopathic effects, playing a natural role in attracting pollinators, healing injured tissues, and repelling insects and parasites (Maffei, 2010; War et al., 2012).

- **Phenolic Compounds**

Phenolic compounds, also referred to as polyphenolics, are characterized by a phenol structure—an aromatic benzene ring with one or more hydroxyl substituents, along with functional derivatives such as methyl ethers, esters, and glycosides (Lattanzio et al., 2006). Their systematic name, hydroxybenzene, reflects this basic structure (Robbins, 2003; Vermerris and Nicholson, 2006). Over 8,000 phenolic compounds have been identified, and this number continues to grow as research advances (Gharras, 2009). Phenolic compounds are primarily classified based on their chemical structures into categories such as phenolic acids, flavonoids, tannins, lignans, and stilbenes (Babbar et al., 2014) (Figure 3). These compounds exhibit two basic structural frameworks: the C6–C3–C6 ring structure, which includes flavonoids, some phenolic acids, and condensed tannins, and the C6–C1 structure, which encompasses other phenolic acids and hydrolyzed tannins (Ringli et al., 2008).

Phenolic compounds play diverse roles in plants, ranging from structural functions to regulatory processes. They are involved in plant development, growth, cell division, seed germination, and pigmentation, as well as in defense mechanisms against biotic and abiotic

stresses (Lattanzio et al., 2012). While phenolics are often associated with plant defense responses, they also contribute to other ecological functions, such as attracting pollinators through pigmentation and scent, providing camouflage, and deterring herbivores through antibacterial and antifungal activities (Alasalvar et al., 2001; Edreva et al., 2007).

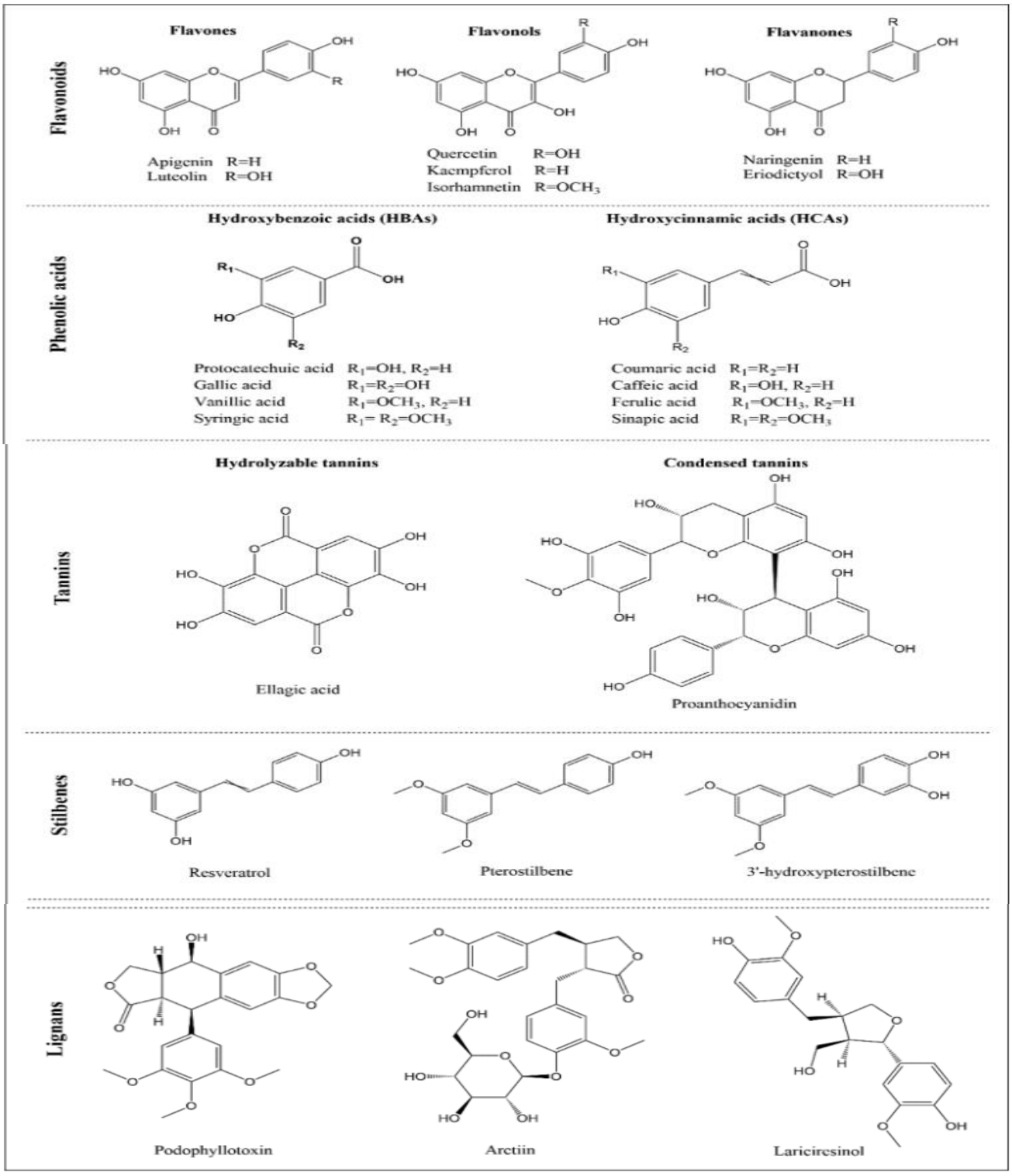

Figure 3: Phenolic compounds found from plants (Zhang et al., 2022)

The molecular structure of phenolic compounds, particularly the position and number of hydroxyl (OH) groups, confers strong bioactivity. Most phenolics possess multiple hydroxyl groups, making them excellent hydrogen donors and potent antioxidants. This structural feature has garnered significant attention in recent years, as plant polyphenols are recognized for their potential to prevent oxidative stress-related diseases, including cancer, and to combat premature aging. Additionally, phenolic compounds exhibit anti-inflammatory, antibiotic, and antiseptic properties.

Due to their antioxidant, antimicrobial, anticarcinogenic, and anti-inflammatory activities, phenolic compounds have become a focal point of research and utilization: Foods rich in phenolics are increasingly valued for their benefits, and these compounds are now widely studied for their potential applications as natural antioxidants and functional food ingredients.

- **Alkaloids**

Alkaloids are a diverse group of nitrogen-containing secondary metabolites, primarily characterized by their basic nitrogen atoms, though some related compounds exhibit neutral or weakly acidic properties (McNaught and Wilkinson, 1997). In addition to carbon, hydrogen, and nitrogen, alkaloids may also contain oxygen, sulfur, and, more rarely, elements such as chlorine, bromine, and phosphorus. The term "alkaloids" (German: *Alkaloide*) was introduced in 1819 by the German chemist Carl Friedrich Wilhelm Meißner, derived from the Latin *alkali*, which traces back to the Arabic *al-qalawī*. However, the term gained widespread use only after the publication of a review article by Oscar Jacobsen in the 1880s (Croteau et al., 2000).

Alkaloids are low-molecular-weight compounds that account for approximately 20% of plant-based secondary metabolites, with over 12,000 alkaloids isolated from diverse plant species to date (Kaur, 2015). These compounds are primarily found in higher plants, particularly in botanical families such as Apocynaceae, Annonaceae, Papaveraceae, and Rubiaceae, among others. Alkaloids are typically named by adding the suffix "-ine" to the species or genus name of the plant from which they are isolated, as exemplified by atropine from *Atropa belladonna*.

Ecologically, alkaloids serve as feeding deterrents and toxins to insects and herbivores, playing a critical role in plant defense mechanisms (Harborne, 1993). While they are generally toxic to mammals (Rattan, 2010), some specialized herbivores have evolved

mechanisms to tolerate or sequester alkaloids from their host plants. Functionally, alkaloids interact with molecular targets in the nervous system, acting as agonists or antagonists to neurotransmitter systems. They achieve this through direct binding to neuroreceptors, interference with neurotransmitter metabolism (e.g., cholinesterase inhibition), and modulation of signal transduction and ion channel function (Wink, 2000).

Alkaloids have been utilized for their medicinal properties for over 4,000 years, highlighting their significance in traditional and modern pharmacology (Amirkia and Heinrich, 2014). Their diverse biological activities, ranging from neuroactive effects to antimicrobial and anticancer properties, make them a focal point of research in natural product chemistry and drug discovery.

Despite their defensive roles, alkaloids have garnered attention for their pharmacological potential, including analgesic, anticancer, and antimicrobial properties, two potential exceptions are caffeine, which is ubiquitously consumed by humans, and nicotine, the psychoactive constituent of tobacco (Figure 4).

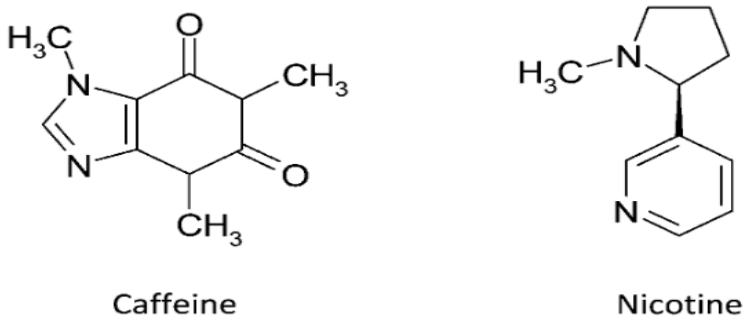

Figure 4: Structure of the alkaloids caffeine and nicotine (Kennedy and Wightman, 2011)

2.3.2.3 Effect of secondary metabolites on animal health and nutrition

The role of secondary metabolites extends beyond plant adaptation, as these compounds have significant potential to influence various physiological processes and functions in animals. Historically, research on plant secondary metabolites focused primarily on their adverse effects when ingested by animals, such as toxicity or antinutritional properties (Colegate et al., 1994; D'Mello, 1997; Cheeke, 1998). However, in recent years, the focus has shifted toward understanding their beneficial effects, particularly in the context of animal health and nutrition. Secondary metabolites have been extensively studied for their applications in ecology, human foods, animal feeds, and pharmaceuticals, owing to their diverse chemical and biochemical properties (Wiseman, 2003; Bento, 2004; Nash, 2004)

The effect of these compounds in animal feeding depends on several factors, including the chemical structure, concentration, and number of each secondary metabolites present in the plant, as well as the animal species, mode of application, and interactions with rumen microbes.

The beneficial effects of secondary metabolites on animal health and nutrition include:

- **Enhanced Digestion and Nutrient Utilization**: Secondary metabolites improve organic matter digestibility (Ma et al., 2017) and prevent digestive disorders (Tedeschi et al., 2021).
- **Antioxidant Properties**: Combat oxidative stress by scavenging free radicals and reducing cellular damage, that protect livestock from oxidative stress caused by environmental stressors.
- **Antimicrobial and Anti-Inflammatory Effects**: Secondary metabolites reduce the incidence of gastrointestinal parasites and infections by lowering parasite loads in grazing animals.
- **Immune-Boosting Mechanisms**: These compounds enhance immune function by modulating immune responses and promoting antibody production.
- **Improved Production Outcomes**: By enhancing nutrient utilization, reducing disease incidence, and supporting overall health, secondary metabolites contribute to better growth rates, lactation performance, and reproductive success in livestock.
- **Enhanced Animal Product Quality**: Secondary metabolites improve the nutritional profile and shelf life of animal products, such as milk and meat, like enhancing the sensory properties and oxidative stability of meat and dairy products.

3 Oxidative stress and antioxidants in livestock system

3.1 Oxidative stress, causes and effects

Oxidative stress is a physiological condition that occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them (Figure 5) through antioxidant defenses (Jain and Shakkarpude, 2024). ROS are oxygen-containing molecules with an odd number of electrons, such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•), which are generated as inevitable byproducts of aerobic metabolism (Hong et al., 2024). The endogenous

production of these reactive intermediates can be substantially amplified by various extrinsic factors:

Environmental stressors: Thermal stress, ultraviolet radiation exposure, and atmospheric pollutants induce excessive ROS formation in animal tissues through multiple mechanistic pathways (Ray et al., 2012). Hyperthermia, in particular, accelerates mitochondrial respiratory chain activity, leading to electron leakage and subsequent superoxide radical generation.

Nutritional deficiencies: Suboptimal dietary intake of micronutrients essential for antioxidant enzyme function—including selenium, zinc, copper, and vitamins—compromises endogenous antioxidant defense systems, thereby exacerbating oxidative damage (Makkar et al., 2007; Fagbohun et al., 2023). These deficiencies impair the biosynthesis and activity of critical antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

Inflammatory and infectious processes: Pathogen invasion and chronic inflammatory conditions induce respiratory burst in phagocytic cells, dramatically increasing ROS production through NADPH oxidase activation, overwhelming antioxidant capacity (Ivanov et al., 2017).

The deleterious effects of oxidative stress on animal physiological functions are multifaceted and profound. At the subcellular level, ROS-mediated oxidative damage manifests as lipid peroxidation of polyunsaturated fatty acids in cell membranes, protein carbonylation leading to structural and functional alterations, and DNA strand breaks with potential mutagenic consequences (Trevisan et al., 2001). These molecular perturbations

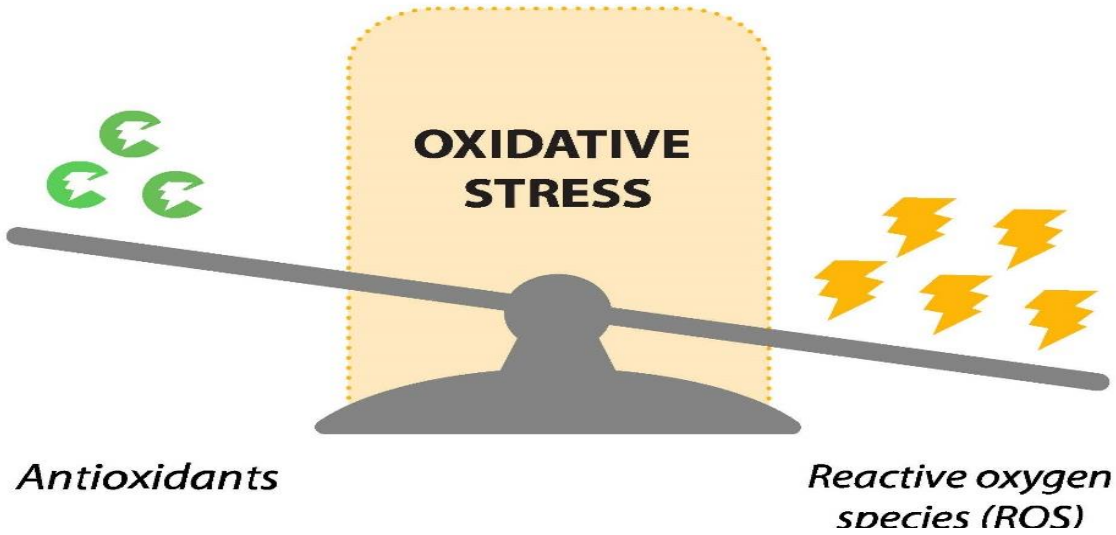

Figure 5: Schematic description of oxidative stress

lead to cell dysfunction and apoptosis (Lykkesfeldt and Svendsen, 2007). In livestock production systems, these biochemical alterations translate into several economically significant outcomes:

Growth performance and productivity impairment: Oxidative stress compromises intestinal epithelial integrity and nutrient transporter function, diminishing feed conversion efficiency and impairing anabolic processes essential for tissue accretion and lactation (Catoni et al., 2008). This results in quantifiable reductions in average daily gain and milk yield parameters.

Immunocompetence deterioration: ROS-mediated damage to immune cell membranes and signaling pathways attenuates both innate and adaptive immune responses, predisposing animals to opportunistic infections and reducing vaccination efficacy (Lykkesfeldt and Svendsen, 2007).

Reproductive inefficiency: Oxidative stress disrupts hypothalamic-pituitary-gonadal axis function, impairs gametogenesis, compromises corpus luteum function, and increases embryonic mortality, collectively manifesting as reduced conception rates and elevated embryonic loss (Nardone et al., 2010).

3.2 Antioxidant classification and mechanism of actions

Antioxidants constitute a diverse array of molecular entities that counteract oxidative processes through electron donation, thereby neutralizing reactive ROS and mitigating cellular oxidative damage. These compounds can be taxonomically categorized into two principal classifications based on their origin and synthesis pathways:

Synthetic antioxidants: These comprise laboratory-synthesized compounds engineered for specific antioxidative properties, such as synthetic vitamin E (Pitino et al., 2021). Despite their documented efficacy in ROS scavenging, regulatory authorities have progressively restricted their utilization in animal nutrition due to emerging evidence of potential hepatotoxicity, carcinogenicity, and bioaccumulation in adipose tissues after prolonged administration.

Natural Antioxidants: These encompass endogenously synthesized compounds and exogenously derived phytochemicals obtained from botanical sources. This category exhibits remarkable structural and functional diversity, including:

Enzymatic antioxidants: Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which catalyze the conversion of ROS to less reactive intermediates through redox reactions.

Non-enzymatic antioxidants: Encompassing carotenoids (β-carotene, lycopene, lutein), polyphenolic compounds (flavonoids, phenolic acids, anthocyanins), organosulfur compounds (allicin, diallyl disulfide), and essential micronutrients (vitamins E and C, selenium, zinc) (Abourashed, 2013). These compounds exhibit superior biocompatibility, biodegradability, and multifaceted antioxidative mechanisms compared to their synthetic counterparts, establishing them as physiologically advantageous alternatives in animal nutrition paradigms (Corino and Rossi, 2021).

3.3 Physiological implications in animal production systems

Antioxidants play a critical role in maintaining animal health and productivity by:

ROS neutralization and cellular protection: Antioxidants function as electron donors, effectively terminating free radical chain reactions through various mechanisms, including hydrogen atom transfer (HAT) and single electron transfer (SET), thereby preserving structural and functional integrity of cellular macromolecules (Ray et al., 2012).

Immunomodulatory effects: Antioxidant supplementation enhances immune responses through stabilization of immune cell membranes, optimization of cytokine production profiles, and potentiation of lymphocyte proliferation and differentiation (Hosseindoust et al., 2020). These immunomodulatory effects translate into enhanced pathogen clearance efficiency and reduced inflammatory tissue damage during immunological challenges.

Reproductive efficiency enhancement: Antioxidants exhibit critical regulatory functions in reproductive physiology, including protection of gamete DNA integrity, optimization of folliculogenesis and spermatogenesis, and prevention of oxidative stress-induced embryonic apoptosis (Jain and Shakkarpude, 2024). These mechanisms collectively contribute to improved conception rates, reduced embryonic mortality, and enhanced reproductive longevity in breeding stock.

Qualitative improvement of animal-derived products: Dietary antioxidant supplementation induces significant enhancements in the nutritional and sensory profiles of animal products through mechanisms including prevention of lipid peroxidation in muscle tissues, stabilization of myoglobin oxidation status in meat products, and enrichment of milk

with bioactive compounds (Salami et al., 2016). These qualitative improvements manifest as extended shelf life, superior organoleptic properties, and enhanced nutritional value of derived products, thereby augmenting their commercial viability and consumer acceptability.

In regions prone to elevated temperatures and pronounced climatic variability, such as semi-arid ecocystems, the role of antioxidants becomes even more critical. Environmentally induced periods of heat stress trigger oxidative stress, impairing livestock productivity and health (Nardone et al., 2010). Incorporating natural antioxidants like selenium and vitamin E into livestock diets has proven effective in mitigating oxidative damage, supporting immune function, and enhancing overall animal resilience to environmental stressors (Chauhan et al., 2014).

4 Extraction of bioactive compounds from medicinal plants

4.1 Definition of extraction

Extraction constitutes a critical preliminary operation in the systematic isolation of bioactive constituents from medicinal plant matrices, serving as the fundamental prerequisite for subsequent analytical characterization, pharmacological evaluation, and therapeutic application. The primary objective of extraction protocols is the selective isolation of target phytochemical entities from complex biological matrices while maintaining their structural integrity and bioactive potential (Sasidharan et al., 2011). Among the diverse methodological approaches available, solvent-based extraction techniques remain predominant in phytochemical investigations due to their methodological simplicity, operational flexibility, and adaptability across diverse plant matrices. The selection of appropriate extraction methodology significantly influences both qualitative phytochemical profiling and quantitative yield determination, thereby representing a critical determinant in the validity and reliability of subsequent analytical outcomes (Sasidharan et al., 2011).

The phytochemical extraction process encompasses four sequential physicochemical phenomena that operate synergistically:

Solvent penetration: Infiltration of the extraction solvent through the complex three-dimensional architecture of the plant cell wall and cellular compartments.

Solute solubilization: Dissolution of target phytoconstituents into the extraction medium based on solubility parameters and partition coefficients.

Intra- and extracellular diffusion: Migration of solubilized phytochemicals through semi-permeable cellular membranes via concentration gradient-mediated passive diffusion.

Solute recovery: Collection and concentration of the solubilized phytoconstituents for subsequent purification and analytical characterization.

4.2 Factors influencing extraction efficiency

Several factors influence the efficiency of the extraction process, primarily by enhancing the diffusivity and solubility of the target compounds during the various extraction phases:

Solvent selection criteria: Solvent determination represents the most critical parameter influencing extraction outcomes. Selection criteria must integrate considerations of solvent selectivity, solubilization capacity, toxicological profile, environmental impact, and economic feasibility. In accordance with the fundamental physicochemical principle of "like dissolves like," solvents exhibiting polarity indices approximating those of target analytes demonstrate superior extraction efficiency. Ethanol (EtOH) and methanol (MeOH) have been extensively employed as universal extraction media in phytochemical investigations due to their intermediate polarity profiles (respective dielectric constants: 24.5 and 32.6) and capacity to solubilize diverse phytochemical classes ranging from moderately polar flavonoids to highly polar glycosides.

Plant material particle size distribution: Reduction in particle size enhances solvent penetration and solute diffusion, thereby improving extraction efficiency. However, excessively fine particles can complicate filtration and lead to the excessive retention of solute in the solid matrix. An optimal particle size must balance efficiency and practicality.

Extraction temperature: Higher temperatures typically increase solubilization and diffusion rates, thus enhancing extraction yield. However, overly high temperatures may cause solvent evaporation, co-extraction of undesirable impurities, or degradation of thermolabile compounds, particularly those containing unsaturated bonds or reactive functional groups.

Extraction duration: Prolonged extraction times can increase yield up to the point of equilibrium, where the solute concentration in the solvent matches that in the plant material.

Beyond this equilibrium, extended extraction provides no additional benefit and may lead to undesirable changes in the extract (oxidative degradation of sensitive phytoconstituents).

Solvent-to-solid ratio: Elevated solvent-to-solid ratio generally improves extraction efficiency by ensuring adequate solvent availability for solute dissolution through maintenance of favorable concentration gradients for continued diffusion. However, excessive solvent use can lead to higher costs, longer concentration times, and diminished practicality.

4.3 Conventional extraction methods

Conventional extraction methods employ organic solvents, heat, and mechanical agitation to facilitate the liberation of phytoconstituents from plant matrices. While demonstrating established efficacy, these methodologies typically require substantial solvent volumes and extended extraction durations. The most commonly employed conventional techniques include Soxhlet extraction, maceration, and hydrodistillation, each exhibiting distinct operational parameters, advantages, and limitations.

4.3.1 Soxhlet extraction

Introduced by Franz Ritter Von Soxhlet in 1879, this semi-continuous extraction methodology was initially developed for lipid extraction but has since been widely used for isolating valuable bioactive compounds from various natural sources (Soxhlet, 1879). The technique serves as a reference standard for comparative evaluation of emerging extraction methodologies due to its operational simplicity and reproducible efficiency parameters. In this process, dried plant material is placed in a thimble, which is inserted into a distillation flask containing the solvent of interest. As the solvent is heated, it evaporates and condenses into the thimble-holder. Once the solution in the thimble reaches an overflow level, it is aspirated by a siphon, carrying the extracted solutes into the bulk liquid. The process is repeated until the extraction is complete, with the solvent continuously recycled through the plant material. While Soxhlet extraction efficiently utilizes solvents and automated operation, it is not suitable for thermolabile compounds due to prolonged heating, which can degrade sensitive bioactive compounds (Nikhal and Dambe, 2010).

4.3.2 Maceration

Maceration is one of the oldest and most cost-effective methods for phytoconstituent extraction, traditionally employed in ethnopharmacological preparations. It is particularly suitable for small-scale extractions and involves several steps. First, plant materials are

ground into smaller particles to increase their surface area, facilitating solvent penetration. The powdered material is then placed in a closed vessel, and an appropriate solvent (menstruum) is added. After the extraction period, the liquid extract is strained, and the solid residue, known as the marc, is pressed to recover the remaining solution. The strained liquid is then filtered to remove impurities. Shaking or continuous stirring during maceration enhances the extraction process by promoting diffusion and allowing fresh solvent to interact with the plant material. This method is particularly advantageous for thermolabile compounds, as it does not involve prolonged heat exposure, but it may have lower efficiency compared to other techniques (Ncube et al., 2008).

4.3.3 Hydrodistillation

Hydrodistillation is a traditional method widely used for extracting essential oils and related bioactive compounds from aromatic plant materials. Unlike other conventional methods, hydrodistillation does not involve organic solvents and can be performed on fresh plant materials before dehydration. The method involves placing plant materials in a still compartment, where water is added or direct steam is injected to heat the plant material. Hot water and steam release bioactive compounds, which are carried with the vapor to a condenser. The condensed mixture of water and oil flows to a separator, where essential oils and other bioactives are separated from the water (Silva et al., 2005). The process involves three main physicochemical mechanisms: hydrodiffusion, hydrolysis, and thermal decomposition. While hydrodistillation is environmentally friendly, high extraction temperatures can lead to the loss of volatile or thermolabile components, limiting its application for certain bioactive compounds classes (Vankar, 2004).

