Bioactive and chemical characterization of medicinal plants

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Abstract

Medicinal plants are used worldwide for the treatment of various diseases, and there is increasing interest in studying their bioactive properties and chemical composition. A deeper understanding of these properties could drive significant advancements in multiple industrial sectors. In this study, we investigated three medicinal plants traditionally used in Brazil and Portugal: *Plantago major* L., *Bidens pilosa* L., and *Pistacia lentiscus* L. The primary objective was to expand scientific knowledge of these species by characterizing their chemical composition and diverse bioactive properties.

The plants were characterized regarding their individual profiles in fatty acids, tocopherols, free sugars, and organic acids through adequate chromatographic methodologies. The hydroethanolic extracts were characterized in terms of phenolic composition and antioxidant and antimicrobial activities. The polyphenolic profile was analyzed by HPLC-DAD-ESI/MS. The antioxidant activity was assessed through the thiobarbituric acid reactive substances (TBARS) formation inhibition. Finally, the antimicrobial activity was assessed by the microdilution method against bacteria and fungi.

P. major exhibited the highest lipid content (1.7 g/100 g dw), followed by *P. lentiscus* and *B. pilosa* (1.2 and 0.6 g/100 g dw, respectively). In all species, palmitic acid (C16:0) was the fatty acid detected in higher relative abundance (between 37.8% and 44.1%). The major tocopherol in *P. major* and *P. lentiscus* was alpha-tocopherol (35.5 and 1.7 mg/100 g, respectively), and gamma-tocopherol in *B. pilosa* (0.09 mg/100 g). *B. pilosa* contained the highest concentration of free sugars (4.5 g/100 g), followed by *P. lentiscus* and *P. major* (3.3 and 0.8 g/100 g, respectively). The most abundant organic acid in *B. pilosa* and *P. lentiscus* was quinic acid (0.76 and 0.46 g per 100 g, respectively). In turn, succinic acid was the most abundant in *P. major* (1.62 g per 100 g), which was also the plant with higher total organic acids. A wide variety of phenolic compounds was identified in all the studied plants. The major phenolic compounds in *P. major*, *B. pilosa*, and *P. lentiscus* were verbascoside, quercetin-dimethyl ether rutinoside, and galloylquinic acid, respectively. Regarding antioxidant activity, the hydroethanolic extract of *P. lentiscus* demonstrated the highest activity, followed by *B. pilosa* and *P. major*. For the antibacterial activity, *P. major* extract exhibited activity against all bacterial strains tested. *P. lentiscus* was effective against *Salmonella enterica*, *Yersinia enterocolitica*, *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*,

while *B. pilosa* was only effective against *Escherichia coli* and *S. enterica*. However, none of the plants showed significant antifungal activity against *Aspergillus braziliensis* and *Aspergillus fumigatus*.

In conclusion, the chemical composition, antioxidant, and antimicrobial activities of *P. major*, *B. pilosa*, and *P. lentiscus* were studied, which contributes to the scientific understanding of their potential. The results obtained in this study enable further investigation into their extracts and the validation of their traditional uses.

Resumo

As plantas medicinais são amplamente utilizadas em todo o mundo para o tratamento de diversas doenças. Por isso, há um interesse crescente no estudo propriedades bioativas e composição química dessas plantas, uma vez que este conhecimento pode impulsionar avanços significativos em vários setores industriais. Neste estudo foram estudadas três plantas medicinais tradicionalmente usadas no Brasil e em Portugal: *Plantago major* L., *Bidens pilosa* L. e *Pistacia lentiscus* L. O objetivo principal foi expandir o conhecimento científico sobre essas espécies, através da caracterização química e avaliação das propriedades bioativas.

As plantas foram caracterizadas quanto aos perfis individuais de ácidos gordos, tocoferóis, açúcares livres e ácidos orgânicos, através das metodologias cromatográficas adequadas. Por sua vez, os extratos hidroetanólicos foram caracterizados em termos de composição fenólica e atividades antioxidante e antimicrobiana. A composição fenólica foi analisada por HPLC-DAD-ESI/MS. A atividade antioxidante foi avaliada através do ensaio de inibição da formação de substâncias reativas ao ácido tiobarbitúrico (TBARS). Por fim, a atividade antimicrobiana foi avaliada pelo método de microdiluições sucessivas em bactérias e fungos.

Quanto ao teor lipídico a *P. major* apresentou o maior teor (1,7 g/100 g extrato), seguido da *P. lentiscus* e da *B. pilosa* (1,2 e 0,6 g/100 g extrato, respectivamente). Em todas as espécies estudadas, o ácido palmítico (C16:0) foi o ácido gordo detetado em maior abundância relativa (entre 37,8% e 44,1%). O alfa-tocoferol foi o tocoferol detetado em maior concentração na *P. major* e na *P. lentiscus* (35,5 e 1,7 mg/100 g, respectivamente), enquanto o gama-tocoferol foi a isoforma que exibiu uma maior concentração na *B. pilosa* (0,09 mg/100 g). A *B. pilosa* exibiu ainda a maior concentração de açúcares livres (4,5 g/100 g), seguida da *P. lentiscus* e da *P. major* (3,3 e 0,8 g/100 g, respectivamente). O ácido orgânico mais abundante na *B. pilosa* e *P. lentiscus* foi o ácido quínico (0,76 e 0,46 g por 100 g, respectivamente). Por outro lado, o mais abundante em *P. major* foi o ácido succínico (1,62 g por 100 g).

Uma ampla variedade de compostos fenólicos foi identificada em todas as plantas estudadas. Os compostos fenólicos detetados em maior concentração no extrato hidroetanolico da *P. major*, *B. pilosa* e *P. lentiscus* foram verbascosídeo, quercetina-dimetil éter rutinosídeo e ácido galoilquínico, respectivamente. Em relação à atividade antioxidante, o extrato hidroetanólico da *P. lentiscus* demonstrou a maior atividade,

seguido da *B. pilosa* e da *P. major*. Quanto à atividade antibacteriana, o extrato de *P. major* exibiu atividade contra todas as cepas bacterianas testadas. A *P. lentiscus* demonstrou atividade contra a *Salmonella enterica*, *Yersinia enterocolitica*, *Bacillus cereus*, *Listeria monocytogenes* e *Staphylococcus aureus*, enquanto a *B. pilosa* foi eficaz apenas contra a *Escherichia coli* e a *S. enterica*. No entanto, nenhuma das plantas mostrou atividade antifúngica significativa contra a *Aspergillus braziliensis* e a *Aspergillus fumigatus*.

Em conclusão, foram estudadas a composição química, as atividades antioxidante e antimicrobiana de *P. major*, *B. pilosa* e *P. lentiscus*, o que contribui para o entendimento científico de seu potencial. Os resultados obtidos neste estudo permitem novas investigações sobre seus extratos e a validação de seus usos tradicionais.

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Abbreviations and acronyms

AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
ABTS	2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Variance analysis
APAP	Acetaminophen
AUC	Area under the curve
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
COX-1	Cyclooxygenase 1
COX-2	Cyclooxygenase 2
DAD	Diode array detector
DNP	2,4-dinitrophenylated
DPPH	2,2-diphenyl-1-picrylhydrazyl
dw	Dry weight
ESI/MS	Electrospray ionization–mass spectrometry
FAME	Fatty acid methyl esters
FRAP	Ferric reducing antioxidant power
HPLC	High-performance liquid chromatography
IL-1α	Interleukin 1α
IL-1β	Interleukin 1β
IL-6	Interleukin