4.4 Non-conventional extraction techniques

Advanced non-conventional extraction methodologies have emerged as innovative technological solutions to address the inherent limitations of traditional techniques, including extended processing durations, substantial solvent requirements, and potential thermal degradation of sensitive phytoconstituents (Luque de Castro and García-Ayuso, 1998). These advanced techniques offer significant advantages, including reducing extraction times, enhanced selectivity, and minimized environmental impact. Aligned with the principles of green chemistry established by the Environmental Protection Agency (EPA), USA (http://www.epa.gov/greenchemistry/pubs/about_gc.html), these methods emphasize less hazardous chemical synthesis, energy efficiency, and the use of renewable feedstocks, among other sustainable practices. Through adherence to these principles, non-

conventional extraction techniques not only improve the efficiency of bioactive compound isolation but also contribute to broader environmental conservation efforts, paving the way for future advancements in phytochemical research, among the most promising non-conventional extraction techniques are:

4.4.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is a modern, efficient technique that utilizes ultrasonic waves (20 kHz to 100 MHz) to enhance the extraction of bioactive compounds from plant materials. The process relies on the phenomenon of cavitation, where the formation, growth, and collapse of bubbles generate localized high temperatures (~5000 K) and pressures (~1000 atm), facilitating the release of compounds from the plant matrix (Suslick and Doktycz, 1990). UAE works by intensifying mass transfer and accelerating solvent access to plant cells, leading to two primary mechanisms: (1) diffusion across cell walls and (2) rinsing of cell contents after wall disruption (Mason et al., 1996). Key factors influencing UAE efficiency include solvent type, particle size, moisture content, temperature, pressure, and sonication time. This method offers significant advantages, such as reduced extraction time, lower energy consumption, minimal solvent use, and improved extraction yields compared to conventional techniques (Otles, 2008). UAE has been successfully applied to extract various bioactive compounds, including phenolic acids, flavonoids, and alkaloids, from medicinal plants, demonstrating its versatility and effectiveness as a green extraction method (Zu et al., 2012).

4.4.2 Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction (EAE) employs specific enzymes such as cellulase, α-amylase, and pectinase to break cell walls and hydrolyze structural polysaccharides, enhancing the release of bioactive compounds bound within plant matrices (Singh et al., 1999). Key factors influencing EAE include enzyme type, concentration, plant particle size, and hydrolysis conditions (Niranjan and Hanmoungjai, 2004). This eco-friendly method uses water as a solvent, making it a sustainable alternative to organic chemicals (Puri et al., 2012). EAE has been applied successfully to extract oils, phenolic antioxidants, and flavonoids. For example, celluclast, pectinex, and novoferm enzymes improved phenolic recovery from grape pomace and citrus peels, with higher yields achieved by optimizing enzyme mixtures (Gómez-García et al., 2012). Compared to conventional methods, EAE-derived oils contain higher free fatty acids and phosphorus contents (Domínguez et al., 1995).

4.4.3 Pulsed-Electric Field Extraction (PEF)

Pulsed-electric field (PEF) extraction enhances the recovery of bioactive compounds by disrupting cell membranes through short electric pulses, increasing permeability and mass transfer efficiency (Bryant and Wolfe, 1987). The process employs electric fields (typically 500–1000 V/cm) to create pores in plant cell membranes, allowing intracellular compounds to be released with minimal temperature increase, preserving heat-sensitive components (Fincan and Dejmek, 2002). PEF can operate in continuous or batch modes depending on the treatment chamber design and is influenced by parameters such as field strength, pulse number, and treatment temperature (Heinz et al., 2003). Studies demonstrate its application as a pretreatment to conventional extraction, reducing effort and time. For instance, PEF pretreatment improved the recovery of betanin from beetroots (Fincan et al., 2004), phytosterols from maize (Guderjan et al., 2005), and anthocyanins from grape skins (Corrales et al., 2008). Additionally, PEF application on grape skins enhanced polyphenol and anthocyanin extraction while reducing maceration time during vinification (Delsart et al., 2012). This innovative, energy-efficient method minimizes degradation of sensitive compounds and improves extraction efficiency, making it an effective tool for modern plant-based bioactive extraction.

4.4.4 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) uses electromagnetic waves (300 MHz–300 GHz) to heat polar materials via ionic conduction and dipole rotation, enabling efficient extraction of bioactive compounds (Vyas et al., 2009). The process involves solute separation from the matrix under increased temperature and pressure, diffusion of the solvent into the matrix, and release of solutes into the solvent (Ani et al., 2012). MAE offers advantages such as faster heating, reduced thermal gradients, smaller equipment size, and higher extract yields compared to conventional techniques (Cravotto et al., 2008). It is recognized as a green technology due to its reduced use of organic solvents. Studies have demonstrated its effectiveness in extracting various compounds, such as polyphenols and caffeine from green tea (Pan et al., 2003). This rapid and selective method provides superior extraction efficiency, making it an ideal alternative to traditional solvent-based methods.

4.4.5 Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE) or enhanced solvent extraction (ESE), uses high pressure to keep solvents in a liquid state above their boiling points, improving extraction efficiency (Nieto et al., 2010). This

method enhances analyte solubility, reduces solvent viscosity and surface tension, and accelerates mass transfer, leading to faster extraction with lower solvent consumption (Ibañez et al., 2012). PLE is widely recognized as a green technology and an alternative to traditional methods like Soxhlet extraction, offering reduced time and solvent use. It is also a viable substitute for supercritical fluid extraction, particularly for polar compounds (Kaufmann and Christen, 2002) and has demonstrated superior performance compared MAE and UAE.

4.4.6 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) uses fluids in their supercritical state—where gas and liquid phases merge—to extract bioactive compounds efficiently. Carbon dioxide (CO_2) is commonly used due to its low critical temperature (31°C) and pressure (74 bar), which allow operation under mild conditions, preserving heat-sensitive compounds (Temelli and Güçlü-Üstündağ, 2005). SFE benefits from CO_2's tunable solvation power, enhanced by modifiers like methanol or ethanol to improve polarity and extraction efficiency for diverse compounds (Lang and Wai, 2001). Key parameters influencing SFE include temperature, pressure, particle size, moisture content, and flow rate, which can be adjusted to optimize extraction outcomes (Reverchon and De Marco, 2006). SFE offers several advantages: faster extraction, high selectivity, minimal organic solvent use, efficient solute-solvent separation via depressurization, and scalability from lab to industrial levels. Additionally, SFE's recyclability and on-line coupling with chromatography enhance its environmental and analytical appeal (Lang and Wai, 2001).

5 Ethnobotany in livestock systems

5.1 Historical background and definition of ethnobotany

Ethnobotany, etymologically derived from the Greek roots *ethnos* (culture) and *botanikos* (of plants), was formally introduced into scientific nomenclature by North American botanist John William Harshberger in 1895 (Suryanullah and Mundofi, 2024), explores the dynamic relationships between people and plants, focusing on the traditional knowledge surrounding the use of plants for food, medicine, and other purposes (Pardo-de-Santayana et al., 2010). The practice of documenting the use of plants predates the term itself, with early records such as Pedanius Dioscorides' *De Materia Medica* providing a foundation for modern ethnobotanical research.

Initially defined as the "science of people's interaction with plants" (Turner, 1998), the study of indigenous plant use, ethnobotany has evolved into a multidisciplinary field incorporating insights from anthropology, botany, ecology, pharmacology, and economics (Martin, 1995). It now encompasses not only the documentation of phytotaxonomic identification and utilization patterns but also the sociocultural and ecological contexts in which these practices occur, making it invaluable for conserving biological and cultural diversity (Muthu et al., 2006).

5.2 Importance of ethnobotanical studies in livestock systems

Ethnobotanical research methodologies demonstrate particular significance within livestock production contexts, functioning as essential heuristic tools for the identification and characterization of autochthonous plant species with dual functionality in animal nutrition and therapeutic applications. This assumes critical importance in agroecological zones characterized by pronounced forage resource constraints and environmental stressors, including semi-arid regions experiencing precipitation volatility and soil fertility limitations. The systematic documentation of indigenous knowledge systems pertaining to multifunctional phytogenetic resources—particularly those exhibiting both nutritional and therapeutic bioactivity—facilitates the establishment of epistemic bridges between traditional ecological knowledge and contemporary scientific research paradigms. This integration enables the development of sustainable management protocols for local biological resources through scientifically validated approaches informed by generations of empirical observation (Dean, 2024). The strategic integration of ethnobotanical insights into livestock systems has numerous benefits:

Identification and of nutri-medicinal plant resources: The systematic documentation of traditional phytotherapeutic applications in animal husbandry establishes foundational data for subsequent scientific investigation of bioactive compounds, nutritional profiles, and therapeutic efficacy through rigorous pharmacological screening protocols.

Enhancement of agroecological sustainability parameters: The prioritization of environmentally adapted autochthonous plant species in livestock feeding systems promotes ecological resilience through reduced dependency on exogenous inputs.

Preservation of indigenous knowledge: Documenting traditional practices ensures the conservation of intangible cultural heritage while providing new perspectives for scientific research and development within sustainable livestock production paradigms.

5.3 Methods for ethnobotanical research in livestock systems

Ethnobotanical research involves qualitative and quantitative methodologies to effectively document traditional knowledge and validate its applications in modern contexts:

5.3.1 Field-based studies and interviews

The primary methodological approach comprises systematic engagement with knowledge custodians including pastoral communities, smallholder livestock producers, and traditional veterinary practitioners to collect comprehensive data regarding phytotherapeutic applications in animal husbandry:

Implementation of semi-structured interview protocols: The development and administration of semi-structured interview instruments enables the systematic collection of ethnobotanical data while simultaneously accommodating the emergent nature of traditional knowledge systems. These protocols typically incorporate quantifiable parameters including plant species identification, preparation methodologies, administration routes, dosage determination mechanisms, and observed therapeutic outcomes.

Application of participant observation techniques: Extended immersion within the studied communities facilitates observational documentation of plant collection procedures, preparation methodologies, and administration techniques, providing critical contextual data that may not emerge through verbal communication alone.

Employment of free listing exercises: This cognitive anthropological technique enables the identification of culturally significant plant species and their relative importance within local ethnopharmacological systems through analysis of frequency, order, and salience indices.

5.3.2 Botanical identification and specimen collection

The accurate taxonomic identification of ethnobotanically significant plant species represents a critical methodological component:

Cross-referencing vernacular nomenclature with taxonomic classifications: Development of comprehensive lexicons correlating local phytonyms with standardized botanical nomenclature according to the International Code of Botanical Nomenclature (ICBN).

Implementation of standardized herbarium collection protocols: Collection of botanical voucher specimens following standardized herbarium preparation methodologies for taxonomic verification, permanent repository establishment, and future reference.

5.3.3 Ethnographic Mapping and Ecological Documentation

The spatial and temporal dimensions of ethnobotanical knowledge require specialized documentation approaches:

Implementation of participatory resource mapping: Collaborative development of spatially explicit representations of important forage and medicinal plant distributions, incorporating indigenous classification systems and management practices.

Documentation of phenological patterns: Systematic recording of seasonal availability patterns, reproductive cycles, and temporal variations in bioactive compound concentrations to identify optimal harvesting periods and sustainable collection practices.

Assessment of conservation status: Evaluation of population dynamics, regeneration patterns, and anthropogenic pressure factors affecting the sustainability of utilized plant resources through standardized ecological survey methodologies.

5.3.4 Ethical considerations

Ethnobotanical research must be conducted with ethical sensitivity, particularly when working with indigenous communities. The International Society of Ethnobiology (ISE, 2022) outlines key principles that ensure the ethical collection, documentation, and application of traditional knowledge. One of the fundamental requirements is prior informed consent, which mandates that researchers obtain explicit approval from local communities before conducting interviews, collecting plant specimens, or publishing findings. This ensures transparency and respect for the rights of knowledge holders.

Equitable benefit sharing is another critical ethical consideration. Communities that contribute their knowledge should receive fair recognition and tangible benefits, whether in the form of co-authorship in scientific publications, financial support for local initiatives, or access to the research findings. Additionally, researchers must respect cultural practices by ensuring that traditional plant use is neither exploited nor misrepresented. The ethical implications of ethnobotanical research extend to concerns about biopiracy, where commercially valuable knowledge is extracted without due compensation to the originating communities.

CHAPTER II: MATERIAL AND METHODS

1 Ethnobotanical study

1.1 Principle

Ethnobotany bridges social and biological sciences, focusing on the relationship between people and plants. Documenting indigenous knowledge through ethnobotanical studies is crucial for the conservation and sustainable utilization of biological resources (Parimelazhagan, 2016).

1.2 Study area characterization

1.2.1 Geographic location

The present investigation was conducted in Bordj Bou Arreridj province (wilaya), situated in the northeastern region of Algeria, geographically delineated by coordinates 36.0698° N, 4.7661° E (Figure 6). The study area encompasses a total surface area of 3,920.42 km² and is administratively bounded by four adjacent provinces: Bejaia to the north, Sétif to the east, M'Sila to the south, and Bouira to the west. These administrative separations coincide with significant biogeographical transitions, particularly along the northern boundary with Bejaia where Mediterranean influences intensify, and the southern boundary with M'Sila where pre-Saharan characteristics become increasingly pronounced.

The region exhibits pronounced topographic heterogeneity, with altitudinal gradients ranging from 302 m to 1,885 m above sea level. That gave characterization to three distinct geomorphological zones: the mountainous Bibans range in the north, a high plateau in the

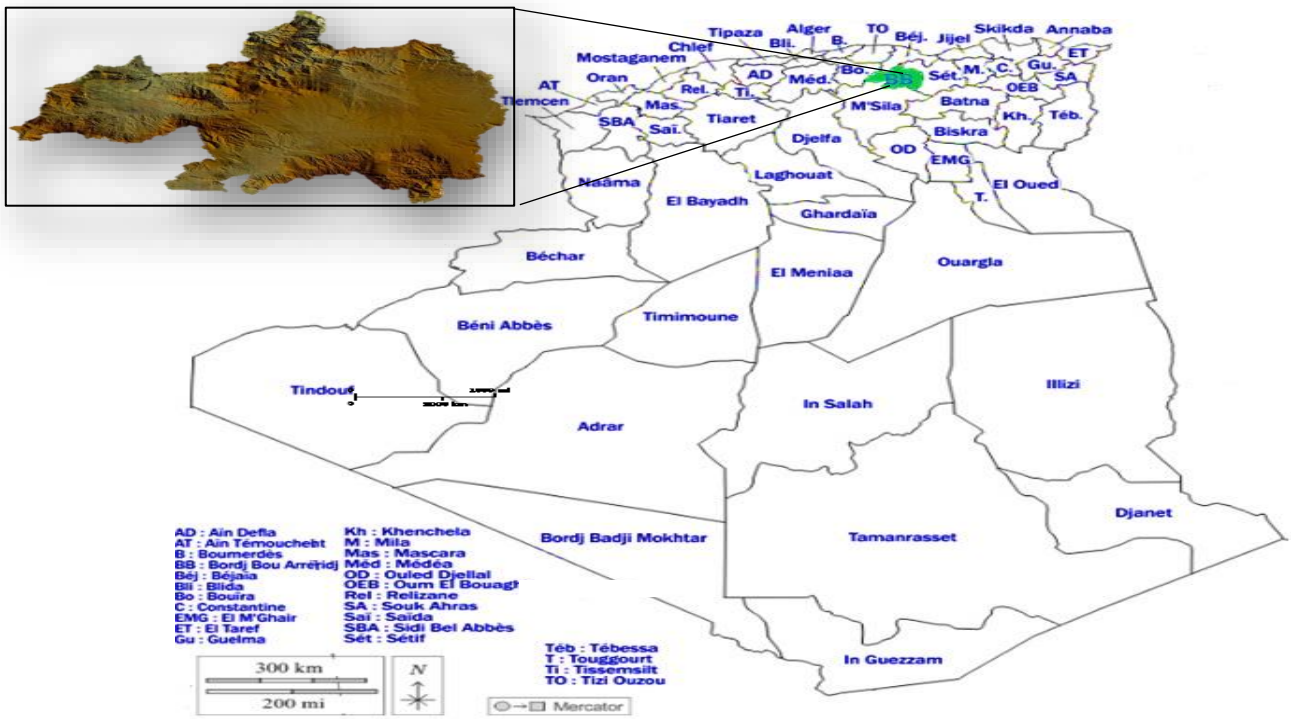

Figure 6: Location map of the study area, Bordj Bou Arreridj

central area that constitutes the major part of the wilaya, and a southwestern section that transitions into a steppe zone.

1.2.2 Climatic parameters

According to Emberger's bioclimatic quotient (Q_2) classification system, Bordj Bou Arreridj is situated predominantly within the semi-arid bioclimatic zone (Figure 7), with cool winter variant (Q_2 values ranging from 32.7 to 45.9). Application of the UNESCO-FAO aridity index (P/PET ratio of 0.28-0.35).

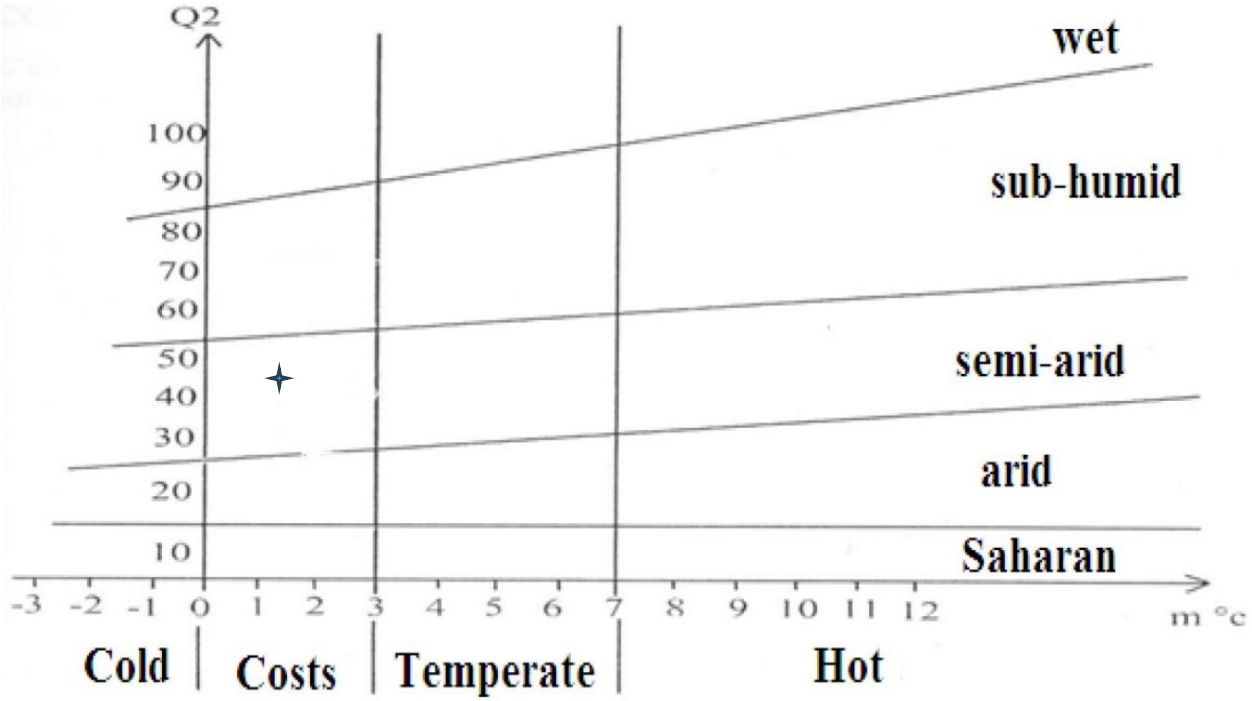

Figure 7: Emberger's bioclimatic quotient (Q_2) classification system of Bordj Bou Arreridj

(BBA), Algeria. The selection of study sites was carried out in February 2022 through a collaborative approach involving key regional authorities. This method ensured a comprehensive and representative inclusion of communes central to livestock farming and rangeland management.

1.3.1 Institutional Support and Field Site Selection

Directorate of Agricultural Services (DSA): The DSA provided detailed data on regions with significant livestock farming activities, highlighting communes with the highest livestock populations. Their assistance included introductions to prominent farms and experienced farmers, ensuring our study focused on areas critical to livestock production.

Directorate-General for Forestry (DGF): The DGF facilitated ecological assessments of potential study sites through their local offices. Their support was instrumental in

understanding the environmental conditions and obtaining official permissions for plant sampling. Additionally, they fostered collaboration with local communities, enhancing the practicality of fieldwork.

High Commission for Steppe Development (HCDS): The HCDS contributed expertise on the steppe ecosystem, providing valuable insights into rangeland conditions and pastoral practices. Their input ensured the selection of communes accurately represented the challenges and dynamics typical of semi-arid regions.

Through this collaborative effort, 13 communes across the Wilaya of BBA were identified as data collection sites: Bir Kasdali, Bordj Zemoura, Djaâfra, El Main, El Mehir, Khelil, Mansoura, Medjana, Ras El Oued, Sidi Embarek, Tassamert, Tixter, and Zemmoura. These communes encompass diverse ecological zones and livestock farming practices, including high plateaus, steppe regions, and mountainous terrains. This diversity provides a robust foundation for ethnobotanical research and ensures that the study addresses a wide range of forage-related challenges typical of semi-arid regions.

Participants were selected using a snowball sampling technique (Naderifar et al., 2017), ensuring the inclusion of individuals with extensive knowledge and experience in livestock management and medicinal plants. The targeted informants included male and female shepherds, farmers, and forest rangers who volunteered to participate in the study. The age of the 84 participants ranged from 25 to 85 years, offering diverse perspectives and intergenerational insights into traditional knowledge and forage management in the region.

1.4 Data collection

Data collection was conducted between February and July 2022 in the 13 selected study sites in Bordj Bou Arréridj (BBA), Algeria, using a combination of ethnobotanical research methods to gather comprehensive information on plants with dual-purpose applications. Semi-structured interviews and questionnaires were the primary tools, following methodologies outlined by Hedberg (1993), Martin (1995), and Cotton (1996). These instruments were designed to obtain detailed insights into the local names, life forms, traditional medicinal and non-medicinal uses, availability seasons, edible parts, palatability for livestock, foraging methods, and animal consumers of the plants. Guided field walks and participatory observations further enriched the data collection process, as informants accompanied researchers to identify, document and take pictures of plants in their natural habitats, ensuring accurate species identification and contextual understanding. All

interactions were conducted in the Algerian Arabic dialect (Darija) to ensure effective communication and cultural relevance. The interviews adhered to the ethical guidelines outlined by the International Society of Ethnobiology (ISE), ensuring that participants felt no pressure, enabling them to respond candidly (ISE, 2022).

Data collection continued until saturation was reached, in accordance with Mason (2010), at which point no new information was being obtained from additional informants.

Ethical considerations were upheld throughout the process, with informed consent obtained from all participants and strict confidentiality maintained. This comprehensive approach provided a robust foundation for analyzing the ethnobotanical knowledge of the region.

1.5 Preparation of herbarium specimens

During the guided fieldwork, plant samples were collected, identified using using the Pl@ntNet plant identification application (https://identify.plantnet.org/), and preserved as voucher specimens at the research laboratory of medicinal and aromatic plants of the University of Blida 1, Algeria. Field notes were meticulously documented, and specimen tags were written in pencil to ensure durability. A unique collection number was assigned to each sample and used as its tag for future reference.

To capture morphological characteristics that may not be visible in dried specimens, photographs were taken during the collection process, focusing on key features such as leaves, flowers, and stems. The collected specimens were pressed in the field using newspaper sheets, ensuring that only one plant was pressed per sheet to avoid confusion or data overlap. Care was taken to prevent unnecessary overlapping of leaves or plant parts, with some leaves turned over to display both upper and lower surfaces for accurate identification.

Once pressed, the specimens were secured in plant presses, and straps were tightened to apply even pressure. The pressed specimens were dried for several days to preserve their structural integrity. Following the drying process, the specimens were carefully mounted on herbarium sheets using glue for added stability. Each herbarium sheet included a label affixed at the lower right corner, containing detailed information such as the scientific name, habitat, date of collection and collection number. These preserved specimens serve as a permanent reference for the study and contribute to the scientific documentation.

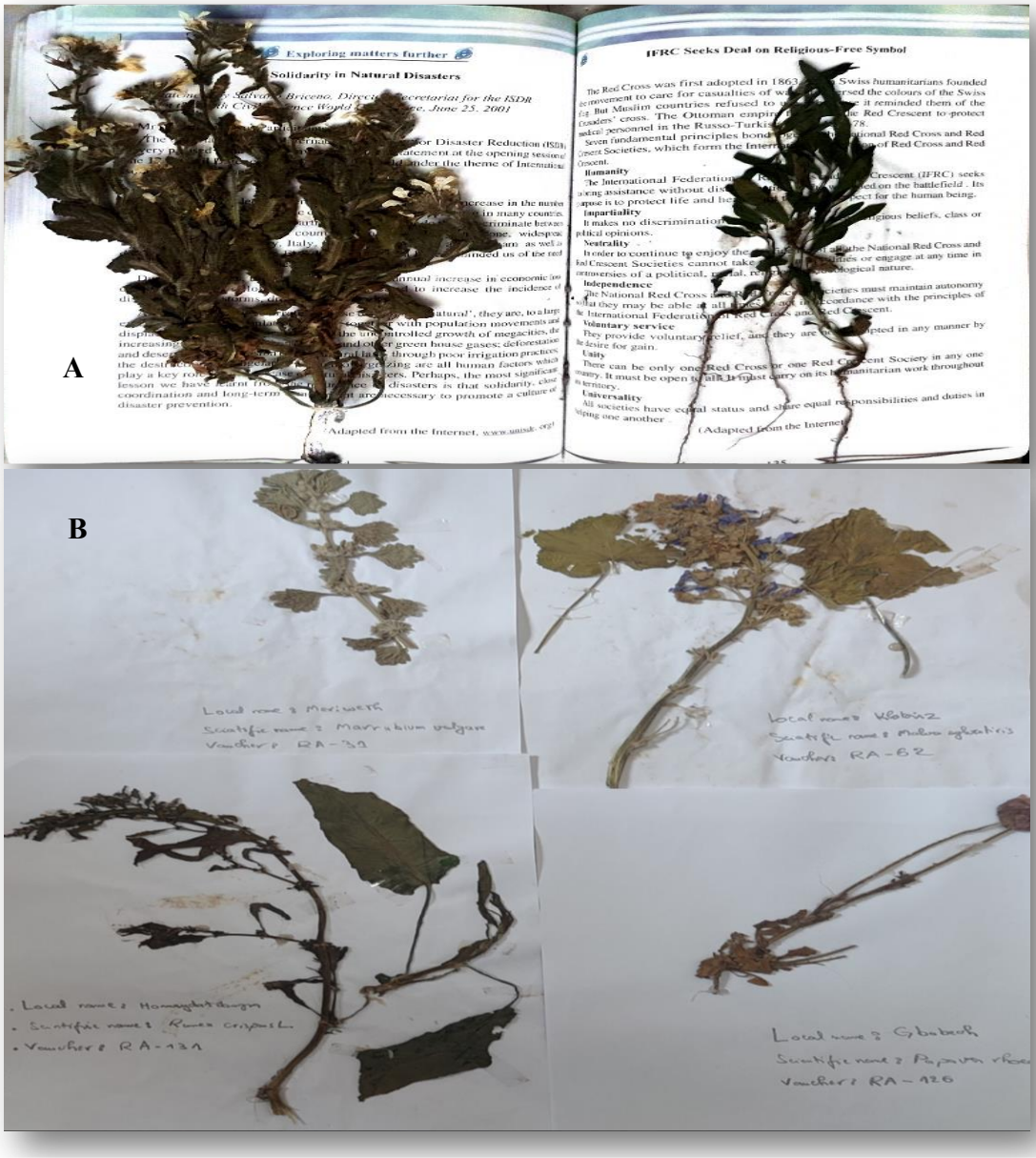

Figure 8: Herbarium preparation: A: Pressing; B: Mounting on sheet.

1.6 Data analysis

The collected ethnobotanical data were systematically organized and analyzed using Microsoft Excel, presented in tabular form to include details such as local and scientific names, family names, palatability, consumed plant parts, life forms, and foraging methods. To evaluate the prominence of each forage species, the Relative Frequency of Citation (RFC) index was calculated using the formula:

$$\mathrm{RFC} = \frac{\mathrm{FC}}{\mathrm{N}}$$

where FC represents the number of participants who mentioned a particular species as fodder, and N denotes the total number of participants in the study. The RFC values range from 0 to 1, with higher values indicating greater recognition and utilization of a species among the community. This method aligns with approaches used in similar ethnobotanical studies (Sujarwo and Caneva, 2016; Harun et al., 2017).

These analyses facilitated a comprehensive understanding of the role of medicinal plants as forage resources within the study area.

2 Phytochemical investigations

2.1 Theoretical framework

Phytochemical characterization represents a critical investigative paradigm for explaining the compositional profiles of bioactive constituents within plant matrices. These secondary metabolites, products of specialized metabolic pathways, function as crucial mediators of plant-environment interactions and possess significant bioactivity potential applicable to pharmacological, nutraceutical, and agricultural domains. Contemporary phytochemical research integrates classical analytical methodologies with advanced chromatographic and spectroscopic techniques, facilitating comprehensive metabolomic profiling and structure elucidation of novel phytoconstituents. This multifaceted approach permits both qualitative identification and quantitative determination of bioactive compounds, establishing structure-activity relationships essential for rational utilization of plant resources in biotechnological applications.

2.2 Plant material acquisition and authentication

Species selection was predicated on the results of the conducted ethnobotanical survey among indigenous livestock agriculturalists inhabiting the semi-arid ecological zone of Bordj Bou Arreridj, Algeria. Which identified plant species exhibiting dual functionality as forage resources and ethnoveterinary therapeutic agents. Six species demonstrating high citation frequency indices were selected for comprehensive investigation: *Artemisia herba-alba, Atriplex canescens, Juniperus phoenicea, Opuntia ficus-indica, Sinapis arvensis,* and *Ziziphus lotus.* The plant specimens were identified and authenticated based on morphological characteristics by expert madame BENHOUHOU Salima taxonomists at the Herbarium Department of Botany, Higher National Agronomic School (ENSA).

Sampling was implemented across geographically distinct locations within each species' distribution range (Table 1), *A. herba-alba*, *A. canescens*, and *Z. lotus* from the

southwestern steppe ecological zone (El Hamadia district); *J. phoenicea* from the southeastern montane region (Taglait commune); and *O. ficus-indica* and *S. arvensis* from the high plateaus' region (Belimour commune).

Table 1: Plant name, life cycle, life form and sampling site of the six investigated plants

Scientific name	Life cycle	Life form	Sampling region
Artemisia herba-alba Asso	Perennial	Chamaephyte	El Hamadia
Atriplex canescens (Pursh) Nut	Perennial	Chamaephyte	El Hamadia
Juniperus phoenicea L	Perennial	Phanerophyte	Taglait
Opuntia ficus-indica (L.) Mill	Perennial	Phanerophyte	Belimour
Sinapis arvensis L	Annual	Therophyte	Belimour
Ziziphus lotus Lam	Perennial	Phanerophyte	El Hamadia

Aerial vegetative structures constituting palatability-appropriate forage components (leaves, young stems, and terminal branches) were harvested from phenologically mature individuals during inflorescence (April–June 2022). Collection parameters were standardized, with 5–10 individuals sampled per species at each location, yielding aggregate biomass of approximately 500 g (of the part consumed by livestock) per species.

Post-collection processing adhered to standardized protocols designed to maintain phytochemical integrity. Plant materials underwent drying through air exposure under ambient temperature in the shadow, with the exception of *O. ficus-indica*, which necessitated thermal dehydration in a ventilated oven (40 ± 1°C) (Moula et al., 2019). Dried materials were subsequently ground using a stainless-steel laboratory grinder and passed through sieve to ensure homogeneity of particle dimensions. The resultant powdered materials were stored in hermetically sealed, glass containers at ambient temperature in the dark with periodic monitoring of moisture content to ensure stability.

2.3 Extraction procedure

Bioactive constituents were extracted using two different solvents: distilled water and 70% (v/v) ethanol in water. For each solvent, 10 g of ground plant material was subjected to sequential maceration three times.

In the first cycle, the plant material is mixed with 100 mL of solvent (sample-to-solvent ratio of 1:10 w/v) and left to macerate with agitation (150 rpm) for 24 hours at

ambient temperature (approximately 20-25°C) under light-protected conditions to prevent potential photodegradation of labile compounds.

After 24 hours, the mixture was filtered through Whatman No. 1 filter paper. The collected filtrate was set aside, and the residual plant material (marc) underwent two additional extraction cycles using 100 mL of fresh solvent each time, following the same maceration parameters (24 h, 150 rpm, ambient temperature, light-protected).

The filtrates obtained from the three sequential extractions for each solvent were combined and then concentrated under reduced pressure using a rotary evaporator (HEI-VAP Heidolph rotary) at a controlled temperature of 40°C to minimize thermal degradation of constituents.

The resulting crude extracts were weighed, and the extraction yield was calculated as a percentage (w/w) relative to the initial mass of the dry plant material. Extracts were stored in labeled, airtight glass bottles at 4°C until required for further analysis.

2.4 Qualitative phytochemical screening

2.4.1 Principle

Phytochemical screening serves as a crucial initial step to qualitatively detect the presence of major classes of secondary metabolites within plant material, compounds often responsible for the plant's biological activities. The principle of these screening tests is based on the specific reactivity of different classes of phytochemicals with particular chemical reagents. When a reagent is added to the plant extract, it interacts with compounds possessing certain functional groups or structural features characteristic of a metabolite class (e.g., alkaloids, tannins, glycosides). This targeted chemical reaction results in a distinct and observable change, most commonly the development of a specific color or the formation of a precipitate, signaling the likely presence of that phytochemical class in the sample (Parimelazhagan, 2016).

2.4.2 Procedures

The ethanolic and aqueous extracts obtained from each plant material were subjected to qualitative phytochemical screening to detect major classes of secondary metabolites, following validated analytical protocols adapted from established methodologies (Thangaraj, 2015; Rani et al., 2018; Shaikh and Patil, 2020; Settaluri et al., 2024). The specific procedures used for the identification of these phytochemical classes are detailed in Table 2.

For each phytochemical class, appropriate positive control standards were analyzed in parallel to validate analytical performance. Results were systematically documented using a semi-quantitative scale reflecting reaction intensity: (-) absent, (+) weakly present, (++) moderately present, and (+++) strongly present.

Table 2: Qualitative Phytochemical Screening Protocols

Secondary metabolite	**Test name**	**Methodology and reagents**	**Positive reaction indicator**
Alkaloids	Dragendorff's Test	2 mL plant extract + 5 mL HCl (1%) + 2 mL Dragendorff's reagent	Formation of orange-red precipitate
Tannins	Ferric Chloride Test	2 mL plant extract + 3 drops $FeCl_3$ (5%)	Development of black or blue-green coloration/precipitate
Flavonoids	Alkaline Reagent Test (Shinoda Test)	2 mL plant extract + few drops concentrated HCl + magnesium ribbon fragments	Appearance of orange to red coloration
Reducing Sugars	Fehling's Test	2 mL plant extract + 1 mL Fehling's solution (A:B, 1:1 ratio) + heated in water bath	Formation of brick-red precipitate
Terpenoids	Salkowski Test	2 mL plant extract + 2 mL chloroform, shaken and allowed to separate + 2 mL H_2SO_4 added along tube wall	Development of reddish-brown coloration at interface
Saponins	Frothing Test	2 mL plant extract + 10 mL distilled water, vigorously shaken for 15 min	Persistence of stable froth for 15 min
Anthraquinone Glycosides	Borntrager's Test	2 mL plant extract + 2 mL H_2SO_4 (5%), heated, filtered + equal volume $CHCl_3$, separated + lower layer + dilute NH_4OH	Formation of rose-pink to red coloration in ammoniacal layer
Anthocyanins	NaOH Test	To 2 ml of the extract + 1 ml of 2N sodium hydroxide, heated for 5 min at 100°C.	bluish green colour indicates the presence of anthocyanin
Phlobatannins	Hydrochloric Acid Test	2 mL plant extract + 1 mL HCl (1%), boiled in water bath	Formation of red precipitate
Coumarins	Alcoholic NaOH Test	1.5 mL plant extract + few drops alcoholic NaOH	Development of yellow coloration
Gums and Mucilages	Alcohol Precipitation Test	10 mL distilled water containing 100 mg extract + 25 mL absolute alcohol with constant stirring	Formation of white or cloudy precipitate

Quinones	Modified Borntrager's Test	2 mL plant extract + 5 mL HCl (20%), heated for 30 min, cooled + extracted with 20 mL $CHCl_3$ + organic layer separated + 0.5 mL NH_4OH (50%) added	Development of red-to-violet coloration in organic layer

2.5 Quantitative analysis

2.5.1 Proximate Composition

Proximate composition analysis quantifies the major constituents of plant materials, including moisture content, crude fiber, crude lipid, crude protein, and ash content. The Association of Official Analytical Chemists (AOAC, 2000) has established standardized methods for these determinations, ensuring accuracy and reproducibility in analytical results. with all values expressed per dry matter (DM) to standardize measurements.

2.5.1.1 Moisture content determination

Moisture content was determined by gravimetric analysis using a hot air oven. Approximately 2 g of the powdered sample was accurately weighed into a pre-weighed moisture dish. The sample was dried in a hot air oven at 105°C until a constant weight was achieved (typically 4–6 hours). After cooling in a desiccator, the dried sample was reweighed. The moisture content was calculated as the percentage weight loss relative to the initial sample weight:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{X } 100$$

2.5.1.2 Crude fiber determination

The sample was first defatted using petroleum ether to remove lipids. The defatted residue was then subjected to sequential digestion with 1.25% sulfuric acid (H_2SO_4) and 1.25% sodium hydroxide (NaOH) solutions under controlled heating conditions. The insoluble residue was filtered, washed thoroughly with distilled water, and dried at 105°C overnight. The dried residue was weighed, ashed at 550°C for 4 hours, and reweighed after cooling in a desiccator. Crude fiber content was calculated as follows:

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Initial sample weight}} \text{X } 100$$

2.5.1.3 Crude lipid content determination

Lipid content was determined using the Soxhlet extraction method. Approximately 5 g of the dried sample was placed in a thimble and extracted with petroleum ether in a Soxhlet apparatus for 6 hours. After extraction, the solvent was evaporated in a fume hood, and the remaining lipid residue was dried and weighed. Lipid content was expressed as a percentage of the initial sample weight:

$$\text{Lipid content (\%)} = \frac{\text{Weight of lipid residue}}{\text{Initial sample weight}} \text{X } 100$$

2.5.1.4 Ash content determination

Ash content was determined by incineration. Approximately 2 g of the sample was placed in a pre-weighed crucible and heated in a muffle furnace at 550°C for 4 hours until a white or light gray ash was obtained. The crucible was cooled in a desiccator and reweighed. Ash content was calculated as follows:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Initial sample weight}} \text{X } 100$$

2.5.1.5 Crude protein determination (Kjeldahl Method)

Crude protein content was determined using the Kjeldahl method, which involves three main steps: digestion, distillation, and titration.

i. **Digestion:** Approximately 0.5–1 g of the sample was accurately weighed and transferred to a digestion flask. Concentrated sulfuric acid (H_2SO_4) and a catalyst mixture (e.g., potassium sulfate and copper sulfate) were added to the flask. The mixture was heated in a digestion block until the solution became clear, indicating complete conversion of organic nitrogen into ammonium sulfate.

ii. **Distillation:** The digested sample was diluted with distilled water and made alkaline with sodium hydroxide (NaOH), releasing ammonia gas (NH_3). The ammonia was distilled and collected in a receiving flask containing boric acid (H_3BO_3) solution.

iii. **Titration:** The collected ammonia was titrated with standardized hydrochloric acid (HCl) using few drops of methyl red to determine the endpoint (when the color turn orange).

The crude protein content was calculated as follows:

$$\text{Nitrogen (\%)} = \frac{(\text{Vacid} - \text{Vblank}) \times \text{Nacid} \times 14.007}{\text{Wsample}} \text{X } 100$$

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Where:

- Vacid: Volume of acid used for titration (mL)
- Vblank: Volume of acid used for blank titration (mL)
- Nacid: Normality of the acid (HCL)
- 14.007: Molecular weight of nitrogen
- Wsample: Weight of the sample (g)

2.5.2 Mineral Analysis Atomic Absorption Spectroscopy

2.5.2.1 Principle

Atomic Absorption Spectroscopy (AAS) represents a highly sensitive spectroanalytical procedure employed for the quantitative determination of specific elemental constituents within biological matrices. The fundamental principle relies on the quantum mechanical phenomenon wherein ground-state atoms absorb electromagnetic radiation at discrete wavelengths characteristic of each element. During analysis, the sample undergoes thermal decomposition, volatilization, and atomization, generating a population of free atoms in the optical path. These atoms selectively absorb radiation emitted from element-specific hollow cathode lamps, with the magnitude of absorption exhibiting a direct proportionality to the concentration of the target element in accordance with the Beer-Lambert law.

2.5.2.2 Sample preparation and digestion

Precisely 1.000 ± 0.001 g of finely grounded plant material was accurately weighed into polytetrafluoroethylene (PTFE) digestion vessels. To each tube, 8 mL of 65% analytical-grade nitric acid (HNO_3) was added as the digestion medium. Sample mineralization was accomplished using a microwave-assisted digestion system (STAR D Microwave Digestion System, CEM Corporation, Matthews, NC) operating under controlled pressure and temperature parameters following protocol of Hseu, (2004). The optimized digestion protocol comprised an initial ramp to 180°C for 10 min, followed by isotherm maintenance at 180 ± 5°C for 20 min, and subsequent cooling to ambient temperature (approximately

22°C) for 10 min. This procedure facilitated complete solubilization of mineral constituents while minimizing potential analyte loss.

2.5.2.3 Post-digestion processing

The digested samples were quantitatively transferred to tubes (25 mL) using sequential aliquots of ultrapure water. The vessels were subjected to multiple rinses to ensure complete transfer of all mineral constituents. Sample solutions were then brought to volume with ultrapure water and stored at 4 ± 1°C in glass tubes until analysis to prevent potential contamination or analyte adsorption.

2.5.2.4 Instrumentation and analytical procedure

Elemental determinations were performed using a PerkinElmer PinAAcle 900H Atomic Absorption Spectrophotometer (PerkinElmer Inc., Waltham, MA) equipped with both flame and graphite furnace atomization systems. Element-specific hollow cathode lamps were employed as radiation sources.

First, multi-point calibration standards were prepared through serial dilution of certified reference standard solutions (1000 mg L^{-1}, Merck KGaA) in matrix-matched diluent (2% HNO_3 v/v). Standard concentrations were strategically selected to encompass the anticipated analytical range while maintaining optimal signal-to-noise ratios:

- Copper (Cu): 0.5, 1.0, 2.5, and 5.0 mg L^{-1}
- Potassium (K): 0.5, 1.0, 2.0, and 4.0 mg L^{-1}
- Phosphorus (P): 10.0, 25.0, 50.0, and 100.0 mg L^{-1}

Calibration curves were constructed via weighted linear regression ($1/x^2$) of absorbance values against known concentrations. Acceptance criteria for calibration included correlation coefficients (r^2) ≥ 0.9995. Then the sample solutions were diluted appropriately to fall within the calibration range of the instrument.

After that each element was analyzed sequentially across all plant samples. The absorbance readings were recorded, and the concentrations of P, K, and Cu were determined by referencing the respective calibration curves. Quality control measures, including the analysis of blank samples and standard reference materials, were implemented to ensure the reliability and accuracy of the results.

2.5.3 Secondary metabolite quantification

2.5.3.1 Principle

Colorimetric methods, analyzed via spectrophotometry, were employed for the quantitative determination of bioactive phytochemicals. These techniques utilize specific chromogenic reactions where selective reagents interact with target analytes based on their chemical functionalities. This process generates measurable chromophores with characteristic maximum absorption wavelengths (λmax). Quantification was achieved using the Beer-Lambert Law, which relates absorbance to concentration, with the help of a calibration curve (y = ax + b) that is constructed in parallel using a standard prepared at varying concentrations under the same experimental conditions as the samples.

2.5.3.2 Total phenolic content

Phenolics, the aromatic compounds with hydroxyl groups, are widespread in plant kingdom. Their estimation involves the reduction of phenols by phosphomolybdic-phosphotungstic components in the Folin-Ciocalteu reagent. Phenolic compounds are oxidized in a basic medium resulting in the formation of superoxide ion, which in turn react with molybdate to form molybdenum oxide. The blue-coloured complex thus developed has a very intensive absorbance at 725 nm.

Analytical protocol: The total phenolic content was determined using the Folin-Ciocalteu colorimetric method, following the procedure described by Medoua et al. (2009). In brief, 0.25 mL of the plant extract was mixed with 0.25 mL of 10% (v/v) Folin-Ciocalteu reagent. After a 5-minute incubation at room temperature, 2 mL of 7.5% sodium carbonate solution was added. The mixture was then incubated in the dark for 30 min at room temperature. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer.

Gallic acid was used as the calibration standard, and results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW). Each sample was analyzed in triplicate.

2.5.3.3 Total flavonoid content

The basic principle of colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols, respectively. In addition, it also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids resulting in pink colour formation, and it is measured at 510 nm. (Parimelazhagan, 2016).

Analytical protocol: Total flavonoid content was assessed using the aluminum chloride colorimetric method, as outlined by Elfalleh et al. (2019). A 1 mL aliquot of the plant extract was mixed with 1 mL of 2% aluminum chloride solution prepared in methanol. The mixture was incubated for 30 min at room temperature, protected from light. Absorbance was recorded at 415 nm using a UV-Vis spectrophotometer.

Quercetin served as the reference standard, and flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW). Analyses were performed in triplicate.

2.5.3.4 Total terpenoid content

Terpenoids constitute a structurally diverse class of lipophilic secondary metabolites derived from isoprene (C_5) units, exhibiting diverse biological activities. Their quantification employs acidic dehydration reactions generating chromophoric condensation products with characteristic absorption properties. Specifically, concentrated sulfuric acid facilitates dehydration and rearrangement of terpenoid structures, yielding conjugated unsaturated systems with enhanced molar absorptivity at 538 nm wavelengths.

Analytical protocol: The total terpenoid content was estimated using a colorimetric assay (Ghorai et al., 2012). Specifically, 200 µL of the plant extract (2 mg/mL in methanol) was combined with 1.5 mL of chloroform and vortexed thoroughly. The mixture was allowed to stand for 3 min at room temperature, followed by the addition of 100 µL of concentrated sulfuric acid. After cooling in an ice bath for 15 min, the mixture was incubated in the dark for 1.5 hours. Absorbance was measured at 538 nm using a UV-Vis spectrophotometer.

Linalool was used as the calibration standard, and terpenoid content was expressed as milligrams of linalool equivalents per gram of dry weight (mg LE/g DW). All measurements were conducted in triplicate.

2.5.3.5 Condensed tannin content

Condensed tannins, also known as proanthocyanidins, are polyphenolic compounds formed through the polymerization of flavonoid monomers, primarily catechins and epicatechins. These secondary metabolites are widely recognized for their antioxidant, anti-inflammatory, and protein-binding properties. Their quantification is typically based on colorimetric methods involving nucleophilic attacks on the flavan-3-ol units under acidic conditions, resulting in the formation of stable chromogenic complexes

Analytical protocol: Condensed tannin content was quantified using the vanillin assay, as described by Awaga et al. (2023). Briefly, 50 µL of each extract was mixed with 1.5 mL of 4% methanolic vanillin solution. After thorough mixing, 750 µL of 36% hydrochloric acid was added, and the mixture was incubated at room temperature for 20 min. Absorbance was measured at 550 nm using a UV-Vis spectrophotometer.

Catechin was used as the standard, and results were expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW). Each analysis was performed in triplicate.

2.6 Analytical techniques

2.6.1 Fourier Transform Infrared (FT-IR) analysis

2.6.1.1 Principle

Fourier Transform Infrared (FT-IR) Spectroscopy is an analytical technique employed to identify and characterize functional groups within a substance. It operates by directing an infrared light beam through a sample, where specific molecular bonds absorb distinct frequencies of this light, corresponding to their vibrational modes. The resulting absorption spectrum serves as a molecular fingerprint, enabling the identification of functional groups such as C=O, C-H, and N-H bonds. FT-IR spectroscopy is highly sensitive and precise, capable of detecting compounds at very low concentrations. Additionally, it is a non-destructive method suitable for analyzing solid, liquid, and gaseous samples, making it a versatile tool in qualitative phytochemical analysis.

FT-IR analysis was conducted on plant powders, hydroethanolic extracts, and aqueous extracts following (Zishan et al., 2024).

2.6.1.2 Sample preparation

Plant powders: Approximately 3 mg of finely ground plant powder was intimately mixed with 20 mg of spectroscopic-grade potassium bromide (KBr) in an agate mortar to ensure homogeneity. This mixture was then compressed into a translucent pellet using a hydraulic press under vacuum conditions, forming a disc suitable for infrared analysis.

Hydroethanolic and aqueous extracts: The dried extracts were triturated with KBr in a similar ratio to achieve uniform dispersion. The resulting mixture was pressed into pellets as described above. This preparation method facilitates the acquisition of clear spectra by minimizing scattering effects and ensuring optimal interaction with the infrared beam.

2.6.1.3 Analytical protocol

The prepared KBr pellets containing the samples were placed in the sample holder of the FT-IR spectrometer. Spectral data were collected over the mid-infrared region, spanning 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}. Each spectrum was obtained by averaging 32 scans to enhance the signal-to-noise ratio. Background spectra were recorded under identical conditions using a pure KBr pellet to correct for atmospheric interference and instrument artifacts. The resulting spectra were analyzed to identify characteristic absorption bands corresponding to various functional groups present in the plant materials and their extracts. IRsolution Shimadzu's FT-IR software was used to analyze and visualize the data.

2.6.2 Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis

2.6.2.1 Principle

Liquid Chromatography-Mass Spectrometry (LC-MS/MS) is an analytical technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (Figure 9). This method is particularly effective for identifying and quantifying phenolic compounds in complex plant extracts. In LC, compounds are separated based on their interactions with the stationary phase and the mobile phase. The separated compounds are then ionized and introduced into the mass spectrometer, where they are detected and quantified based on their mass-to-charge ratios. This combination allows for high sensitivity and specificity in analyzing complex mixtures.

2.6.2.2 Sample Preparation

The 70% ethanolic extract, selected for its superior phenolic content (compared to the aqueous extract), underwent a purification process prior to LC-MS/MS analysis. One mg of the extract was dissolved in 25 mL of distilled water and stirred for 10 min. The solution was defatted by extracting three times with hexane in a separatory funnel, discarding the upper hexane layer each time. The defatted aqueous layer was then fractionated with ethyl acetate; the upper ethyl acetate layer was collected in each of three extractions. The combined ethyl acetate fractions were concentrated using a rotary evaporator at 40 °C under reduced pressure. The resulting residue was dissolved in 8 mL of methanol and filtered through a 0.22 µm syringe filter.

2.6.2.3 Analytical protocol

Following established protocols by Ignat et al. (2011), the analysis was conducted using a Shimadzu 8040 UPLC-ESI-MS/MS system equipped with UFMS technology and a binary pump Nexera XR LC-20AD. Chromatographic separation was achieved on a Restek Ultra C18 column (150 mm × 4.6 mm, 3 µm particle size) maintained at 25 °C. The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (methanol), delivered at a flow rate of 0.4 mL/min. The injection volume was 5 µL. The gradient elution was as follows: 0–2 min, 95% A; 2–15 min, 95% A; 15–18 min, 5% A; 18–20 min, 5% A; 20–25 min, 95% A.

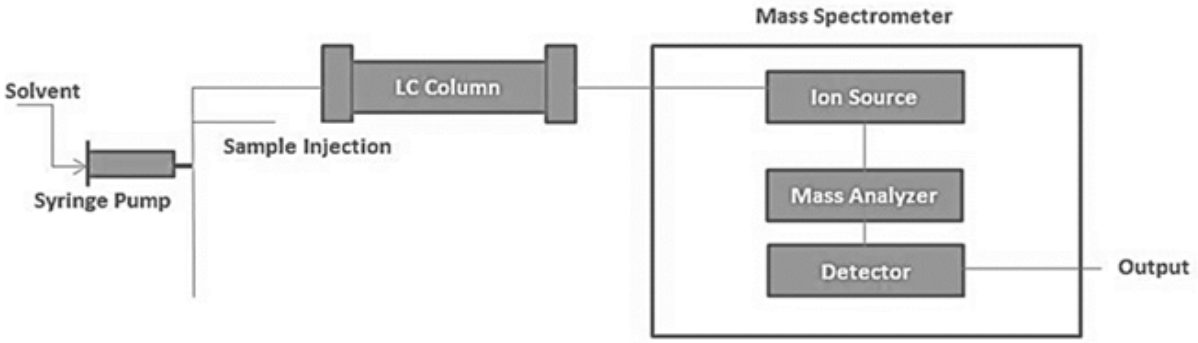

Figure 9: Simple schematic of LC-MS system

The mass spectrometer operated with an electrospray ionization (ESI) source. The conditions were: collision-induced dissociation (CID) gas at 230 kPa; conversion dynode at −6.00 kV; interface temperature at 350 °C; desolvation line (DL) temperature at 250 °C; nebulizing gas flow at 3.00 L/min; heat block temperature at 400 °C; and drying gas flow at 15.00 L/min. Polyphenol standards were optimized using direct injection without a column. The mass spectrometer operated in both negative and positive ion modes with multiple reaction monitoring (MRM) to detect and quantify the phenolic compounds present in the sample.

2.7 In Vitro antioxidant assays

In the study we used two different methods for the evaluation in vitro of the antioxidant activity of the plants' extracts.

2.7.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

2.7.1.1 Principle

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay evaluates the antioxidant capacity of a sample by measuring its ability to donate hydrogen atoms or electrons to neutralize free radicals. DPPH is a stable free radical characterized by a deep violet color in solution, with a strong absorption at approximately 517 nm. When an antioxidant is present, it donates electrons or hydrogen atoms to DPPH, reducing it to 2,2-diphenyl-1-picrylhydrazine, which results in a color change from violet to pale yellow (Figure 10). This decolorization can be quantitatively measured by a decrease in absorbance at 517 nm, providing an estimate of the sample's free radical scavenging activity.

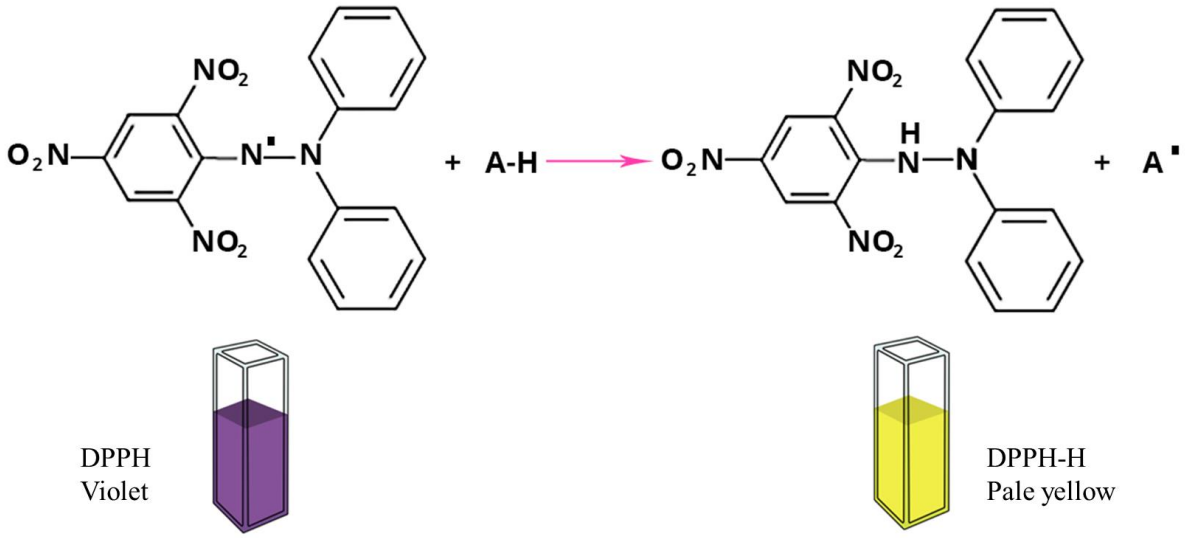

Figure 10: Principle of DPPH antioxidant assay

2.7.1.2 Analytical protocol

The antioxidant activity of the plant extracts was assessed using the DPPH free radical scavenging assay, following the procedure outlined by Bersuder et al. (1998). Plant extracts were prepared in methanol at concentrations ranging from 3 to 500 µg/mL. A freshly prepared DPPH solution (0.024 mg/mL in methanol) was mixed with 1 mL of extract and 2.7 mL of the DPPH solution. The mixture was incubated in the dark at room temperature (25 °C) for 60 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\text{Percentage Inhibition } (\%) = \frac{\textit{Blank Absorbance} - \textit{Sample Absorbance}}{\textit{Blank Absorbance}} \times 100$$

The IC_{50} value, representing the concentration of extract required to scavenge 50% of DPPH radicals, was determined by plotting the percentage inhibition against the extract concentrations. Ascorbic acid was used as a reference standard for antioxidant activity. All measurements were performed in triplicate, and results are expressed as $IC_{50} \pm$ standard deviation.

2.7.2 Ferric Reducing Antioxidant Power (FRAP) assay

2.7.2.1 Principle

The Ferric Reducing Antioxidant Power (FRAP) assay quantifies the antioxidant potential of a sample by measuring its capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (Figure 11). In this method, antioxidants present in the sample reduce the ferric-tripyridyl-s-triazine (Fe^{3+}-TPTZ) complex to its ferrous form, resulting in the formation of a blue-colored Fe^{2+}-TPTZ complex. The intensity of the blue color, measured spectrophotometrically at 593 nm, is directly proportional to the reducing power of the sample, thereby reflecting its total antioxidant capacity.

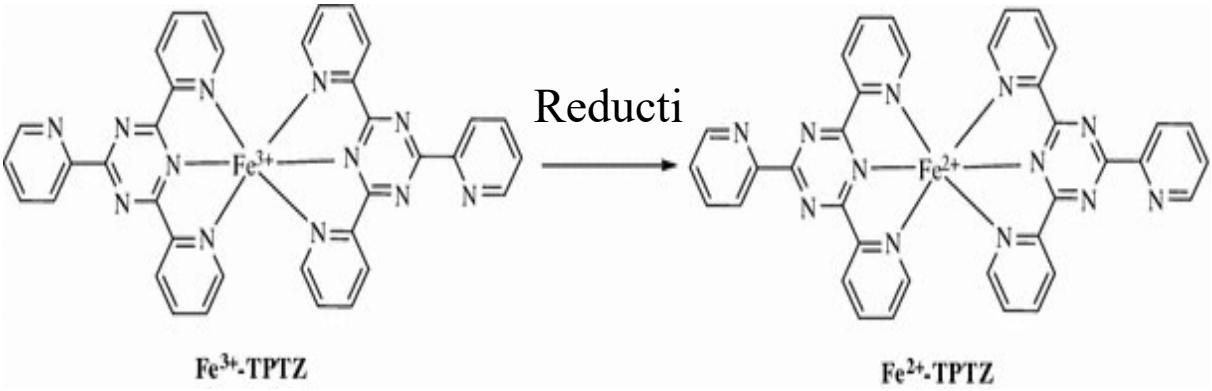

Figure 11: Principle of FRAP antioxidant assay

2.7.2.2 Analytical protocol

The antioxidant capacity of the plant extracts was evaluated using the FRAP assay, following the protocol by Pulido et al. (2000) with slight modifications. Plant extracts were dissolved in 70% ethanol to achieve a final concentration of 2 mg/mL. The FRAP reagent was freshly prepared by mixing 200 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$ solution in a 10:1:1 (v/v/v) ratio. In a typical assay, 100 µL of the plant extract was combined with 2 mL of the FRAP reagent. The mixture was vortexed and incubated at 25 °C in the dark for 30 min. Absorbance was measured at 593 nm using a UV-Vis spectrophotometer. A calibration curve was constructed using Trolox standards ranging from 0.1 to 1.0 mM. The FRAP values were expressed as mmol Trolox equivalents per gram of plant extract (mmol TE/g) using the formula:

$$\text{FRAP value (mol Trolox equivalent/g)} = \frac{Ct}{C}$$

where Ct is the concentration of Trolox equivalent determined from the calibration curve, and C is the concentration of the tested sample solution. All measurements were performed in triplicate to ensure accuracy and reproducibility.

2.8 Statistical analysis

All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation. To assess significant differences among the parameters, a one-way analysis of variance (ANOVA) was performed. Subsequently, Duncan's Multiple Range Test (DMRT) was employed for post hoc comparisons to identify specific group differences. A significance level of $P < 0.05$ was considered for all statistical tests. Data analyses were carried out using IBM SPSS Statistics software, version 21.

CHAPTER III: RESULTS AND DISCUSSION

1 Ethnobotanical study

1.1 Result

1.1.1 Livestock distribution and knowledge holders' profile

In the high plateau regions, livestock management practices incorporate structured feeding protocols wherein breeders confine animals to stalls and administer nutritional supplements comprising natural fodder augmented with cereal derivatives and seasonal grazing opportunities. Conversely, in steppe ecosystems, grazing on indigenous phytomass predominates, many of which were considered medicinal, such as *Artemisia herba-alba* (Chih), *Stipa tenacissima* (Halfa), and *Atriplex canescens* (Gtaf).

Ethnobotanical data acquisition involved 84 respondents (71 males, 13 females) with an age distribution of 5 (25-35 years), 18 (36-45 years), 29 (46-55 years), 22 (56-65 years), and 10 (66-85 years). Professional demographics encompassed 45 shepherds, 12 veterinarians, 9 forest rangers, and 18 agricultural practitioners (Figure 12). Ethnobotanical knowledge demonstrated significant heterogeneity across demographic variables, with elderly respondents exhibiting comprehensive ecological knowledge despite limited formal education. Gender-differentiated knowledge patterns revealed that male participants

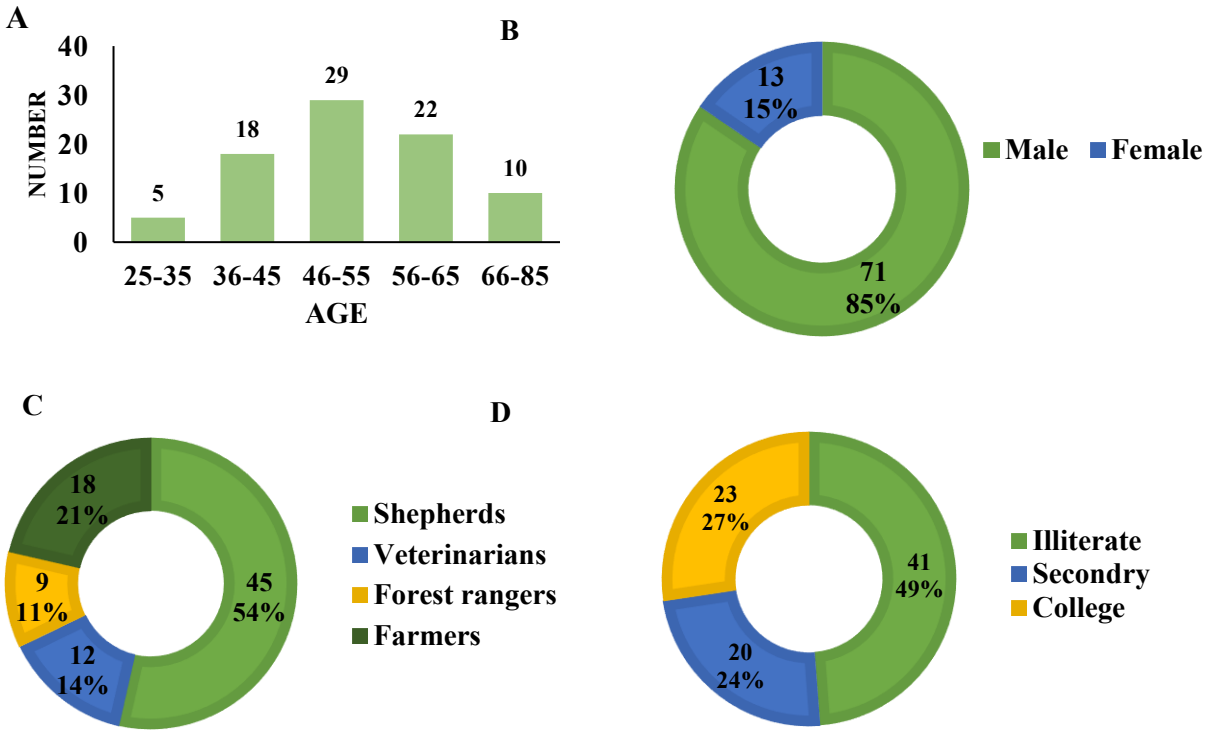

Figure 12: Informants profiles (A) the age, (B) gender, (C) profession and (D) educational level

possessed extensive taxonomic familiarity with forage species, while female respondents demonstrated specialized expertise regarding medicinal phytotaxa.

The regional livestock population comprised three primary taxonomic categories—ovine, caprine, and bovine—with spatially heterogeneous distribution patterns. Bovine populations demonstrated predominance in the high plateau zones of Ras El Oued (eastern sector) and Medjana (central region). Caprine populations exhibited preferential distribution in steppe ecosystems of El Ach and Rabta (southern sector), while ovine populations demonstrated ecological plasticity with predominant occurrence in the southern steppe regions of El Hamadia and El Ach.

In the high plateau region, breeders bring their livestock to stalls, where they mow natural fodder mixed with corn grains and wheat bran or even freshly chopped cultivated fodder or hay, supplemented by seasonal grazing. In contrast, in the steppe areas, the primary mode of livestock feeding involved grazing on indigenous wild plants, many of which were considered medicinal, such as *Artemisia herba-alba* (White Wormwood or Chih in Arabic), *Stipa tenacissima* (Esparto Grass or Halfa), and *Atriplex canescens* (Four-winged Saltbush or Gtaf), was the main form of livestock feeding.

1.1.2 Diversity, life forms, parts consumed, and medicinal properties of forage plants

Taxonomic documentation yielded 133 forage plant species, of which 92 (69%) exhibited documented medicinal properties (Annex 2). Systematic classification revealed 90 genera distributed across 43 families, with Asteraceae (27 species), Poaceae (17 species), Fabaceae (11 species), and Apiaceae (8 species) exhibiting highest taxonomic representation (Figure 13). These four families constituted 47% of the documented phytodiversity, while 25 families (19%) were monospecific. The remaining familial taxa contributed between 2 and 5 species each, totaling 34% of documented biodiversity.

Morphological analysis of consumed plant parts indicated predominant utilization of aerial parts (96 species), followed by leaves and tender branches (21 species), whole plant consumption (9 species), and selective leaf utilization (7 species) (Figure 14). Life form categorization (Figure 15) demonstrated herbaceous predominance (76%), with shrubs (14%) and arborescent species (10%) comprising the remaining biodiversity component.

1.1.3 Accessibility and palatability

Consumption patterns revealed selective utilization of nutritionally advantageous and tender plant parts, with temporal availability dictated by plant's growth cycle. Seasonal accessibility analysis of forage availability revealed that the highest number of species (42) were available in spring (Figure 16) including *Sinapis arvensis* and *Convolvulus durandoi.* In addition, 32 species were available all year round (e.g., *Artemisia herba-alba* and *Atriplex canescens*), while a smaller portion exhibited seasonal accessibility: spring to summer (19 species, e.g., *Moricandia arvensis* and *Rosa canina*), spring to autumn (18 species, e.g., *Ziziphus lotus* and *Cichorium intybus)*, summer only (13 species, e.g., *Chenopodium album*), and summer to autumn (9 species, e.g., *Tribulus terrestris*). In terms of palatability, the majority of forage plants used were classified as highly palatable or moderately palatable (61 and 47 respectively) with species like *Cynodon dactylon*, *Avena fatua* and *Sonchus oleraceus*, with only a small number classified as less palatable (25) like *Sinapis pubescens.*

1.1.4 Forage plant ranking based on RFC

Quantitative ethnobotanical analysis of the 133 documented plants utilizing the relative frequency of citation (RFC) score methodology yielded values ranging from 0.01 to 0.57. With 44 species exceeding the average RFC of 0.06, and 89 species have RFC values below this average. The most cited species were *Artemisia herba-alba* (RFC: 0.57), *Sinapis arvensis* (0.4), *Stipa tenacissima* (0.29), *Zizyhus lotus* (0.27), *Atriplex canescens* (0.21), and *Juniperus phoenicea* (0.21). In addition, seven other plant species have RFC values above 0.14, such as *Opuntia ficus-indica*, *Quercus ilex*, *Medicago sativa*, and *Anthemis nobilis*

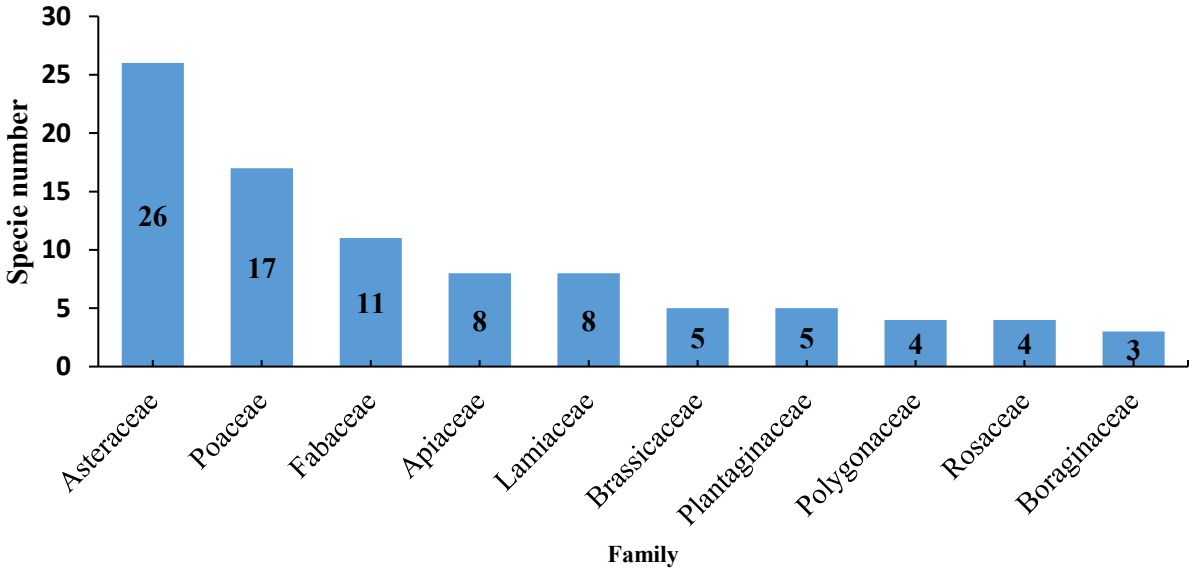

Figure 13. Most cited families of spontanes forage plants in Bordj Bou Arreridj.

(0.19 each), while *Papaver rhoeas*, *Trifolium repens*, and *Sinapis pubescens* each has RFC value of 0.17. The least cited species were *Xanthium strumarium*, *Centaurea pullata*, *Acacia saligna, Dittrichia viscosa*, and *Matthiola lunata*, each with an RFC value of 0.01.

1.2 Discussion

The topographical heterogeneity of Bordj Bou Arreridj contributes to significant phytodiversity, establishing the region as an agroecological zone characterized by extensive traditional ethnobotanical knowledge (Miara et al., 2019). These wild plants serve not only to provide valuable forage resources for livestock, potentially comparable to or even exceeding the quality of some cultivated forage resources (Kadi and Zirmi-Zembri, 2016; Zirmi-Zembri and Kadi, 2016) but also play a crucial role in their health. However, a recent investigation has shown that most of this knowledge is held by older smallholder farmers, who rely on wild fodder plants to feed their animals.

Recent decades have witnessed substantial shifts toward modernized livestock production methodologies among younger demographic followers, incorporating exotic forage taxa with intensive irrigation requirements, thereby compromising sustainability parameters. This transitional process has precipitated the marginalization of traditional ethnobotanical knowledge concerning indigenous forage species, with related reduction in small-scale livestock production activities, further endangering ethnobotanical knowledge preservation.

1.2.1 Influence of forage diversity on herbivore diet selection

The documented 133 indigenous forage taxa, with predominant representation of

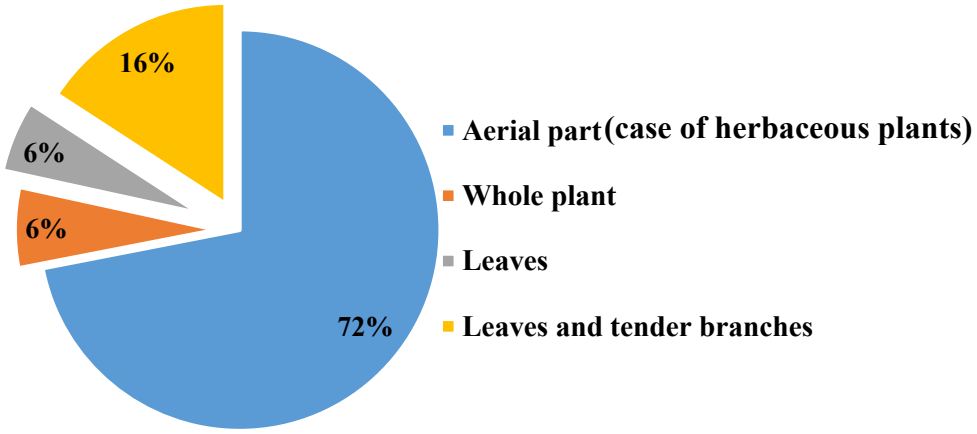

Figure 14. Parts consumed of recorded spontaneous forage plants in Bordj Bou Arreridj.

Asteraceae, Poaceae, and Fabaceae, corroborate previous investigations by Neffar et al. (2016) and (Merdas et al. (2017), reporting similar familial dominance in semi-arid halophytic pastures and arid steppe ecosystems, respectively. Notably, Asteraceae demonstrated highest species richness, confirming Mediterranean distribution patterns previously reported by Carpino et al. (2003), Jeanmonod et al. (2011), and Neffar et al. (2018). Closely following were the Poaceae and Fabaceae families, both well-known for containing valuable forage plants. The majority of identified plants were herbaceous therophytes (annuals), which depend on rainfall for their life cycle. This finding aligns with observations by Merdas et al. (2017), who observed therophytes as the dominant life form in the Algerian arid steppes. These characteristics suggest that these therophytes are likely well adapted to the challenging environmental conditions of the region.

The selection of plant life forms for forage varies considerably across herbivore groups within a particular region. Herbivores show discernible preferences, and free-grazing animals tend to select the best plants while avoiding toxic or less nutritious ones (Soder et al., 2009). Cattle primarily target grasses, such as *Trifolium repens*, with occasional reports of consumption of tree leaves such as *Olea europaea.* Goats show a preference for browsing on woody plants, such as *Atriplex canescens* and *Anabasis articulata*, while sheep occupy an intermediate position, consuming a mixture of grasses and other plant materials, including shrubs and trees. Caprine herbivores demonstrate exceptional dietary plasticity, consuming all documented plant species within the Bordj Bou Arreridj ecosystem. Palatability assessments indicate preferential selection of 100% of reported plant species by caprine herbivores, 93% by ovine herbivores, and 42% by bovine herbivores. Differences in palatability were observed across various plant parts. Specifically, only 7% of forage species

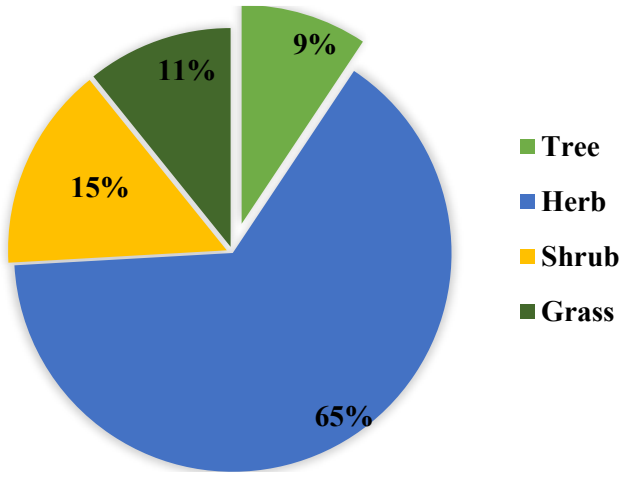

Figure 15. Life forms of wild forage plants in Bordj Bou Arreridj.

were consumed as whole plants, such as *Cynodon dactylon*, and *Lolium multiflorum.* In contrast, 72% of the plants were consumed for their aerial parts, while 16% were selectively consumed as leaves and tender branches. The preference for consuming these species as whole plants can be attributed to their small size and tender herbaceous texture. These findings align with similar results reported in the existing literature (Geng et al., 2017).

1.2.2 Seasonality and palatability of forage plants

Temporal availability patterns of forage species constitute critical parameters for effective herbivore nutrition management (Yang et al., 2021). Therophytes showed seasonality in their availability, with peak use occurring during the rainy spring season. Perennial woody plants, in contrast to herbaceous species, show a more continuous availability throughout the year. This characteristic is particularly relevant during the dry season when herbaceous plants are less available. Consequently, perennial woody plants may become the primary forage source for herbivores during these periods (Lo et al., 2022).

According to local people, some low-palatability plants, namely *Sinapis pubescens*, is known to cause undesirable flavors in animal milk when consumed. As a result, farmers have taken to drying these plants before feeding them to their livestock. Additionally, farmers have found that certain spiny plants, such as *Astragalus spinosus*, can be made more palatable by cooking them to remove their spines. Other plants, such as *Silybum marianum*, have a low palatability when raw, so they are dried before feeding to improve their taste. It has been reported that the palatability of some forage species can decrease after flowering, resulting in reduced consumption by herbivores. These plants are therefore collected before they start to flower. Moreover, some less palatable species, such as *Thymelaea hirsuta*, have

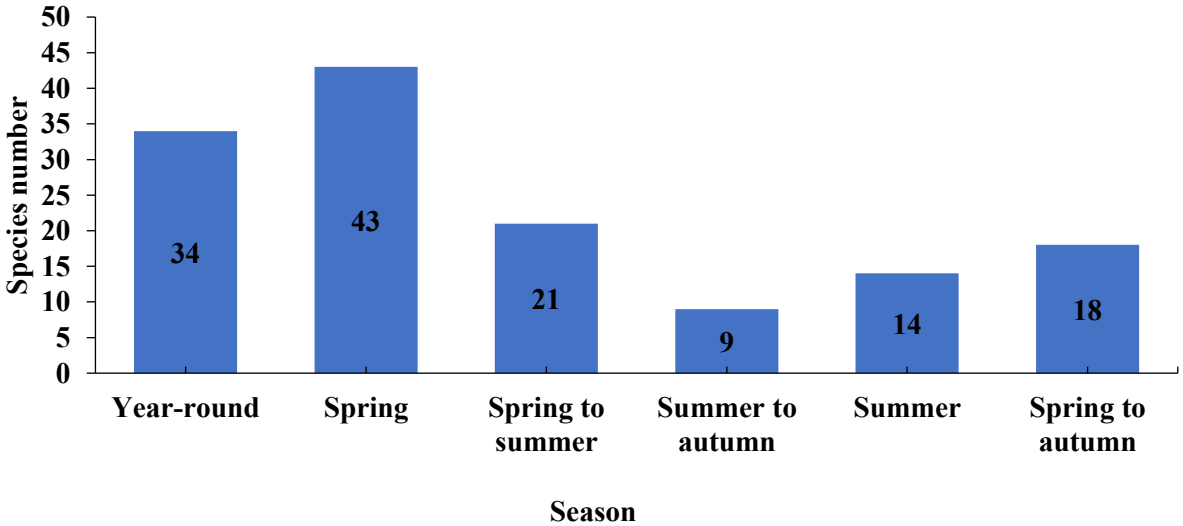

Figure 16. Seasonality of spontaeous forage species in Bordj Bou Arreridj.

been identified by ecologists and forest rangers as indicators of overgrazing, and are therefore used for monitoring purposes.

1.2.3 Potential for livestock intoxication by wild plants

Veterinary professionals operating within the study region have expressed concerns regarding the likelihood of livestock being adversely affected by the consumption of certain wild plants. Susceptibility appears elevated in recently introduced herbivores or those experiencing initial grazing periods following confinement. Although indigenous livestock are not completely immune, they appear to be less likely to be affected (Parton and Bruere, 2002). This could be attributed to their established foraging patterns and their recognition of the potential toxicity of certain plants. It is worth noting that the risk of intoxication tends to increase during periods of drought-induced pasture scarcity and overgrazing events (Desta, 2019; Mossie and Yirdaw, 2023). Further phytotoxicological investigations are necessary to identify specific causative species, potentially including *Thapsia garganica*.

1.2.4 A sustainable approach to livestock health management through medicinal forage plants

A significant overlap was observed between plants identified as forage and their report to be medicinal. Specifically, 69.2% (92 out of 133) of the documented forages were also reported as having medicinal value. Furthermore, species ranked within the topmost consumed forages based on the RFC ranking were also reported medicinal: *Artemisia herba-alba, Opuntia ficus-indica* and *Atriplex canescens*. This strong alignment resonates with recent investigations concerning multifunctional phytoresources (Ali et al., 2021; Abbas et al., 2022; Martins et al., 2023), suggesting a potential link between forage value and inherent medicinal benefits. Integrating such medicinal forage species into grazing systems leverages the natural medicinal properties of these plants. This approach has the potential to reduce dependency on synthetic veterinary drugs, and potentially mitigating the risk of drug resistance in livestock populations (French, 2018). Furthermore, the incorporation of medicinal forage species may offer additional environmental benefits, such as reducing methane emissions (Villalba et al., 2019).

2 Qualitative phytochemical screening

2.1 Results

The extraction yields of the six studied plants varied between the aqueous and hydroethanolic methods (Figure 17). For aqueous extraction, the yields ranged from

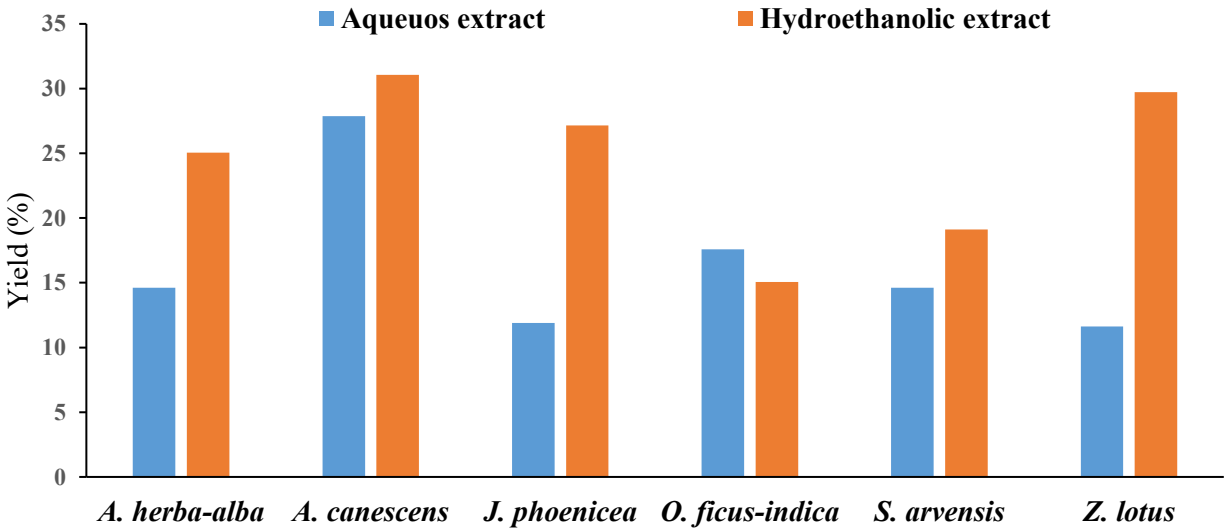

Figure 17. Extraction yield of maceration

11.621% to 27.872%, with *A. canescens* showing the highest efficiency (27.872%), followed by *O. ficus-indica* (17.59%). The hydroethanolic extraction generally resulted in higher yields, ranging from 15.07% to 31.067%, with *A. canescens* again providing the highest yield (31.067%), followed by *Z. lotus* (29.73%) and *J. phoenicea* (27.143%). Notably, for *O. ficus-indica*, the aqueous extraction (17.59%) proved more efficient than the hydroethanolic method (15.07%).

The qualitative phytochemical screening of the hydroalcoholic and aqueous extracts of the six studied plants revealed the presence of key secondary metabolites, including alkaloids, phenolics (tannins, flavonoids, quinones, coumarins), sugars, steroids, and saponins. The distribution and intensity of these metabolites varied depending on the plant species and extraction method, as summarized in Table 3.

Hydroalcoholic extracts were more effective in extracting alkaloids, triterpenoids, and glucosides, whereas aqueous extracts exhibited a higher concentration of tannins, reducing sugars, and mucilages. Among the tested plants, *J. phoenicea* and *Z. lotus* displayed the richest phenolic profiles, with high levels of tannins and flavonoids, while *A. canescens* and *A. herba-alba* were notable for their coumarin and saponin content. *O. ficus-indica* exhibited a moderate presence of alkaloids and flavonoids, suggesting a balanced phytochemical composition. Some metabolites, such as anthocyanins, were only detected in the aqueous extracts of *A. herba-alba* and *A. canescens*, while phlobatannins were completely absent in all tested samples. Additionally, saponins were not detected in the hydroalcoholic extracts of *O. ficus-indica* and *S. arvensis*, highlighting variations in solubility and extraction efficiency.

Table 3: Phytochemical screening result of the six species

Metabolites/Plants		*A. herba-alba*		*A. canescens*		*J. phoenicea*		*O. ficus-indica*		*S. arvensis*		*Z. lotus*	
		HA	**AQ**	**HA**	**AQ**	**HA**	**AQ**	**HA**	**AQ**	**HA**	**AQ**	**HA**	**AQ**
Alkaloids		++	++	+	+	++	++	++	++	++	+	+	++
Phenolics	Tannins	+	+++	++	++	+++	+++	++	++	+	++	++	+
	Quinones	++	-	++	+	-	++	++	+	-	+	++	+
	Flavonoids	+	++	+	+	+	++	+	+	+	+	+++	+
	Anthocyanins	-	+	+	-	-	-	-	-	-	-	-	-
	Phlorotannin	-	-	-	-	-	-	-	-	-	-	-	-
	Coumarins	+	++	+++	++	+	++	++	-	+	-	++	+
Sugars	Reducing sugar	++	+++	+++	+	+	+++	++	+	+	+	++	+
	Mucilage	-	+	-	+	-	++	-	+	-	+	-	+
	Glucosides	+++	+++	+++	+++	++	++	+++	+++	++	++	++	++
Steroids	Triterpenoids	++	+	+	+	+++	-	+	+	++	-	+	+
	Saponins	++	++	+	++	-	+	+	-	-	++	+++	+++

HA: Hydroacoholic (70% ethanol) solvent; AQ: Aqueous solvent.

2.2 Discussion

The phytochemical screening results demonstrate substantial diversity in bioactive compound composition across the six investigated plant species, with extraction solvent polarity significantly influencing metabolite recovery efficiency. The differential detection patterns between HA and AQ extraction methodologies reflect compound-specific solubility characteristics (Iloki-Assanga et al., 2015). HA solvents demonstrated superior extraction efficiency for quinones and triterpenoid, whereas AQ extraction yielded enhanced recovery of saponins and mucilaginous substances (Pote et al., 2024).

The presence of alkaloids, phenolics (tannins, flavonoids, quinones, coumarins), sugars, steroids, and saponins suggests that these plants offer multiple functional benefits, particularly in improving animal productivity and digestive efficiency in semi-arid environments. The bioactivity of these phytochemicals plays a crucial role in ruminant nutrition and performance (Tedeschi et al., 2021). Saponins, which were abundant in *Z. lotus, A. herba-alba,* and *S. arvensis*, have been shown to reduce rumen protozoa populations, thereby enhancing bacterial activity and nitrogen utilization, leading to better animal growth, increased milk production, and improved feed efficiency (Maxime et al., 2005; Maxime and Benarbia, 2022). Alkaloids, present in *J. phoenicea, A. herba-alba*, and *O. ficus-indica*, have been reported to modulate rumen fermentation, reduce methane emissions, and enhance protein degradation, which supports sustainable livestock production (Mickdam et al., 2016). Flavonoids, richly present in *Z. lotus*, offer several advantages, including reducing the risk

of acidosis, lowering the acetate-to-propionate ratio, and enhancing the digestibility of organic matter, which ultimately leads to improved milk production and overall metabolic efficiency (Balcells et al., 2012; Ma et al., 2017; Olagaray and Bradford, 2019). Steroids and triterpenoids, particularly abundant in *J. phoenicea*, have growth-promoting effects, contributing to higher milk yields, better rumen fermentation, and enhanced microbial biomass, while also reducing ammonia and lactate accumulation in the rumen (Xi et al., 2014; Patel and Savjani, 2015). Additionally, reducing sugars and glucosides, highly present in *A. herba-alba, A. canescens*, and *J. phoenicea*, improve nutrient utilization by increasing energy availability, thereby supporting metabolic efficiency and enhancing feed conversion rates (Cosgrove et al., 2009).

The observed phytochemical diversity substantiates the dual functionality of these plant species as both medicinal resources and nutritional fodder. While their bioactive constituents provide significant health-promoting and nutritional benefits for livestock, certain compounds, particularly tannins and saponins, may exhibit anti-nutritional effects at elevated concentrations. Therefore, quantitative metabolite profiling is essential to determine actual concentration parameters and assess suitability for forage applications in sustainable livestock management systems.

3 Quantitative phytochemical composition

3.1 Results

The comprehensive quantitative phytochemical analysis of six medicinal plant species revealed significant interspecific variations and solvent-dependent extraction efficiencies for total phenolic content (TPC), total flavonoid content (TFC), total terpenoid content (TT), and condensed tannins (CT). Differential metabolite concentrations were observed between hydroalcoholic (HA) and aqueous (AQ) extraction methodologies, as systematically presented in Table 4.

J. phoenicea demonstrated superior phytochemical accumulation, exhibiting maximal TPC (169.31 ± 3.88 mg GAE/g DW) and TT (7.84 ± 0.18 mg LE/g DW) concentrations in HA extracts, coupled with elevated TFC and CT levels. *Z. lotus* similarly displayed robust phytochemical profiles, particularly within HA extracts, registering maximal TFC (24.18 ± 0.22 mg QE/g DW) among all investigated taxa. Notably, its AQ extracts exhibited substantially reduced TPC, TFC, and TT concentrations, underscoring the critical importance of extraction solvent selection in phytochemical investigations.

O. ficus-indica presented an anomalous solvent-dependency pattern, with AQ extracts yielding significantly enhanced TPC (190.12 mg GAE/g DW) and TFC (14.71 mg QE/g DW) compared to HA extracts. This solvent-specific variation suggests unique phytochemical solubility characteristics and structural properties within this taxon. *Artemisia herba-alba* and *A. canescens* exhibited intermediate phytochemical accumulation, with HA extracts generally demonstrating superior TPC and TFC concentrations relative to AQ extracts. However, *A. herba-alba* displayed elevated CT concentrations in AQ extracts, while *A. canescens* consistently exhibited reduced metabolite concentrations across all quantified compound classes. *Sinapis arvensis* demonstrated minimal phytochemical accumulation, with comparatively reduced concentrations in both extraction methodologies, although slightly elevated TPC and TFC levels were detected in HA extracts.

In summary, HA extraction protocols generally facilitated enhanced recovery of TPC, TFC, and TT, with the notable exception of *O. ficus-indica*, which exhibited superior metabolite extraction in AQ solvents. These findings illuminate the critical influence of extraction parameters on phytochemical recovery efficiency and highlight species-specific variations in secondary metabolite profiles.

Table 4: Quantitative phytochemical result of the six species

Plants	CT (mg CE/g DW)		TT (mg LE/g DW)		TFC (mg QE/g DW)		TPC (mg GAE/g DW)	
	HA	AQ	HA	AQ	HA	AQ	HA	AQ
A. herba-alba	12,67 ± 2,03 cd	31,9 ± 0,50 a	2,59 ± 0,51 c	3,48 ± 0,11 b	13,4 ± 0,32 c	16,79 ± 0,18 a	127,03 ± 1,76d	100,07 ± 0.92 b
A. canescens	14,93 ± 0,21 cd	22,47 ± 2,64 b	3,08 ± 0,07 c	1,37 ± 0,08 d	10,08 ± 0,18 d	12,05 ± 0,85 c	26,33 ± 0,71 f	19,01 ± 3.87 f
J. phoenicea	27,14 ± 3,71 a	22,29 ± 2,28 b	7,84 ± 0,18 a	9,73 ± 0,22 a	15,63 ± 0,22 b	10,71 ± 1,41 d	169,31 ± 3,88 a	70,47 ± 2.49 c
O. ficus-indica	9,57 ± 0,29 d	29,28 ± 0,14 a	1,42 ± 0,09 d	2,83 ± 0,08 c	10,21 ± 0,22 d	14,71 ± 0.09 b	135,1 ± 1,76 c	190,12 ± 0.10 a
S. arvensis	9,86 ± 1,45 d	29,14 ± 0,89 a	1,51 ± 0,45 d	1,15 ± 0,04 e	10,15 ± 0,34 d	15,02 ± 0,31 b	60,13 ± 2,46 e	42,87 ± 0.97 d
Z. lotus	23,86 ± 0,29 b	11,14 ± 0,49 c	5,85 ± 0,09 b	1,53 ± 0,22 d	24,18 ± 0,22 a	3,81 ± 0,03 e	142,76 ± 1,27 b	33,87 ± 0.96 e

AQ: Aqueous extract; HA: hydroalcoholic extract; TPC: total phenolic content; TFC: total flavonoid content; TT: total terpenoid content; CT: condensed tannins

Note: Different letters (a, b, c, etc.) within each column indicate statistically significant differences between groups as determined by Duncan's Multiple Range Test ($P < 0.05$). Groups sharing the same letter are not significantly different from each other.

3.2 Discussion

The substantial secondary metabolites content of these plants highlights their potential to positively influence livestock performance and the quality of derived products (Cabiddu et al., 2019). Phenolic compounds have been shown to significantly impact rumen fermentation, milk yield and composition, while also exerting anti-inflammatory effects (Vasta et al., 2019). In nitrogen-deficient diets, phenolic compounds modulate the ruminal protozoan populations, enhancing nitrogen metabolism and improving nitrogen utilization efficiency in ruminants (De Paula et al., 2016). Furthermore, dietary phenolic supplementation increases total volatile fatty acid (VFA) concentrations and modifies acetate molar proportions during ruminal fermentation, consequently enhancing digestive efficiency parameters (Huang et al., 2023). Moreover, research by Cools et al. (2014) demonstrated that bovine subjects receiving phenolic-enriched diets exhibited elevated phenolic compound concentrations in both serum and milk matrices, suggesting potential quality enhancement in animal-derived product. Moreover, phenolic compounds provide environmental sustainability benefits by reducing methane (CH_4) emissions through improved nitrogen utilization, shifting nitrogen excretion from urine to feces and reducing nitrogen pollution risks (Landau et al., 2023).

Flavonoid compounds, beyond their established antioxidant properties associated with enhanced milk production parameters (Garavaglia et al., 2015; Orzuna-Orzuna et al., 2023) and improved liver health through lipid metabolism modulation (Liu et al., 2011), represent a viable non-antibiotic alternative for calves, particularly for subjects receiving medicated milk replacer formulations. . Flavonoid supplementation has demonstrated enhancement of intestinal glucose absorption mechanisms, lactase enzymatic activity, and endogenous glucose production in colostrum-deprived bovine neonates (Gruse et al., 2015; Kehoe and Carlson, 2015). They also contribute to rumen health optimization by reducing the intensity and duration of diarrheal episodes (Teixeira et al., 2015), selectively inhibiting Gram-positive bacterial proliferation, consequently decreasing methane production and improving energy digestibility (Cushnie and Lamb, 2005).

Terpenoid constituents are recognized for their organoleptic property enhancement, including flavor profile and pigmentation characteristics, potentially stimulating feed consumption in livestock species (Tedeschi et al., 2021). In addition to their sensory benefits, terpenoids offer numerous nutritional benefits, including the prevention of digestive disorders when used as feed additives (Tedeschi et al., 2011). Their anti-inflammatory

properties are also significant, as they have been shown to inhibit colon carcinogenesis and colitis (Kawabata et al., 2012). Moreover, certain terpenoids serve as precursors to provitamin A, an essential nutrient for vision, immune function, and overall health (Kotake-Nara and Nagao, 2011).

The six species examined in this study fall within the range of low to moderate condensed tannin content, which is not considered an antinutritional factor (Patra and Saxena, 2011). Condensed tannins have been associated with increased milk yield and improved milk compositional characteristics, including protein, lactose, and lipid content (Soltan, 2009; Dey and De, 2014; Anantasook et al., 2015). Additionally, condensed tannins have been shown to reduce blood and milk urea nitrogen concentrations, contributing positively to animal health (Huang et al., 2018).

4 Antioxidant Activity

4.1 Results

The antioxidant capacity of the six investigated medicinal plant species was assessed utilizing complementary methodological approaches: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Significant interspecific variations and solvent-dependent differentials were observed between hydroalcoholic (HA) and aqueous (AQ) extraction protocols, as comprehensively delineated in Table 5.

Remarkably *J. phoenicea* exhibited the strongest antioxidant activity, with the lowest IC_{50} values in the DPPH assay (7.11 ± 1.5 µg/mL for HA; 15.84 µg/mL for AQ) and the highest ferric-reducing power in the FRAP assay (812.71 mM TE/g for AQ; 601.86 ± 6.06 mM TE/g for HA). However, *Z. lotus* also demonstrated high antioxidant capacity, particularly in the HA extract (IC_{50} = 13.65 ± 3.16 µg/mL; FRAP = 577.36 ± 24.83 mM TE/g). However, its AQ extract showed significantly lower activity (IC_{50} = 254.07 µg/mL; FRAP = 202.93 mM TE/g). *A. herba-alba* displayed moderate antioxidant activity, with better performance in the HA extract (IC_{50} = 31.31 ± 4.26 µg/mL; FRAP = 578.53 ± 21.29 mM TE/g) compared to the AQ extract (IC_{50} = 96.52 µg/mL; FRAP = 442.93 mM TE/g).

A. canescens showed lower antioxidant capacity overall, with higher activity in the HA extract (IC_{50} = 160.97 ± 11.44 µg/mL; FRAP = 237.61 ± 13.32 mM TE/g) than in the AQ extract (IC_{50} = 637.18 µg/mL; FRAP = 212.15 mM TE/g). In contrast, *O. ficus-indica* exhibited an interesting exception to the general trend, with superior antioxidant

activity in the aqueous extract (IC_{50} = 109.94 µg/mL; FRAP = 560.93 mM TE/g) compared to the HA extract (IC_{50} = 212.64 ± 15.87 µg/mL; FRAP = 296.78 ± 35.49 mM TE/g). This unique solvent-dependent behavior suggests distinct phytochemical properties. *Sinapis arvensis* displayed relatively weak antioxidant activity compared to other species, with IC_{50} values of 199 ± 12.21 µg/mL (HA) and 227.06 µg/mL (AQ) in the DPPH assay and moderate FRAP values (HA: 251.03 ± 24.62 mM TE/g; AQ: 301.48 mM TE/g).

In summary, hydroalcoholic extracts generally exhibited stronger antioxidant activity, except for *O. ficus-indica*, which performed better in the aqueous extract.

Table 5: Antioxidant activity results

Plants	DPPH, IC50 (µg/mL)		FRAP (mM TE/g extract)	
	HA	AQ	HA	AQ
A. herba-alba	31,31 ± 4,26 c	96,52 ± 23,58c	578,53 ± 21,29 a	442,93 ± 41,76 c
A. canescens	160,97 ± 11,44 b	637,18 ± 152,98 a	237,61 ± 13,32 c	212,15 ± 75,97 e
J. phoenicea	7,11 ± 1,5 d	15,84 ± 3,07 c	601,86 ± 6,06 a	812,71 ± 37,31 a
O. ficus-indica	212,64 ± 15,87 a	109,94 ±9,82 c	296,78 ± 35,49 b	560,93 ± 94,60 b
S. arvensis	199 ± 12,21 a	227,06 ± 31,86 b	251,03 ± 24,62 c	301,48 ± 4,29 d
Z. lotus	13,65 ± 3,16 d	254,07 ± 30,06 b	577,36 ± 24,83 a	202,93 ± 4,70 f

AQ: Aqueous extract; HA: hydroalcoholic extract.

Note: Different letters (a, b, c, etc.) within each row/column indicate statistically significant differences between groups as determined by Duncan's Multiple Range Test ($P < 0.05$). Groups sharing the same letter are not significantly different from each other.

4.2 Discussion

4.2.1 Antioxidant mechanisms and physiological implications

The medicinal plant species analyzed in this investigation exhibit significant antioxidant potential, as evidenced by their electron-donating capacity in FRAP assay protocols and hydrogen atom transfer capabilities in DPPH radical neutralization assays. This dual antioxidant mechanism highlights their potential as natural agents for mitigating oxidative stress in livestock, a critical issue in semi-arid environments where animals are frequently exposed to high temperatures, nutritional insufficiencies, and metabolic stressors.

The strong antioxidant capacity of these plants can be attributed to their rich phytochemical composition (Rammohan et al., 2023).

Phenolic compounds, abundant in these plants, play a central role in counteracting oxidative stress. They act as hydrogen and electron donors, directly neutralizing reactive oxygen species (ROS) and preventing lipid peroxidation in biological tissues (Agati et al., 2012). Additionally, phenolics stimulate endogenous antioxidant defenses by enhancing enzymatic activity of critical antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Masella et al., 2005). This dual action, direct ROS scavenging and indirect enzyme activation, reinforces the animal's natural defense system, improving cellular viability and resilience under oxidative stress. Flavonoids, partticularly quercetin and kaempferol, further amplify these benefits by modulating the expression of antioxidant enzymes (Jeon et al., 2003) and activating Nrf2-mediated pathways (Daddam et al., 2023), which protect against heat-induced metabolic imbalances and maintain homeostasis during environmental stress (Ramírez-Mares and González de Mejía, 2003). These mechanisms collectively enhance immune function, reduce oxidative damage to milk and meat, and support reproductive health, thereby improving overall livestock productivity and product quality.

The integration of these antioxidant-rich medicinal plant species into livestock nutritional regimens represents a biologically rational and sustainable approach to oxidative stress mitigation in semi-arid production contexts. This strategic phytotherapeutic application not only enhances animal health parameters and welfare status but also potentially improves nutritional composition and organoleptic properties of animal-derived products, including dairy and meat commodities.

5 Proximate and mineral composition

5.1 Results

Comprehensive proximate composition analysis revealed significant interspecific variation among the six selected nutri-medicinal plant species, providing essential data regarding their nutritional constituents (Figure 18). Crude fiber content varied significantly among species, ranging from 31.00% in *J. phoenicea* to 42.40% in *S. arvensis*, with most species exhibiting moderate to high fiber levels. The highest crude lipid content was recorded in *J. phoenicea* (11.70%), while *S. arvensis* had the lowest (2.80%). Crude protein levels also differed considerably, with *O. ficus-indica* displaying the highest protein content

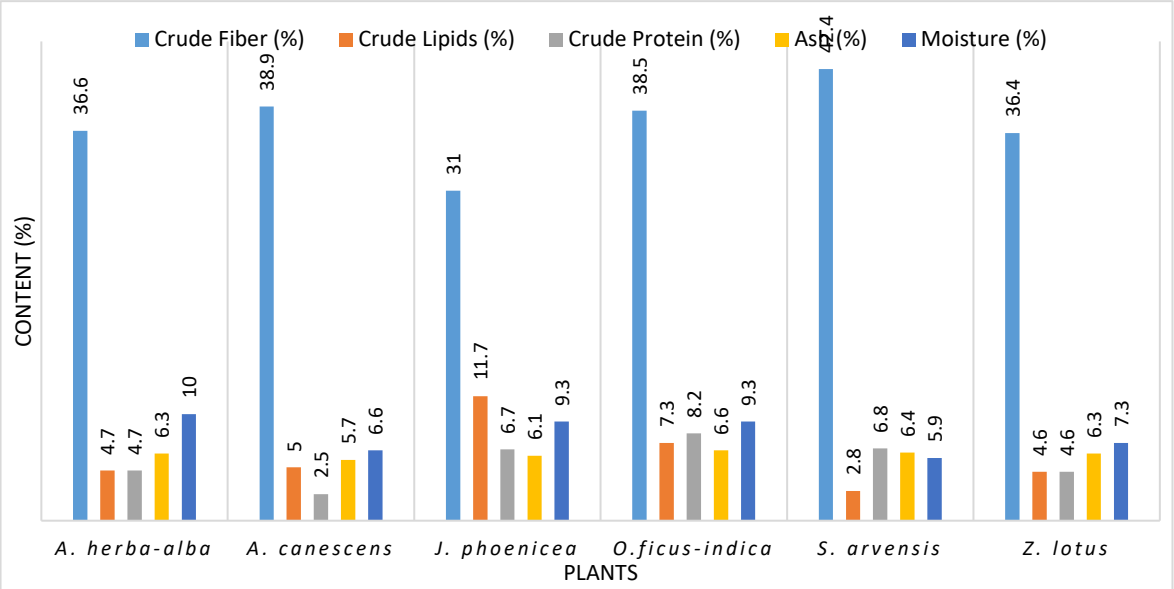

Figure 18: Proximate composition of plants per dry matter

(8.20%), followed by *S. arvensis* (6.80%) and *J. phoenicea* (6.70%), whereas *A. canescens* exhibited the lowest value (2.50%). Moisture content ranged from 5.90% in *S. arvensis* to 10.00% in *A. herba-alba*. Ash content, an indicator of total mineral composition, showed slight variation, with *O. ficus-indica* having the highest value (6.60%) and *A. canescens* the lowest (5.70%).

The mineral composition analysis (Figure 19) focused on essential macrominerals such as phosphorus (P) and potassium (K), as well as trace elements like copper (Cu), which are vital for animal health and metabolic functions. *A. canescens* demonstrated the highest potassium concentration (47.376 mg/g). In contrast, *O. ficus-indica* exhibited the lowest phosphorus content (0.096 mg/g), while *S. arvensis* recorded the highest phosphorus

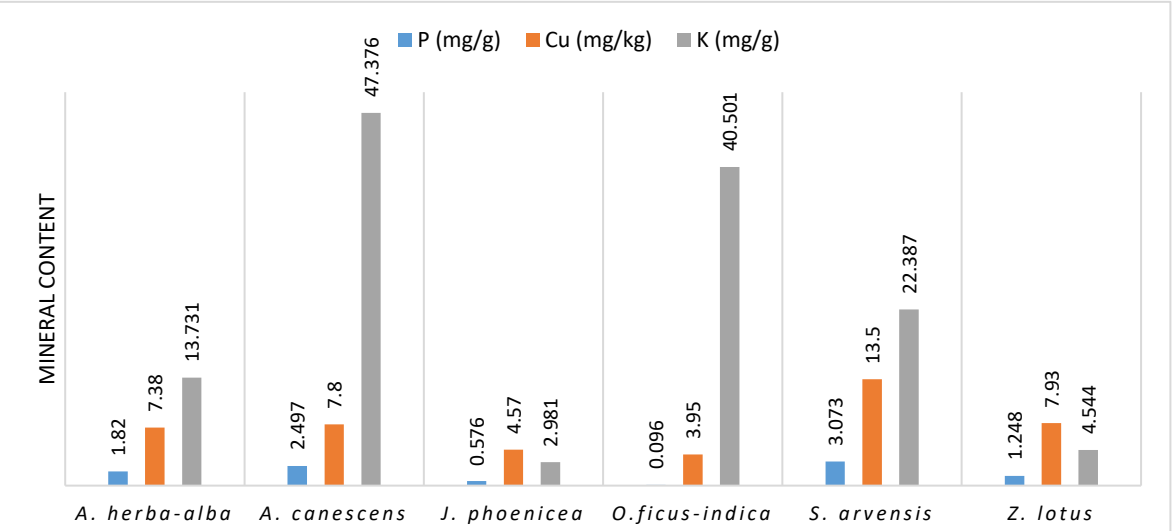

Figure 19: Mineral content per dry matter

concentration (3.073 mg/g). Additionally, *S. arvensis* showed the highest copper content (0.0135 mg/g), an important trace element for enzymatic reactions and immune function in livestock.

5.2 Discussion

5.2.1 Nutritional significance and comparative analysis of proximate composition

The results revealed considerable richness and variability among the studied plants, which grants them significant potential as forage resources in livestock nutrition. Their diverse nutrient profiles and bioactive compounds contribute to improved digestion, metabolism, and overall animal health.

The elevated crude fiber concentrations identified across all investigated species, particularly in *S. arvensis* (42.40%), contribute significantly to ruminant gastrointestinal health by promoting optimal ruminal function and microbial fermentation. These values exceed those reported by Bashir et al. (2021) and Aberoumand (2011), yet remain below the concentrations documented by Ahmed et al. (2013), suggesting that variations in developmental stage, environmental parameters, and genotypic factors may influence fiber biosynthesis and accumulation. The substantial fiber fractions identified in this investigation align with Li et al. (2014) and Montserrat-Malagarriga et al. (2024), who documented enhanced digestive efficiency and physiological well-being in ruminants maintained on fiber-enriched diets.

Lipid analysis revealed exceptional concentrations in *J. phoenicea* (11.70%), positioning this species as a valuable energy source in ruminant nutrition. The observed lipid values correspond with those reported by Aberoumand (2011) while exceeding the concentrations documented by El-Amier et al. (2022). However, for *A. herba-alba* the values remain below those reported by Houmani et al. (2004). As elucidated by Ponnampalam et al. (2024), dietary lipids constitute a concentrated energy source while simultaneously providing essential fatty acids that enhance the nutritional quality of animal-derived products through modified lipid profiles in meat and dairy constituents.

The crude protein concentrations documented in this investigation demonstrate similarity with values reported by Aberoumand (2011) and Louhaichi et al. (2021), while exhibiting lower concentrations than those documented by Ahmed et al. (2013). Notably, *O. ficus-indica* exhibited superior protein content (8.20%) compared to previously reported values by Moula et al. (2019) in Algeria. While the protein content of these species remains

below that of leguminous forages, the values are comparable to gramineous species (Castro-Montoya et al., 2023) and meet the minimum nutritional requirements for ruminant maintenance (7-9%) as established by El-Shatnawi et al. (2000). This finding substantiates their potential application as viable nutritional resources, particularly in environments characterized by climatic variability and restricted forage availability.

Moisture content plays a critical role in determining forage preservation and storage stability, with lower moisture levels being preferable for prolonged shelf life (Offia-Olua, 2014). The values obtained in this study are consistent with those reported by Qayyum et al. (2012) and Al-Rowaily et al. (2019), as well as those observed by El-Amier et al. (2022) in other wild species adapted to arid environments. However, these moisture levels were lower than those reported by Aberoumand (2011).

The nutritional profiles of these plants are not only comparable to but, in some cases, superior to commonly used wild forage resources such as *Arthrocnemum macrostachyum* and *Tamarix nilotica* (El-Amier et al., 2022). Additionally, they exhibit higher nutrient density than supplementary plants like *Emblica officinalis*, *Terminalia bellirica*, and *Terminalia chebula* (Bostami et al., 2021). Beyond their nutritional value, these plants hold significant medicinal importance in livestock management. Their condensed tannin (CT) content remains below the threshold of 72 g/kg DM for antinutritional effects in ruminants (Rossi et al., 2023), indicating that these compounds may positively influence protein utilization and nutrient absorption.

The mineral profiles of the investigated species demonstrate significant variability, with potential implications for nutritional supplementation strategies in livestock management systems. The documented mineral concentrations align with established requirements for ruminant nutrition as outlined by the Agricultural Research Council (ARC, 1980) and the National Research Council (NRC, 2006), particularly regarding the nutritional demands of gestating and lactating bovines. These findings support those reported by Vejnovic et al. (2018), highlighting the potential application of these species as natural mineral supplements in livestock nutrition.

5.2.2 Ecological resilience and environmental adaptability

It is essential to emphasize that the study was conducted in Bordj Bou Arreridj, a region that experienced a severe drought in 2022, with total rainfall amounting to only 267.79 mm—substantially below the annual average of 321.9 mm (Meteorological Station

BBA, 2024). Despite these extreme conditions, the studied nutri-medicinal plants exhibited notable resilience and gave these significant finding. This suggests that, beyond their nutritional and medicinal benefits, these plants contribute to agricultural resilience in drought-prone areas. Their integration into livestock diets offers a sustainable means of supporting rural livelihoods while providing adaptive solutions to climate-induced forage scarcity in both developing and developed regions.

Incorporating these plants into livestock diets presents a balanced nutritional approach by offering varied protein, energy, and bioactive compound levels, including flavonoids and polyphenols. These compounds collectively enhance livestock productivity by improving digestion, immune function, and metabolic efficiency. Furthermore, their long history of use in ethnoveterinary medicine for promoting livestock health (Miara et al., 2019) underscores their dual functionality as both nutritional and therapeutic agents. This reinforces the importance of further exploring and integrating these plants into modern sustainable livestock systems.

6 FT-IR analysis

6.1 Results

Fourier Transform Infrared (FT-IR) spectroscopic analysis was conducted to elucidate the functional group characteristics and phytochemical profile of six selected nutri-medicinal species. Spectral data acquisition was performed in the mid-infrared region (4000-500 cm^{-1}) utilizing KBr pellet methodology. Three preparative forms were comparatively analyzed: powdered plant material, aqueous extracts, and ethanolic extracts (Figure 20).

The spectroscopic investigation revealed differential absorption patterns across sample types, with powdered samples exhibiting pronounced spectral complexity characterized by multiple merged absorption bands, whereas extract preparations demonstrated more distinct and separated peaks attributable to selective phytochemical extraction. Comprehensive spectral interpretations and functional group assignments are summarized in Table 6.

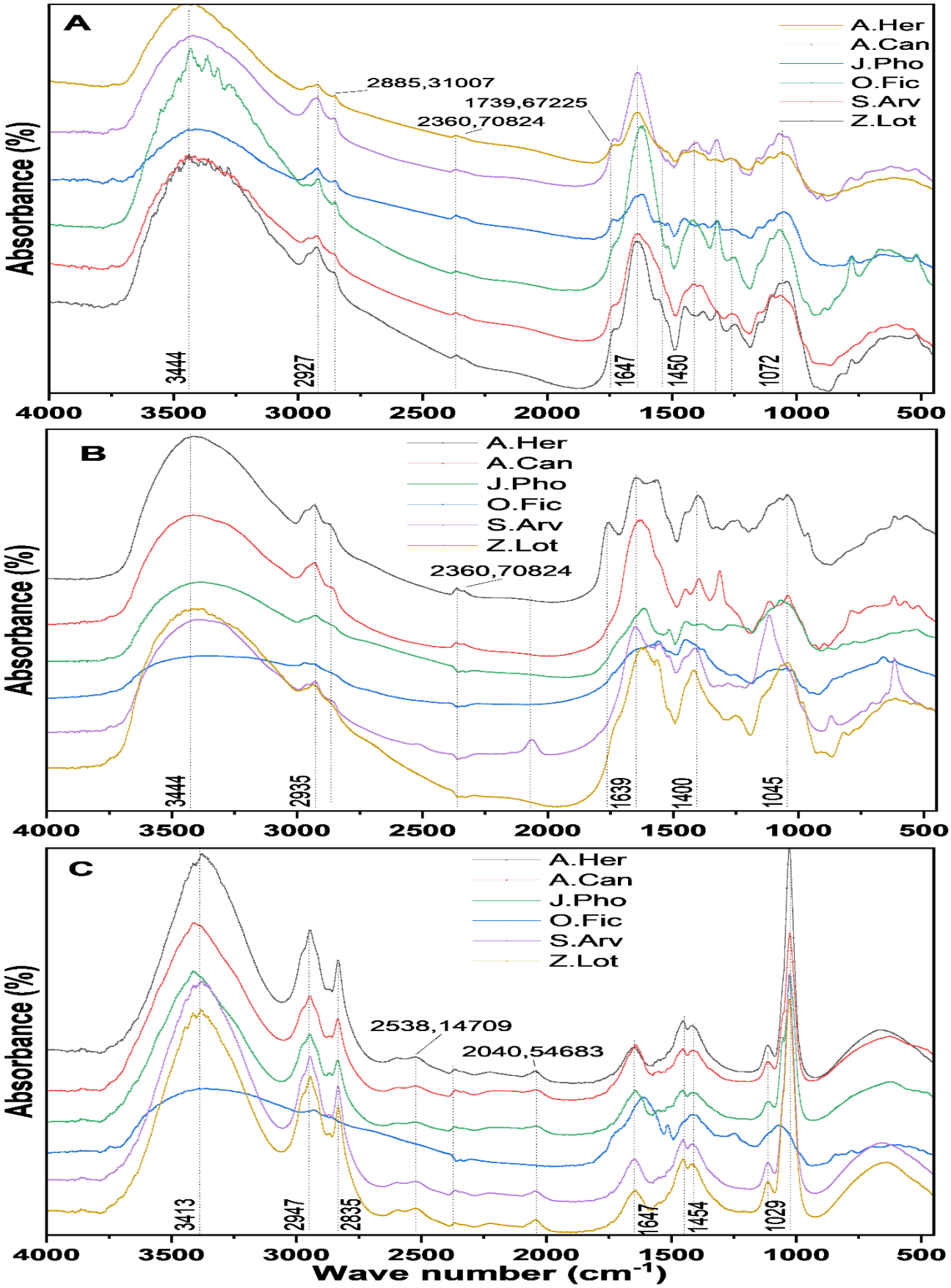

Figure 20: FT-IR spectre results of the six species: (A) Powdered sample; (B) Aqueous extract; (C) Hydroalcoholic extract.

Table 6: FT-IR absorption bands and functional groups

Functional Group	Wave Number Range (cm^{-1})	Powdered Samples (cm^{-1})	Aqueous Extracts (cm^{-1})	Hydroethanolic Extracts (cm^{-1})
O-H (Hydroxyl, Phenols)	3200–3600	3444	3444	3413
C-H (Aliphatic Compounds)	2800–3000	2927	2935	2947
	2500–3200	2885	-	2835
Atmospheric CO_2		2360	2067	2040
C=O (Carbonyl, Amides, Carboxylic Acids, Esters, Ketones)	1600–1750	1739	1647	1644
C=C (Aromatic Rings, Alkenes)	1450–1686	1647	1450	1647
O-N (Nitriles)	1475–1550	1519	-	-
C-N (Amides, Amines)	1335–1250 1250–1020	1319,1253, 1149	1250,1118	1129
C-O (Carbohydrates, Glycosides, Alcohols, Esters, Ethers)	1000–1200 1050–1450	1072,1450	1045,1400,1450	1029,1454, 1415

Spectroscopic data revealed pronounced variations in functional group distribution patterns among the different sample preparations. The hydroxyl (O-H) stretching vibration band (3200–3600 cm^{-1}) demonstrated universal presence across all sample types, although with differential intensity profiles. Notably, powdered *O. ficus-indica* exhibited maximal peak intensity in this spectral region, while *J. phoenicea* demonstrated minimal absorption. Conversely, in extract formulations, *O. ficus-indica* presented diminished hydroxyl band intensity relative to other species.

Aliphatic hydrocarbon (C-H) stretching vibrations (2800–3000 cm^{-1}) were consistently detected across all samples, with ethanolic extracts demonstrating superior band intensity. Carbonyl (C=O) stretching absorption (1600–1750 cm^{-1}) exhibited maximal intensity in powdered preparations. Aromatic carbon-carbon double bond (C=C) stretching vibrations (1450–1686 cm^{-1}) appeared across all samples but displayed significant intensity variations across sample types.

Carbon-oxygen (C-O) stretching vibrations (1000–1200 cm^{-1}, 1050–1450 cm^{-1}) were detected in all spectral profiles, with ethanolic extracts demonstrating pronounced

absorption intensity, particularly in *A. herba-alba* preparations. These spectral variations reflect differential solubility characteristics of phytochemical constituents in extraction solvents of varying polarity.

6.2 Discussion

6.2.1 Functional group distribution

The FT-IR spectroscopic analysis of powdered samples, aqueous extracts, and hydroethanolic extracts from the six investigated plant species provided valuable insights into phytochemical composition and solvent-dependent extraction efficiency. The powdered sample preparations demonstrated superior spectral complexity characterized by numerous merged absorption bands, indicative of a heterogeneous phytochemical profile representative of intact plant material. In contrast, both aqueous and hydroethanolic extracts exhibited reduced spectral band complexity, suggesting selective phytoconstituent isolation governed by solvent polarity parameters. These observations align with previous investigations by Iloki-Assanga et al. (2015), which demonstrated that extraction solvent polarity significantly influences phytochemical yield and compositional profiles, with no single solvent capable of extracting the complete spectrum of bioactive constituents present in plant matrices.

The prominent hydroxyl (O-H) stretching vibration observed in the 3200–3600 cm^{-1} region across all sample types confirms the presence of phenolic compounds and hydroxylated metabolites (Edison and Sethuraman, 2013), which typically contribute to antioxidant capacity. The more pronounced hydroxyl absorption bands in powdered samples compared to extract preparations suggests that the intact plant matrix contains a more comprehensive profile of hydroxyl-bearing compounds, some of which may demonstrate limited solubility in the employed extraction solvents. Similarly, aliphatic hydrocarbon (C-H) stretching vibrations in the 2800–3000 cm^{-1} region were consistently detected across all sample preparations, with hydroethanolic extracts exhibiting superior band intensity. This observation indicates that 70% ethanol demonstrates enhanced efficiency in extracting terpenoids, steroids, and fatty acid constituents compared to aqueous solvent systems.

The pronounced carbonyl (C=O) stretching absorption observed in the 1600–1750 cm^{-1} region of powdered sample spectra indicates abundant presence of carbonyl-containing compounds including unsaturated esters, ketones, aldehydes, and carboxylic acids within the crude plant matrix (Shameem Rani et al., 2021). The attenuated intensity of this absorption

band in both aqueous and hydroethanolic extract preparations suggests selective loss or reduced solubility of specific carbonyl-bearing constituents during the extraction process (Baümler et al., 2016).

The nitrile (O-N) stretching vibration (1475–1550 cm^{-1}), detected exclusively in powdered samples at approximately 1519 cm^{-1}, is attributable to nitro-group-containing secondary metabolites (Chandran, 2014). The absence of this absorption band in both aqueous and hydroethanolic extract preparations suggests that nitrogenous constituents demonstrate limited solubility in these extraction media or are present at concentrations below the detection threshold in the extracted fractions.

Aromatic carbon-carbon double bond (C=C) stretching vibrations (1450–1686 cm^{-1}) were detected across all sample preparations, indicative of flavonoids, polyphenols, and lignin-derived compounds (Bulut and Özacar, 2009). The enhanced intensity of these absorption bands in hydroethanolic extracts corroborates the superior efficiency of 70% ethanol in extracting aromatic phytochemicals (Thummajitsakul and Silprasit, 2022), suggesting its suitability for subsequent LC-MS characterization of phenolic constituents.

Carbon-nitrogen (C-N) stretching vibrations (1335–1250 cm^{-1} and 1250–1020 cm^{-1}) were detected in powdered samples (1319, 1253, and 1149 cm^{-1}), aqueous extracts (1250 and 1118 cm^{-1}), and hydroethanolic extracts (1129 cm^{-1}), indicating the presence of aliphatic amine functionalities (Shameem Rani et al., 2021). These spectral features are typically associated with alkaloids, proteins, and peptides, suggesting that nitrogenous compounds contribute significantly to the phytochemical composition of the investigated species.

Carbon-oxygen (C-O) stretching vibrations (1000–1200 cm^{-1} and 1050–1450 cm^{-1}), associated with glycosides, esters, flavonoid glycosides, and terpenoids (Jain et al., 2016), demonstrated maximal intensity in hydroethanolic extracts, particularly in *A. herba-alba* preparations. This observation suggests enhanced concentration of these metabolite classes in *A. herba-alba* (Mohamed et al., 2010) and superior extraction efficiency of 70% ethanol for such constituents.

The diminished absorption intensity observed in *O. ficus-indica* extract preparations is attributable to its high mucilage content, which forms viscous hydrocolloids that impede solvent penetration and diffusion through the plant matrix, thereby reducing extraction efficiency (Elshewy et al., 2023). This physical barrier effect decreases the solubilization

and subsequent extraction of phytochemical constituents, resulting in attenuated absorption band intensity in the corresponding FT-IR spectra.

These spectral variations demonstrate the critical influence of solvent polarity in determining phytochemical extraction profiles. While powdered samples represent the complete phytochemical complexity of the plant material, aqueous extracts predominantly contain hydrophilic constituents such as polysaccharides and glycosides, whereas hydroethanolic extracts are enriched in moderately polar and aromatic compounds (Yusof and Mahmood, 2021). This differential extraction behavior underscores the importance of solvent selection in phytochemical investigations and bioactivity studies.

7 LC-MS/MS analysis

7.1 Results

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was conducted on ethyl acetate fractions derived from the six investigated nutri-medicinal to characterize their phenolic constituents on a combination of chromatographic retention time (RT), mass-to-charge ratio (m/z) obtained in positive (+) or negative (-) electrospray ionization (ESI) modes, and comparison with reference standards or fragmentation patterns.

The analysis led to the tentative identification of 28 distinct compounds across the six species (Table 7). These included nine flavonoids (e.g., quercetin, catechin hydrate, kaempferol), twelve phenolic acids (e.g., ferulic acid, caffeic acid, chlorogenic acid), one carotenoid (beta-carotene), one monoterpene phenol (thymol), one coumarin derivative (4-hydroxycoumarin), one vitamin (folic acid), and kojic acid. Chromatographic analysis revealed distinct interspecific variations in phenolic constituent profiles (Figure 21).

A. herba-alba exhibited a profile dominated by ferulic acid (RT 9.26 min; peak area: 60,437,913), with substantial relative abundances of myricetin (RT 16.94 min; peak area: 26,553,915) and kaempferol (RT 12.29 min; peak area: 8,168,951).

A. canescens exhibited significant accumulation of kaempferol (RT 12.291 min; 11,627,939) and myricetin (RT 16.941 min; 11,817,787), with additional contributions from caffeic acid (RT 8.168 min; 8,962,709) and chlorogenic acid (RT 15.5 min; 2,614,502).

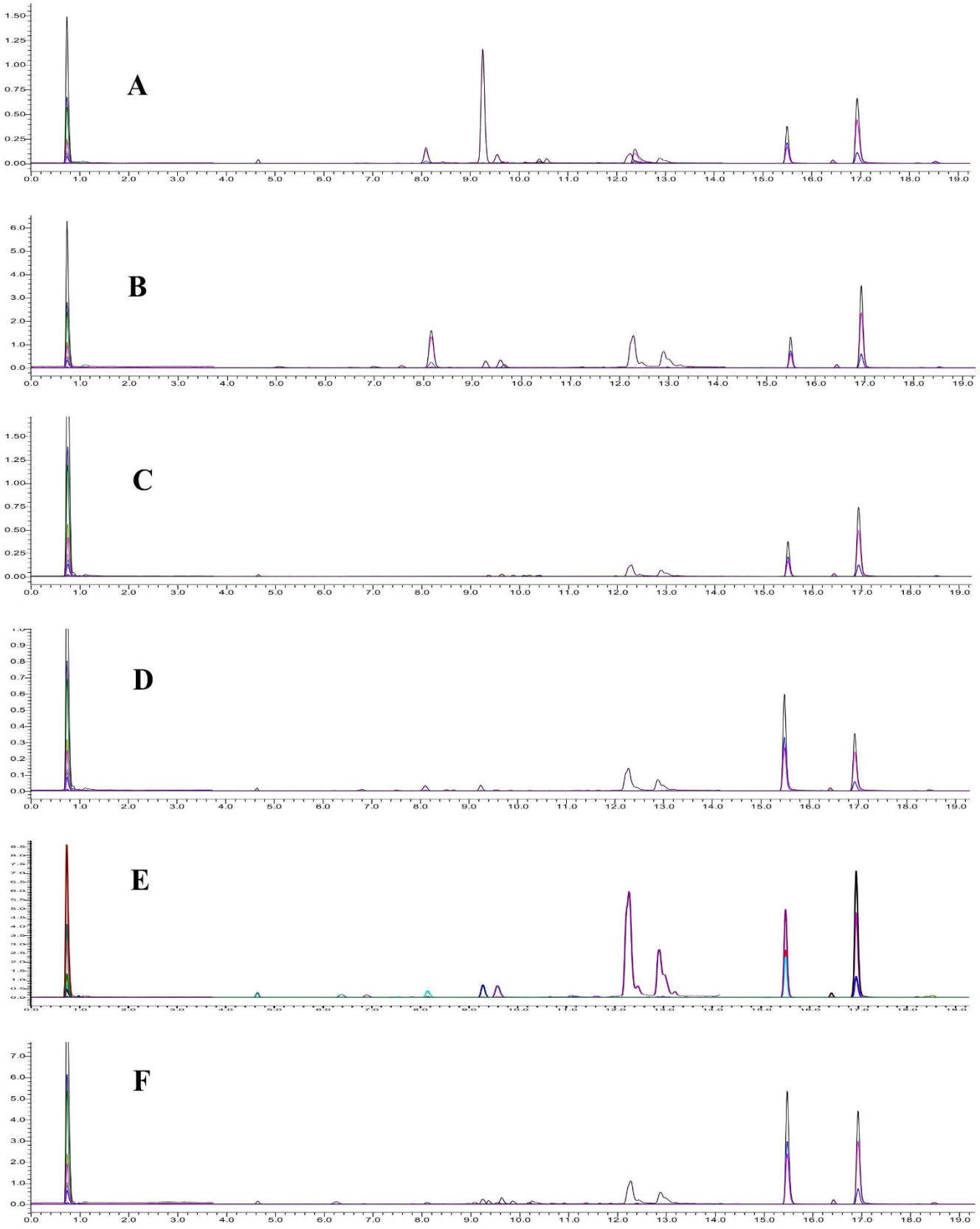

Figure 21: LC-MS/MS chromatograms of ethanolic extracts from six nutri-medicinal plants: (A) *A. herba-alba*, (B) *A. canescens*, (C) *J. phoenicea*, (D) *O. ficus-indica*, (E) *S. arvensis*, (F) *Z. lotus*.

Showed high relative levels of kaempferol (RT 12.29 min; peak area: 11,627,939) and myricetin (RT 16.94 min; peak area: 11,817,787). Caffeic acid (RT 8.17 min; peak area: 8,962,709) and chlorogenic acid (RT 15.50 min; peak area: 2,614,502) were also prominent.

J. phoenicea was distinguished by a very high relative abundance of p-coumaric acid (RT 0.76 min; peak area: 47,343,561). Myricetin (RT 16.95 min; peak area: 29,626,015), kaempferol (RT 12.29 min; peak area: 19,842,388), and chlorogenic acid (RT 15.50 min; peak area: 8,078,502) were also major constituents. Notably, thymol (RT 0.76 min; peak area: 613,391), although detected in all species (Table 3), showed a comparatively notable peak area in this species.

O. ficus-indica displayed a relatively balanced distribution among its principal phenolic constituents, including kaempferol (RT 12.29 min; peak area: 13,829,004), myricetin (RT 16.93 min; peak area: 13,730,562), and chlorogenic acid (RT 15.49 min; peak area: 13,529,202).

S. arvensis exhibited the highest relative abundance of kaempferol (RT 12.30 min; peak area: 49,680,480) among all species studied. Significant contributions from myricetin (RT 16.96 min; peak area: 24,400,217) and chlorogenic acid (RT 15.51 min; peak area: 10,287,056) were also observed.

Z. lotus presented a diverse phenolic profile with kaempferol (RT 12.28 min; peak area: 17,141,905), myricetin (RT 16.93 min; peak area: 16,142,570), and chlorogenic acid (RT 15.48 min; peak area: 11,496,675) as major components. Ferulic acid (RT 10.26 min; peak area: 2,045,887) and caffeic acid (RT 8.11 min; peak area: 553,946) were present at lower relative abundances.

Several compounds were ubiquitous across all six species including hydroxyquinolin, thymol, 2-methoxybenzoic acid, 4-methoxybenzoic acid, p-coumaric acid, ferulic acid, kaempferol, quercetin, valinin, chrysin, myricetin, folic acid, rutin, catechin hydrate, 3,5-dihydroxybenzoic acid, caffeic acid, cis-p-coumaric acid, salicylic acid, gallic acid, luteolin, and chlorogenic acid. Conversely, certain compounds showed a more restricted distribution; kojic acid, naringenin, beta-carotene, sinapic acid, 4-hydroxycoumarin, syringic acid, and hesperetin were not detected (ND) in the ethyl acetate fraction of *Z. lotus* under the employed analytical conditions

Table 7. LC-MS-MS detection of phenolic compounds in the six plants

Compound Name	Molecular Weight	ESI Mode (+/-)	m/z	*A. herba-alba*	*A. canescens*	*O. ficus-indica*	*J. phoenicea*	*S. arvensis*	*Z. lotus*
Hydroxy quinolin	145.16	+	146.0500>101.0000	D	D	D	D	D	D
Thymol	150.22	+	151.7500>88.1000	D	D	D	D	D	D
2-Methoxybenzoic Acid	152.15	+	153.0500>135.0500	D	D	D	D	D	D
4-Methoxybenzoic Acid	152.15	+	153.0500>70.7500	D	D	D	D	D	D
p-Coumaric Acid	164.16	+	165.1000>101.2000	D	D	D	D	D	D
Kojic Acid	142.11	+	143.0000>69.0500	D	D	D	D	D	ND
Ferulic Acid	194.18	+	194.9000>177.1500	D	D	D	D	D	D
Naringenin	272.25	+	272.9500>177.0000	D	D	D	D	D	ND
Beta Carotene	536.87	+	537.2000>23.1000	D	D	D	D	D	ND
Kaempferol	286.24	+	287.1000>255.2500	D	D	D	D	D	D
Quercetin	302.23	+	303.1000>262.2000	D	D	D	D	D	D
Valinin	152.15	+	153.1000>65.1500	D	D	D	D	D	D
Chrysin	254.24	+	255.0500>153.0500	D	D	D	D	D	D
Myricetin	318.23	+	336.2500>46.1500	D	D	D	D	D	D
Folic Acid	441.14	+	442.9000>323.4500	D	D	D	D	D	D
Rutin	610.5	+	611.2000>73.2000	D	D	D	D	D	D
Catechin Hydrate	308.28	-	306.9500>169.1000	D	D	D	D	D	D
Sinapic Acid	224.21	-	223.0000>208.1500	D	D	D	D	D	ND
4-Hydroxy Coumarin	162.14	-	160.8000>117.1000	D	D	D	D	D	ND
3,5-Dihydroxybenzoic Acid	154.12	-	153.1000>109.1000	D	D	D	D	D	D
Caffeic Acid	180.16	-	179.1500>135.0500	D	D	D	D	D	D
Cis-p-Coumaric Acid	164.16	-	163.1500>119.1500	D	D	D	D	D	D
Syringic Acid	198.17	-	196.9500>182.0000	D	D	D	D	D	ND
Salicylic Acid	138.12	-	137.1000>93.1500	D	D	D	D	D	D
Gallic Acid	170.12	-	169.1000>125.0500	D	D	D	D	D	D
Luteolin	286.24	-	284.9500>133.0000	D	D	D	D	D	D
Hesperetin	302.28	-	300.9000>255.2500	D	D	D	D	D	ND
Chlorogenic Acid	354.31	-	352.9000>177.1500	D	D	D	D	D	D

D: Detected; ND: Not detected

7.2 Discussion

This LC-MS/MS analysis provided valuable comparative insights into the phytochemical profiles of six nutri-medicinal plants. We acknowledge this study is semi-quantitative; relative abundance was assessed via peak area, necessitating future work with calibration standards for absolute quantification. The 28 identified compounds, based on available standards, represent a significant but not exhaustive profile of each species' constituents.

7.2.1 Ecological significance of phytochemical variation

The distinct phytochemical signatures observed across species likely reflect adaptive strategies to semi-arid conditions. Pronounced accumulation of specific compounds, such as ferulic acid in *A. herba-alba*, p-coumaric acid in *J. phoenicea*, and kaempferol in *S. arvensis*, suggests specialized metabolic adaptations (Linić et al., 2021). Furthermore, the ubiquitous presence of flavonoids (e.g., quercetin, catechin hydrate, kaempferol) and hydroxycinnamic acids (ferulic, coumaric, caffeic) likely confers ecological advantages through known roles in mitigating UV stress, enhancing drought resilience, and pathogen defense (Mouradov and Spangenberg, 2014; Ramzan et al., 2024).

7.2.2 Potential health and nutraceutical applications for livestock

The broad detection of health-promoting compounds across all six species underscores their potential value in livestock nutrition and health management. Gallic acid, universally detected, possesses well-documented antioxidant, antimicrobial, and immunomodulatory activities (Mektrirat et al., 2023). The presence of beta carotene, as essential precursor of vitamin A critical for vision, immune function (Gammone et al., 2015).

Specifically relevant to nutraceutical applications, the universal presence of chlorogenic acid highlights a shared potential for antioxidant effects, possibly leading to reduced oxidative stress and improved nutrient utilization in ruminants (Dai et al., 2024). Thymol, also detected in all species (though notably prominent in *J. phoenicea*), is recognized for its potential to modulate rumen fermentation and mitigate enteric methane production (Ku-Vera et al., 2020; Fukuda et al., 2024), aligning with goals for more sustainable livestock farming.

These findings contribute to a phytochemical database for these species and highlight the importance of conserving such botanical resources in arid ecosystems, which are reservoirs of valuable bioactive compounds. The data support integrating these plants as functional forages into livestock systems. This approach could enhance agricultural sustainability and resilience in arid regions by providing nutritional support alongside health-promoting phytochemicals.

CONCLUSION AND PERSPECTIVES

Conclusion

Maquis and garrigue ecosystems represent significant reservoirs of medicinal plants with high forage potential. The region of Bordj Bou Arréridj, situated in the semi-arid zone, is no exception and constitutes a representative example among many in Algeria. Through detailed analysis of primary and secondary metabolites, as well as the antioxidant activity of these plant species, this research provides an original contribution to the phytochemistry of medicinal plants. It not only establishes a precise chemical profile of these plants but also evaluates, based on their biochemical compositions, their relevance as innovative feed ingredients capable of enhancing animal nutrition and health while contributing to the sustainability of livestock production systems.

The ethnobotanical survey, employing rigorous snowball sampling methodology with 84 participants (25-85 years), documented extensive regional knowledge of forage resources, identifying 133 plants of forage interest distributed across 90 genera and 43 families. Notably, 92 species possessed dual medicinal-forage properties, with Asteraceae, Poaceae, Fabaceae, and Apiaceae emerging as predominant families. This ethnobotanical foundation facilitated the strategic selection of six species unanimously judged to have high forage potential— *Opuntia ficus-indica, Atriplex canescens, Juniperus phoenicea, Artemisia herba-alba, Ziziphus lotus, and Sinapis arvensis*—for comprehensive phytochemical and nutritional characterization.

Phytochemical analyses revealed distinctive metabolic profiles according to extraction methodologies, with *J. phoenicea* and *Z. lotus* exhibiting superior contents of phenolic compounds (169.31 ± 3.88 mg GAE/g DM) and flavonoids (24.18 ± 0.22 mg QE/g DM) in hydroalcoholic extracts, while *O. ficus-indica* demonstrated solvent-dependent extraction efficacy, producing optimal phenolic concentrations (190.12 mg GAE/g DM) in aqueous extracts. These results underscore the methodological importance in protocols for recovery and characterization of bioactive compounds. Antioxidant capacity evaluations via DPPH and FRAP assays demonstrated that *J. phoenicea* and *Z. lotus* exhibit exceptional free radical scavenging potential.

Nutritional profiling revealed moderate crude protein levels (6.8–8.2%), with *O. ficus-indica* and *S. arvensis* exhibiting relatively higher protein content compared to the other studied species. Mineral composition analysis identified *A. canescens* as an exceptional reservoir of potassium (47.376 mg/g), essential for electrolyte homeostasis in ruminant

physiology. Advanced spectroscopic and chromatographic techniques confirmed the presence of specific bioactive constituents, notably chlorogenic acid, β-carotene, and thymol, compounds with documented antimicrobial and antioxidant properties.

Based on the comprehensive phytochemical and nutritional datasets, we recommend differential utilization strategies for the studied species according to specific nutritional and medicinal objectives. *O. ficus-indica* demonstrates optimal parameters with 8.20% crude protein coupled with exceptional aqueous phenolic content (190.12 ± 0.10 mg GAE/g DM), suggesting its potential as a dual-purpose nutritional-medicinal supplement. Where mineral supplementation is critical, particularly concerning potassium requirements, *A. canescens* represents an exceptional resource (47.376 mg/g K), significantly surpassing other studied species. *J. phoenicea* emerges as good candidate for applications requiring potent antioxidant activity (DPPH assay: 7.11 ± 1.5 µg/mL with hydroalcoholic extract; FRAP assay: 812.71 ± 37.31 mM TE/g in aqueous extracts). The integration of these complementary phytochemical and nutritional profiles within livestock feeding systems would optimize physiological benefits while addressing specific nutritional deficiencies prevalent in semi-arid production systems.

Future perspectives should prioritize in vivo nutritional and feeding trials to validate laboratory results, determine optimal inclusion doses in animal rations, and explore potential synergistic interactions between different plant species. Furthermore, investigations into the available biomass of these plants, as well as a better understanding of their impact on livestock health, are necessary. Studies aimed at optimizing their cultivation conditions and agronomic management would also enable consideration of large-scale utilization. An interdisciplinary approach, combining traditional ecological knowledge and modern analytical methodologies, would constitute a promising pathway toward the development of sustainable, resilient, and resource-efficient livestock systems, particularly adapted to semi-arid zones.

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ANNEXES

Annex 01: Questionnaire used to collect data in the ethnobotanical study

<table>
<tr><td>Informats' details</td><td>Male ☐
Female ☐
Age:
Study:</td><td colspan="4">Work
Livestock farmer ☐
Veterinarian ☐
Herbalist ☐
Forest conservation agent ☐
Other</td><td colspan="4">Livestock number
Bovine ☐
Ovine ☐
Caprine ☐</td></tr>
<tr><td>Region</td><td>Region's name</td><td colspan="4">Livestock farming in the region
Bovine ☐
Ovine ☐
Caprine ☐</td><td colspan="4">Topology
Mountain ☐
Hight plateau ☐
Steppe ☐</td></tr>
<tr><td>Plant</td><td>Scientific name</td><td>Vernacular name</td><td>Type(H :herb, A :shrub, B: tree)</td><td>Abandance/disponibility</td><td>Consumation saison</td><td>Consumed part</td><td>Animal(s) consumer</td><td>Palatability</td><td>Medicinal : yes/no</td></tr>
</table>

Annex 02: List of the wild forage plants in the semi-arid region of Bordj Bou Arreridj, Algeria

Family	Arabic name	Scientific name	Type	Class	Life form	Season	Palatability	Foraging method	Uses in medicin	RFC	Voucher
Amaranthaceae	Hamrani	*Amaranthus blitoides*	H	Ther	Annual	summer to autumn	***	Both	Non	0,01	RA-40
	-	*Amaranthus retroflexus L.*	H	Ther	Annual	Summer	***	Both	Med	0,01	RA-115
	Gtaf	*Atriplex canescens*	S	Cham	Perennial	Year-round	***	Grazing	Med	0,21	RA-116
Anacardiaceae	Darw	*Pistacia lentiscus*	T	Phan	Perennial	Year-round	**	Grazing	Med	0,09	RA-21
Apiaceae	Talghouda	*Bunium fontanesii*	H	Cryp	Perennial	Spring to autumn	**	Grazing	Med	0,10	RA-9
	Zrodiya lberiya	*Daucus carota L.*	H	Ther	Biennial	Spring	**	Both	Med	0,01	RA-14
	Makhleb doriss	*Eryngium campestre L*	H	Hemi	Perennial	Spring	*	Grazing	Med	0,01	RA-71
	Basbas lberi	*Foeniculum vulgare Mill.*	H	Ther	Perennial	Spring	**	Both	Med	0,01	RA-95
	Zayata	*Helosciadium nodiflorum (L.) Wimm. & Grab.*	H	Ther	Annual	Spring to autumn	***	Both	Med	0,03	RA-97
	Timachta	*Scandix pecten-veneris L*	H	Hemi	Annual	Spring	***	Both	Non	0,10	RA-103
	Bounafa'	*Thapsia garganica L*	H	Hemi	Perennial	Spring	*	Grazing	Med	0,03	RA-109
	Tabelawt	*Turgenia latifolia L*	H	Ther	Annual	Spring	*	Grazing	Non	0,01	RA-112
Apocynaceae	Defla	*Nerium oleander L.*	S	Phan	Perennial	Year-round	*	Grazing	Med	0,01	RA-22
Asparagaceae	Zitot	*Muscari comosum L*	H	Cryp	Perennial	Spring	**	Grazing	Non	0,04	RA-110
Asteraceae	Boumlel	*Anthemis nobilis L.*	H	Ther	Perennial	summer to autumn	***	Both	Med	0,19	RA-1
	Achbet meryem	*Artemisia absinthium L.*	H	Cham	Perennial	summer to autumn	*	Grazing	Med	0,01	RA-11
	Chi7	*Artemisia Ha-alba Asso*	S	Cham	Perennial	Year-round	***	Grazing	Med	0,57	RA-13
	-	*Atractylis cancellata L.*	H	Ther	Perennial	spring to summer	*	Grazing	Med	0,03	RA-18
	Ladad	*Atractylis gummifera L.*	H	Cham	Perennial	summer	*	Grazing	Med	0,06	RA-19
	-	*Bombycilaena erecta (L.)*	H	Ther	Annual	summer	**	Grazing	Non	0,01	RA-20
	Eljamra	*Calendula arvensis L.*	H	Ther	Annual	Spring	**	Grazing	Med	0,01	RA-27
	Chdak ljmal	*Carduus pycnocephalus L.*	H	Hemi	Biennial	summer to autumn	*	Grazing	Med	0,03	RA-29
	Grayn jdi	*Carthamus tinctorius L.*	H	Ther	Annual	Spring	**	Grazing	Med	0,09	RA-34
	Giz	*Catananche caerulea L.*	H	Hemi	Perennial	Spring	***	Grazing	Med	0,01	RA-35

Annex 2: Suite 1

	-	*Centaurea pullata L.*	H	Ther	Annual	Spring	**	Grazing	Med	0,01	RA-38
	Hendeeba	*Cichorium intybus L.*	H	Hemi	Perennial	Spring to autumn	***	Both	Med	0,01	RA-45
	-	*Cirsium arvense (L.) Scop.*	H	Ther	Perennial	summer	*	Grazing	Non	0,03	RA-46
	Ja'da	*Cotula cinerea L.*	H	Ther	Annual	spring	**	Grazing	Med	0,13	RA-52
	Khorchef lberi	*Cynara cardunculus L.*	H	Hemi	Perennial	Summer	**	Grazing	Med	0,01	RA-58
	Magraman	*Dittrichia viscosa L.*	H	Hemi	Perennial	Year-round	*	Grazing	Med	0,01	RA-63
	Chouk lejmal	*Echinops spinosissimus Turra*	H	Hemi	Perennial	spring to summer	*	Grazing	Med	0,06	RA-66
	-	*Launaea nudicaulis (L.) Cass.*	H	Ther	Annual	Spring	**	Grazing	Med	0,03	RA-70
	Feryas	*Onopordum macracanthum Schousb.*	H	Ther	annual	spring to summer	*	Both	Non	0,10	RA-98
	Gernina	*Scolymus hispanicus L.*	H	Ther	biennial	Spring	**	Both	Med	0,13	RA-102
	Talma	*Scorzonera laciniata L.*	H	Cham	annual	Spring	***	Both	Med	0,07	RA-117
	Boul dib	*Senecio vulgaris L.*	H	Ther	Annual	Spring	***	Both	Med	0,01	RA-118
	Khanfoufa	*Silybum marianum (L.) Gaertn.*	H	Ther	Biennial	Spring	**	Both	Med	0,11	RA-120
	Tifef	*Sonchus oleraceus L.*	H	Ther	Annual	spring to summer	**	Both	Med	0,11	RA-121
	Darset la'jouz	*Taraxacum officinale*	H	Hemi	Perennial	Spring	***	Grazing	Med	0,06	RA-122
	Haska	*Xanthium strumarium L.*	H	Ther	Annual	spring to summer	*	Grazing	Med	0,01	RA-128
Boraginaceae	Lsan thour	*Borrago officinalis L.*	H	Ther	Annual	summer to autumn	*	Grazing	Med	0,04	RA-37
	-	*Echium creticum L.*	H	Ther	Biennial	Spring	**	Grazing	Med	0,01	RA-69
	Gozayeh	*Pituranthos chloranthus*	H	Cham	Perennial	spring to summer	**	Grazing	Non	0,03	RA-124
Brassicaceae	Hara lbayda	*Capsella bursa-pastoris*	H	Ther	Annual	Spring	**	Both	Non	0,03	RA-42
	Rjel ljaja	*Matthiola lunata (DC.) Steud.*	H	Hemi	Annual	Spring	**	Grazing	Non	0,01	RA-43
	kromb	*Moricandia arvensis (L.) DC.*	H	Ther	Biennial	spring to summer	**	Both	Non	0,09	RA-65
	Hara	*Sinapis arvensis L.*	H	Ther	Annual	Spring	**	Both	Med	0,40	RA-84
	Werdal	*Sinapis pubescens L.*	H	Hemi	Perennial	Year-round	*	Both	Non	0,17	RA-106
Cactaceae	Hendi	*Opuntia ficus-indica (Mill.) F.A.C.Weber ex Diguet*	S	Phan	Perennial	Year-round	***	Both	Med	0,19	RA-47
Caprifoliaceae		*Scabiosa stellata L.*	H	Ther	Annual	spring to summer	**	Grazing	Non	0,03	RA-136

Annex 2: Suite 2											
Caryophyllaceae	Kasar lhjar	*Paronychia argentea Lam.*	H	Hemi	Perennial	Spring to autumn	***	Grazing	Med	0,04	RA-56
		Silene pratensis (L.) Forssk.	H	Ther	Perennial	Spring	**	Grazing	Non	0,01	RA-137
Chenopodiaceae	Ajram	*Anabasis articulata*	S	Ther	Perennial	Year-round	***	Grazing	Med	0,01	RA-2
	Ramram	*Chenopodium album L*	H	Ther	Annual	Summer	***	Both	Med	0,01	RA-81
Convolvulaceae	Lawaya	*Convolvulus duraudoi*	H	Cryp	Perennial	Spring	***	Both	Med	0,03	RA-67
		Cuscuta americana L.	H	Ther	Annual	Spring	**	Grazing	Non	0,03	RA-123
Cucurbitaceae	Fegouss lehmir	*Ecballium elaterium (L.) A.Rich.*	H	Hemi	Annual	summer	*	Grazing	Med	0,03	RA-28
Cupressaceae	Sarwel	*Cupressus sempervirens L.*	T	Phan	Perennial	Year-round	**	Grazing	Med	0,01	RA-4
	Taga	*Juniperus oxycedrus L.*	T	Phan	Perennial	Year-round	**	Grazing	Med	0,11	RA-87
	A'rar	*Juniperus phoenicea L.*	T	Phan	Perennial	Year-round	***	Grazing	Med	0,21	RA-96
Euphorbiaceae	Hlib daba	*Euphorbia helioscopia L.*	H	Ther	Annual	Spring	*	Grazing	Med	0,01	RA-48
Fabaceae	-	*Acacia saligna*	T	Phan	Perennial	Year-round	*	Grazing	Med	0,01	RA-33
	kdad	*Astragalus spinosus*	S	Cham	Perennial	Year-round	**	Grazing	Med	0,13	RA-53
	Gendoul	*Calicotome spinosa (L) Lamk*	S	Ther	Perennial	Year-round	**	Grazing	Non	0,10	RA-57
	kharoub	*Ceratonia siliqua L.*	T	Phan	Perennial	Year-round	***	Grazing	Med	0,01	RA-59
	Jelbana	*Lathyrus sylvestris L.*	H	Ther	Perennial	Spring	***	Both	Non	0,11	RA-80
	-	*Medicago hispida Schrank*	H	Ther	Annual	spring to summer	***	Both	Non	0,03	RA-82
	Safsfa	*Medicago sativa L.*	H	Hemi	Perennial	spring to summer	***	Both	Med	0,19	RA-85
	-	*Ononis reclinata L.*	H	Ther	Annual	Spring to autumn	***	Both	Non	0,01	RA-100
	Ratma	*Retama sphaerocarpa Boiss.*	S	Phan	Perennial	Year-round	**	Mowing	Med	0,13	RA-113
	Tartag	*Spartium junceum L.*	S	Phan	Perennial	Year-round	**	Grazing	Med	0,03	RA-129
	Nfel	*Trifolium repens L*	H	Ther	Perennial	Year-round	***	Both	Non	0,17	RA-130
Fagaceae	Balout	*Quercus ilex*	T	Phan	Perennial	Year-round	***	Grazing	Med	0,19	RA-7
Iridaceae	Sif ghrab	*Gladiolus illyricus*	H	Ther	Perennial	Spring	***	Both	Non	0,03	RA-91
Juncaceae	Smar	*Juncus acutus*	H	Cryp	Perennial	Year-round	*	Grazing	Non	0,04	RA-93
Lamiaceae	chendgoura	*Ajuga iva (L)Schreber.*	H	Cham	Perennial	spring to summer	***	Grazing	Med	0,07	RA-17
	Meriweth	*Marrubium vulgare*	H	Hemi	Perennial	spring	*	Grazing	Med	0,09	RA-31
	Flio	*Mentha pulegium*	H	Ther	Perennial	Summer	***	Grazing	Med	0,01	RA-36
	Na'na	*Mentha viridis*	H	Ther	Perennial	spring to summer	**	Grazing	Med	0,04	RA-60

	Annex 2: Suite 3										
	Za'ter	*origanum froribundum*	H	Hemi	Perennial	Summer	***	Grazing	Med	0,10	RA-73
	Mezir	*Rosmarinus officinalis L*	S	Phan	Perennial	Year-round	***	Grazing	Med	0,10	RA-75
	Khayata	*Teucrium polium L*	H	Cham	Perennial	summer to autumn	***	Grazing	Med	0,10	RA-76
	Gomayech	*Thymus Munbyanus*	H	Cham	Perennial	spring to summer	**	Grazing	Med	0,14	RA-108
Liliaceae	Barwag	*Asphodelus microcarpus Salzm. & Viv*	H	Hemi	Perennial	Summer	*	Grazing	Med	0,04	RA-8
Malvaceae	khobiz	*Malva sylvestris*	H	Ther	Biennial	Spring to autumn	***	Both	Med	0,10	RA-62
Moraceae	Karma	*Ficus carica*	T	Phan	Perennial	Spring to autumn	***	Mowing	Med	0,03	RA-55
Myrtaceae	kalitous	*Eucalyptus globulus*	T	Phan	Perennial	Year-round	**	Grazing	Med	0,01	RA-54
Oleaceae	Zitoun	*Olea europaea*	T	Phan	Perennial	Year-round	***	Mowing	Med	0,09	RA-111
Papaveraceae	-	*Fumaria officinalis L.*	H	Ther	Annual	Spring	***	Both	Med	0,01	RA-32
	Roz' ljaj	*Fumaria parviflora*	H	Ther	Annual	Spring	***	Both	Med	0,03	RA-125
	Gbobech	*Papaver rhoeas L*	H	Ther	Annual	Spring	***	Both	Med	0,17	RA-126
Pinaceae	Sanawber	*Pinus halepensis*	T	Phan	Perennial	Year-round	**	Grazing	Med	0,03	RA-86
Plantaginaceae	Taselgha	*Globularia alypum L.*	S	Cham	Perennial	Spring to autumn	*	Grazing	Med	0,11	RA-61
	-	*Plantago lagopus L.*	H	Ther	Annual	spring	***	Grazing	Non	0,01	RA-101
	-	*Plantago lanceolata L.*	H	Hemi	Annual	Year-round	***	Grazing	Med	0,03	RA-132
	khnouft lgata	*Veronica hederifolia L.*	H	Ther	Annual	spring to summer	***	Both	Non	0,01	RA-133
	-	*Veronica persica Poir.*	H	Ther	Annual	spring to summer	***	Both	Non	0,01	RA-139
Poaceae	Habet lhajla	*Aegilops triuncialis L.*	G	Ther	Annual	Spring	***	Both	Non	0,03	RA-10
	Diss	*Ampelodesmos mauritanicus (Poir.) T.Durand & Schinz*	G	Cryp	Perennial	Year-round	**	Grazing	Non	0,14	RA-16
	Gssab	*Arundo donax L.*	G	Cryp	Perennial	Year-round	***	Grazing	Med	0,03	RA-23
	Khortal lberi	*Avena fatua L.*	G	Ther	Annual	Spring	***	Both	Non	0,03	RA-24
	-	*Bromus rubens L.*	H	Ther	Annual	Spring	***	Both	Non	0,01	RA-26
	Nejem	*Cynodon dactylon (L.) Pers.*	G	Hemi	Perennial	Year-round	***	Grazing	Non	0,11	RA-39
	Bechna	*Eleusine indica (L.) Gaertn.*	G	Ther	Annual	summer to autumn	***	Both	Non	0,03	RA-41
	Sboult lfar	*Hordeum murinum L.*	G	Ther	Annual	Spring	***	Both	Non	0,11	RA-64

	Annex 2: Suite 4										
	Dil lferouch	*Lagurus ovatus L.*	G	Ther	Perennial	Spring to autumn	***	Both	Non	0,01	RA-72
	Medhoun	*Lolium multiflorum Lam.*	G	Ther	Annual	spring to summer	***	Both	Non	0,07	RA-79
	Senegh	*Lygeum spartum L.*	G	Cryp	Perennial	Year-round	**	Grazing	Non	0,07	RA-88
	Brika	*Phalaris brachystachys Link*	G	Ther	Annual	spring to summer	***	Both	Non	0,01	RA-90
	-	*Poa annua L.*	G	Hemi	Annual	spring	***	Both	Non	0,01	RA-94
	Dil lkelb	*Setaria longiseta P.Beauv.*	G	Cham	Perennial	spring to summer	***	Both	Non	0,01	RA-114
	Soma'a	*Stipa capensis Thunb.*	G	Ther	Annual	Spring	***	Grazing	Non	0,06	RA-119
	-	*Stipa parviflora Desf.*	G	Hemi	Perennial	spring to summer	***	Both	Med	0,03	RA-134
	Halfa	*Stipa tenacissima L.*	G	Cryp	Perennial	Year-round	**	Grazing	Med	0,29	RA-138
Polygonaceae	-	*Persicaria maculosa Gray*	H	Ther	Annual	Spring to autumn	***	Both	Non	0,01	RA-49
	-	*Polygonum aviculare L.*	H	Cham	Annual	summer to autumn	***	Both	Med	0,01	RA-50
	Homayda	*Rumex acetosella L.*	H	Ther	Perennial	Spring to autumn	***	Both	Med	0,09	RA-131
	Homydat lbagra	*Rumex crispus L.*	H	Hemi	Perennial	Spring to autumn	***	Both	Med	0,01	RA-135
Portulacaceae	Rejla	*Portulaca oleracea L.*	H	Ther	Annual	Summer	***	Both	Med	0,06	RA-83
Resedaceae	Dil lkhrouf	*Reseda alba L.*	H	Hemi	Annual	Spring	**	Both	Med	0,09	RA-25
Rhamnaceae	Sedra	*Ziziphus lotus (L.) Lam.*	S	Phan	Perennial	Spring to autumn	***	Grazing	Med	0,27	RA-89
Rosaceae	Za'rour	*Crataegus azarolus L.*	S	Phan	Perennial	Spring to autumn	**	Grazing	Med	0,04	RA-12
	Boumkari	*Crataegus sanguinea Pall.*	S	Phan	Perennial	Spring to autumn	**	Grazing	Med	0,01	RA-77
	Nasrin	*Rosa canina L.*	S	Phan	Perennial	spring to summer	**	Grazing	Med	0,01	RA-104
	Tout lo'lig	*Rubus fruticosus L.*	S	Cham	Perennial	Summer	**	Grazing	Med	0,01	RA-107
Rubiaceae	Losayga	*Galium aparine L.*	H	Ther	Biennial	Spring	**	Both	Med	0,03	RA-68
Rutaceae	-	*Hertia cheirifolia L.*	H	Hemi	Perennial	Spring to autumn	**	Grazing	Med	0,01	RA-30
	Fijl	*Ruta chalepensis L.*	S	Cham	Perennial	Summer	*	Grazing	Med	0,04	RA-127
Salicaceae	Trambl	*Populus alba L.*	T	Phan	Perennial	Spring to autumn	**	Grazing	Med	0,01	RA-105

Annex 2: Suite 4											
Solanaceae	Sikran	*Hyoscyamus niger L.*	H	Ther	Biennial	spring to summer	*	Grazing	Med	0,01	RA-3
	A'wsej	*Lycium europaeum L.*	S	Phan	Perennial	Year-round	**	Grazing	Med	0,03	RA-5
	A'neb dhib	*Solanum nigrum L.*	H	Ther	Annual	Summer	**	Both	Med	0,01	RA-92
Tamaricaceae	Tarfa	*Tamarix gallica L.*	S	Phan	Perennial	Year-round	***	Grazing	Med	0,13	RA-99
Thymelaeaceae	Azaz	*Daphne gnidium L.*	S	Cham	Perennial	Year-round	*	Grazing	Med	0,01	RA-6
	Methnan	*Thymelaea hirsuta (L.)*	S	Phan	Perennial	Year-round	*	Grazing	Med	0,04	RA-74
Ulmaceae	Nchem	*Ulmus minor Mill.*	T	Phan	Perennial	Spring to autumn	***	Grazing	Non	0,03	RA-78
Urticaceae	Horayeg	*Urtica dioica L.*	H	Hemi	Perennial	Spring	*	Grazing	Med	0,10	RA-51
Zygophyllaceae	Harmel	*Peganum harmala L*	H	Cham	Perennial	Spring	*	Grazing	Med	0,06	RA-15
	Bounagar	*Tribulus terrestris L.*	H	Ther	Annual	summer to autumn	***	Both	Med	0,01	RA-44

H= Herb; T=Tree; S= Shrub; Ther= Thermophyte; Hemi= Hemicriptophyte; Cryp= Cryptophyte; Cha=Chamaephyte; Pher= Phanerophyte; * = Weakly palatable; ** = Fairly palatable; *** = Highly palatable; Med=Medicinal; Non= Not medicinal.

Annex 03: LC-MS/MS Phytochemical Profiling Data of *A. herba-alba* phenolics

Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Retention Time	Height	Area
hydroxy quinolin	C9H7NO	145.16	(+)	146.0500>101.0000	7.814	4705	33772
thymol	C10H14O	150.22	(+)	151.7500>88.1000	0.74	66771	250877
2-Methoxybenzoic Acid	C8H8O3	152.15	(+)	153.0500>135.0500	0.74	703669	2594420
4-mythoxybenzoic Acid	C8H8O3	152.15	(+)	153.0500>70.7500	0.74	1212483	4394581
acide coumarique	C9H8O3	164.16	(+)	165.1000>101.2000	0.74	5675251	20768730
kojic Acid	C6H6O4	142.11	(+)	143.0000>69.0500	15.078	1511	15081
Ferulic Acid	C10H10O4	194.18	(+)	194.9000>177.1500	9.263	11574903	60437913
Naringenin	C15H12O5	272.25	(+)	272.9500>177.0000	8.292	8163	74923
beta carotene	C40H56	536.87	(+)	537.2000>23.1000	10.467	5195	45570
keampferol	C15H10O6	286.24	(+)	287.1000>255.2500	12.285	967639	8168951
Quercitine	C15H10O7	302.23	(+)	303.1000>262.2000	12.546	8906	56463
valinin	C8H8O3	152.15	(+)	153.1000>65.1500	8.52	18888	161932
Chrysin	C15H10O4	254.24	(+)	255.0500>153.0500	0	0	0
merycetin	C15H10O8	318.23	(+)	336.2500>46.1500	16.936	4442013	26553915
folic acid	C19H19N7O6	441.14	(+)	442.9000>323.4500	17.5	1483	9861
Rutine	C27H30O16	610.5	(+)	611.2000>73.2000	18.541	168037	1153426
Catechin Hydrate	C15H16O7	308.28	(-)	306.9500>169.1000	13.462	340	1845
Sinapic Acid	C11H12O5	224.21	(-)	223.0000>208.1500	9.565	770	6227
4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000>117.1000	0.74	7408	32202
3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000>109.1000	4.66	383123	1330296
caffiec Acid	C9H8O4	180.16	(-)	179.1500>135.0500	8.097	1329015	7999607
Cis-p.coumaric Acid	C9H8O3	164.16	(-)	163.1500>119.1500	9.225	26596	148417
Syringic Acid	C9H10O5	198.17	(-)	196.9500>182.0000	0	0	0
salysilic Acid	C7H6O3	138.12	(-)	137.1000>93.1500	9.557	818604	5543081
gallic Acid	C4H4O4	170.12	(-)	169.1000>125.0500	9.835	5656	33658
Luteonil	C15H10O6	286.24	(-)	284.9500>133.0000	12.383	1046665	10764792
Hespertin	C16H14O6	302.28	(-)	300.9000>255.2500	0	0	0
chlorogenique acide	C16H18O9	354.31	(-)	352.9000>177.1500	15.499	1672720	8394474

Annex 04: LC-MS/MS Phytochemical Profiling Data of *A. canescens* phenolics

Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Retention Time	Height	Area
hydroxy quinolin	C9H7NO	145.16	+	146.0500>101.0000	2.102	3840	12584
thymol	C10H14O	150.22	+	151.7500>88.1000	0.739	28963	106140
2-Methoxybenzoic Acid	C8H8O3	152.15	+	153.0500>135.0500	0.738	322611	1272770
4-mythoxybenzoic Acid	C8H8O3	152.15	+	153.0500>70.7500	0.738	470237	1626269
acide coumarique	C9H8O3	164.16	(+)	165.1000>101.2000	0.737	2362060	8282005
kojic Acid	C6H6O4	142.11	(+)	143.0000>69.0500	15.163	3870	21025
Ferulic Acid	C10H10O4	194.18	(+)	194.9000>177.1500	9.659	137660	1027049
Naringenin	C15H12O5	272.25	(+)	272.9500>177.0000	0	0	0
beta carotene	C40H56	536.87	(+)	537.2000>23.1000	12.319	3376	27536
keampferol	C15H10O6	286.24	(+)	287.1000>255.2500	12.291	1365670	11627939
Quercitine	C15H10O7	302.23	(+)	303.1000>262.2000	12.546	12570	86680
valinin	C8H8O3	152.15	(+)	153.1000>65.1500	8.542	17873	142021
Chrysin	C15H10O4	254.24	(+)	255.0500>153.0500	12.955	1090	5938
merycetin	C15H10O8	318.23	(+)	336.2500>46.1500	16.941	2352396	11817787
folic acid	C19H19N7O6	441.14	(+)	442.9000>323.4500	17.483	12204	73863
Rutine	C27H30O16	610.5	(+)	611.2000>73.2000	18.534	35417	224563
Catechin Hydrate	C15H16O7	308.28	(-)	306.9500>169.1000	13.443	4290	27242
Sinapic Acid	C11H12O5	224.21	(-)	223.0000>208.1500	9.833	190	1928
4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000>117.1000	0.732	3797	17794
3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000>109.1000	5.066	50186	653210
caffiec Acid	C9H8O4	180.16	(-)	179.1500>135.0500	8.168	1321787	8962709
Cis-p.coumaric Acid	C9H8O3	164.16	(-)	163.1500>119.1500	9.275	269145	1558263
Syringic Acid	C9H10O5	198.17	(-)	196.9500>182.0000	7.332	667	2558
salysilic Acid	C7H6O3	138.12	(-)	137.1000>93.1500	9.579	308205	2270597
gallic Acid	C4H4O4	170.12	(-)	169.1000>125.0500	11.836	1415	11362
Luteonil	C15H10O6	286.24	(-)	284.9500>133.0000	12.428	1670	16530
Hespertin	C16H14O6	302.28	(-)	300.9000>255.2500	11.658	2134	17848
chlorogenique acide -	C16H18O9	354.31	(-)	352.9000>177.1500	15.5	589699	2614502

Annex 05: LC-MS/MS Phytochemical Profiling Data of *J. phoenicea* phenolics

Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Retention Time	Height	Area
hydroxy quinolin	C9H7NO	145.16	+	146.0500>101.0000	9.661	700	11273
thymol	C10H14O	150.22	+	151.7500>88.1000	0.756	141149	613391
2-Methoxybenzoic Acid	C8H8O3	152.15	+	153.0500>135.0500	0.756	1288943	5416473
4-mythoxybenzoic Acid	C8H8O3	152.15	+	153.0500>70.7500	0.755	2381811	9986197
acide coumarique	C9H8O3	164.16	(+)	165.1000>101.2000	0.755	11851303	47343561
kojic Acid	C6H6O4	142.11	(+)	143.0000>69.0500	15.077	3181	53490
Ferulic Acid	C10H10O4	194.18	(+)	194.9000>177.1500	11.972	57419	1205517
Naringenin	C15H12O5	272.25	(+)	272.9500>177.0000	9.078	3567	93742
beta carotene	C40H56	536.87	(+)	537.2000>23.1000	0	0	0
keampferol	C15H10O6	286.24	(+)	287.1000>255.2500	12.291	1223623	19842388
Quercitine	C15H10O7	302.23	(+)	303.1000>262.2000	12.548	13085	93025
valinin	C8H8O3	152.15	(+)	153.1000>65.1500	8.536	20219	383554
Chrysin	C15H10O4	254.24	(+)	255.0500>153.0500	12.963	1248	20324
merycetin	C15H10O8	318.23	(+)	336.2500>46.1500	16.946	4933017	29626015
folic acid	C19H19N7O6	441.14	(+)	442.9000>323.4500	17.495	10986	67574
Rutine	C27H30O16	610.5	(+)	611.2000>73.2000	18.546	76318	511698
Catechin Hydrate	C15H16O7	308.28	(-)	306.9500>169.1000	13.5	150	472
Sinapic Acid	C11H12O5	224.21	(-)	223.0000>208.1500	0	0	0
4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000>117.1000	0	0	0
3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000>109.1000	4.656	204909	746785
caffiec Acid	C9H8O4	180.16	(-)	179.1500>135.0500	8.118	13211	147853
Cis-p.coumaric Acid	C9H8O3	164.16	(-)	163.1500>119.1500	9.26	42895	330536
Syringic Acid	C9H10O5	198.17	(-)	196.9500>182.0000	8.005	1506	9070
salysilic Acid	C7H6O3	138.12	(-)	137.1000>93.1500	9.601	11518	445434
gallic Acid	C4H4O4	170.12	(-)	169.1000>125.0500	11.839	782	13725
Luteonil	C15H10O6	286.24	(-)	284.9500>133.0000	12.425	22352	396607
Hespertin	C16H14O6	302.28	(-)	300.9000>255.2500	11.188	1506	56381
chlorogenique acide -	C16H18O9	354.31	(-)	352.9000>177.1500	15.5	1657298	8078502

Annex 06: LC-MS/MS Phytochemical Profiling Data of *O. ficus-indica* phenolics

Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Retention Time	Height	Area
hydroxy quinolin	C9H7NO	145.16	+	146.0500>101.0000	7.819	3441	22601
thymol	C10H14O	150.22	+	151.7500>88.1000	0.746	78339	279089
2-Methoxybenzoic Acid	C8H8O3	152.15	+	153.0500>135.0500	0.746	826721	2971413
4-mythoxybenzoic Acid	C8H8O3	152.15	+	153.0500>70.7500	0.745	1350416	4898635
acide coumarique	C9H8O3	164.16	(+)	165.1000>101.2000	0.745	6874144	24571026
kojic Acid	C6H6O4	142.11	(+)	143.0000>69.0500	15.056	2390	15396
Ferulic Acid	C10H10O4	194.18	(+)	194.9000>177.1500	13.069	33310	218864
Naringenin	C15H12O5	272.25	(+)	272.9500>177.0000	8.558	3886	23666
beta carotene	C40H56	536.87	(+)	537.2000>23.1000	12.358	5217	49194
keampferol	C15H10O6	286.24	(+)	287.1000>255.2500	12.285	1382771	13829004
Quercitine	C15H10O7	302.23	(+)	303.1000>262.2000	12.541	3839	19038
valinin	C8H8O3	152.15	(+)	153.1000>65.1500	8.525	6778	60208
Chrysin	C15H10O4	254.24	(+)	255.0500>153.0500	12.941	670	5187
merycetin	C15H10O8	318.23	(+)	336.2500>46.1500	16.934	2403851	13730562
folic acid	C19H19N7O6	441.14	(+)	442.9000>323.4500	17.47	1478	7938
Rutine	C27H30O16	610.5	(+)	611.2000>73.2000	18.471	61908	437088
Catechin Hydrate	C15H16O7	308.28	(-)	306.9500>169.1000	13.443	3590	24427
Sinapic Acid	C11H12O5	224.21	(-)	223.0000>208.1500	9.821	290	1303
4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000>117.1000	0.744	7208	34452
3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000>109.1000	4.649	178006	590658
caffiec Acid	C9H8O4	180.16	(-)	179.1500>135.0500	8.107	275948	1803843
Cis-p.coumaric Acid	C9H8O3	164.16	(-)	163.1500>119.1500	9.247	315975	1674540
Syringic Acid	C9H10O5	198.17	(-)	196.9500>182.0000	7.291	444	2228
salysilic Acid	C7H6O3	138.12	(-)	137.1000>93.1500	9.562	67323	608736
gallic Acid	C4H4O4	170.12	(-)	169.1000>125.0500	11.815	2894	19980
Luteonil	C15H10O6	286.24	(-)	284.9500>133.0000	12.411	5531	43691
Hespertin	C16H14O6	302.28	(-)	300.9000>255.2500	11.896	3582	24306
chlorogenique acide -	C16H18O9	354.31	(-)	352.9000>177.1500	15.489	2656645	13529202

Annex 07: LC-MS/MS Phytochemical Profiling Data of *S. arvensis* phenolics

Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Retention Time	Height	Area
hydroxy quinolin	C9H7NO	145.16	+	146.0500>101.0000	7.836	9143	59428
thymol	C10H14O	150.22	+	151.7500>88.1000	0.984	106590	353879
2-Methoxybenzoic Acid	C8H8O3	152.15	+	153.0500>135.0500	0.74	453955	1718680
4-mythoxybenzoic Acid	C8H8O3	152.15	+	153.0500>70.7500	0.741	710205	2642898
acide coumarique	C9H8O3	164.16	(+)	165.1000>101.2000	0.741	3136361	11900423
kojic Acid	C6H6O4	142.11	(+)	143.0000>69.0500	15.172	23561	126445
Ferulic Acid	C10H10O4	194.18	(+)	194.9000>177.1500	12.894	43099	373249
Naringenin	C15H12O5	272.25	(+)	272.9500>177.0000	8.59	4427	37987
beta carotene	C40H56	536.87	(+)	537.2000>23.1000	12.364	5063	43141
keampferol	C15H10O6	286.24	(+)	287.1000>255.2500	12.297	5936429	49680480
Quercitine	C15H10O7	302.23	(+)	303.1000>262.2000	12.521	20201	136914
valinin	C8H8O3	152.15	(+)	153.1000>65.1500	8.556	4940	32809
Chrysin	C15H10O4	254.24	(+)	255.0500>153.0500	14.324	3256	21384
merycetin	C15H10O8	318.23	(+)	336.2500>46.1500	16.964	4760858	24400217
folic acid	C19H19N7O6	441.14	(+)	442.9000>323.4500	17.501	3233	42208
Rutine	C27H30O16	610.5	(+)	611.2000>73.2000	18.535	95772	1075807
Catechin Hydrate	C15H16O7	308.28	(-)	306.9500>169.1000	13.471	3310	20674
Sinapic Acid	C11H12O5	224.21	(-)	223.0000>208.1500	9.596	1310	7388
4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000>117.1000	0.739	5529	25410
3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000>109.1000	4.659	277883	1116071
caffiec Acid	C9H8O4	180.16	(-)	179.1500>135.0500	8.156	331583	2181219
Cis-p.coumaric Acid	C9H8O3	164.16	(-)	163.1500>119.1500	9.292	645868	3647712
Syringic Acid	C9H10O5	198.17	(-)	196.9500>182.0000	6.153	385	1610
salysilic Acid	C7H6O3	138.12	(-)	137.1000>93.1500	9.589	605822	4468232
gallic Acid	C4H4O4	170.12	(-)	169.1000>125.0500	11.618	2293	36285
Luteonil	C15H10O6	286.24	(-)	284.9500>133.0000	12.452	6601	53498
Hespertin	C16H14O6	302.28	(-)	300.9000>255.2500	12.176	3189	29897
chlorogenique acide -	C16H18O9	354.31	(-)	352.9000>177.1500	15.512	2283557	10287056

Annex 08: LC-MS/MS Phytochemical Profiling Data of *Z. lotus* phenolics

Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Retention Time	Height	Area
hydroxy quinolin	C9H7NO	145.16	+	146.0500>101.0000	7.813	4654	25940
thymol	C10H14O	150.22	+	151.7500>88.1000	0.742	58449	269226
2-Methoxybenzoic Acid	C8H8O3	152.15	+	153.0500>135.0500	0.743	627673	2596147
4-mythoxybenzoic Acid	C8H8O3	152.15	+	153.0500>70.7500	0.743	1017571	3870884
acide coumarique	C9H8O3	164.16	(+)	165.1000>101.2000	0.742	5337391	19949607
kojic Acid	C6H6O4	142.11	(+)	143.0000>69.0500	0	0	0
Ferulic Acid	C10H10O4	194.18	(+)	194.9000>177.1500	10.259	143435	2045887
Naringenin	C15H12O5	272.25	(+)	272.9500>177.0000	13.089	7771	154208
beta carotene	C40H56	536.87	(+)	537.2000>23.1000	12.329	3744	162864
keampferol	C15H10O6	286.24	(+)	287.1000>255.2500	12.278	1084372	17141905
Quercitine	C15H10O7	302.23	(+)	303.1000>262.2000	12.537	3020	17885
valinin	C8H8O3	152.15	(+)	153.1000>65.1500	8.526	5700	145166
Chrysin	C15H10O4	254.24	(+)	255.0500>153.0500	13.194	3570	46410
merycetin	C15H10O8	318.23	(+)	336.2500>46.1500	16.929	2959152	16142570
folic acid	C19H19N7O6	441.14	(+)	442.9000>323.4500	17.446	1283	8694
Rutine	C27H30O16	610.5	(+)	611.2000>73.2000	18.486	66144	494915
Catechin Hydrate	C15H16O7	308.28	(-)	306.9500>169.1000	13.434	2460	16259
Sinapic Acid	C11H12O5	224.21	(-)	223.0000>208.1500	9.561	330	7778
4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000>117.1000	0.745	6046	52625
3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000>109.1000	4.644	144952	619942
caffiec Acid	C9H8O4	180.16	(-)	179.1500>135.0500	8.108	73530	553946
Cis-p.coumaric Acid	C9H8O3	164.16	(-)	163.1500>119.1500	9.248	215226	1470655
Syringic Acid	C9H10O5	198.17	(-)	196.9500>182.0000	6.166	-225	-698
salysilic Acid	C7H6O3	138.12	(-)	137.1000>93.1500	9.556	78965	736660
gallic Acid	C4H4O4	170.12	(-)	169.1000>125.0500	11.788	898	28367
Luteonil	C15H10O6	286.24	(-)	284.9500>133.0000	12.403	32685	309210
Hespertin	C16H14O6	302.28	(-)	300.9000>255.2500	10.774	8987	117194
chlorogenique acide -	C16H18O9	354.31	(-)	352.9000>177.1500	15.479	2374267	11496675

Annex 09: Hydroalcoholic extracts One-Way ANOVA summary table

Variable	SS Between	df Between	MS Between	SS Within	df Within	MS Within	F Value	p Value
DPPH_HA	140725.81	5	28145.16	1124.30	12	93.69	300.40	0.000
FRAP_HA	479632.95	5	95926.59	6299.65	12	524.97	182.73	0.000
Tannins_HA	829.61	5	165.92	40.47	12	3.37	49.20	0.000
Flavonoids_HA	453.41	5	90.68	0.80	12	0.07	1363.98	0.000
Phenolics_HA	44997.14	5	8999.43	58.60	12	4.88	1842.80	0.000
Terpenoids_HA	99.95	5	19.99	1.02	12	0.09	234.27	0.000

Annex 10: Aqueous extract One-Way ANOVA summary table

Variable	SS Between	df Between	MS Between	SS Within	df Within	MS Within	F Value	p Value
FRAP_AQ	836830.35	5	167366.07	22817.11	12	1901.43	88.02	0.000
DPPH_AQ	732670.25	5	146534.05	51216.21	12	4268.02	34.33	0.000
phenolic_AQ	59262.33	5	11852.47	90.49	12	7.54	1571.78	0.000
Flavonoid_AQ	323.71	5	64.74	1.49	12	0.12	520.51	0.000
Terpenoid_AQ	159.37	5	31.87	0.16	12	0.01	2411.81	0.000
Tannin_AQ	859.82	5	171.96	27.05	12	2.25	76.29	0.000

LISTE OF PUBLICATIONS AND COMMUNICATIONS

Papers

1. Riah, A., Saidj, D., Ghanai, R., & Mefti Korteby, H. (2025). Ethnobotanical study of wild forage plants in the semi-arid region of Bordj Bou Arreridj, Algeria. Egyptian Journal of Botany, 65(1), 171–181. https://doi.org/10.21608/ejbo.2024.288314.2841
2. Riah, A., Saidj, D., Riahi, L., Ghanai, R., Mefti Korteby, H., Boussebaa, W., and Driouich Chaouachi, R. Phytochemical, nutritional and antioxidant properties of six nutri-medicinal plants growing in the semi-arid area of Algeria for alternative livestock forage use (got acceptation with minor revision in the journal of *Euro-Mediterranean Journal for Environmental Integration (EMJE)*

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1. Riah, A., Saidj, D., Ghanai, R., Mefti Korteby, H. *Opuncia ficus-indica* (L): Medicinal plant with promicing potential as a fodder resource in semi-arid regions. 09/12/2023. Webinaire, Madrid, Spain.
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3. Riah, A., Saidj, D., Ghanai, R., Mefti Korteby, H. Intégration des plantes médicinales comme fourrages naturels. 19/02/2021. Webinaire. Oran, Algérie.
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1. Riah, A., D., Ghanai, R., Mefti Korteby, H. Analyses qualitatives de 3 plantes médicinales d'intérêt fourrager. 05/06/2023. Blida, Algérie.
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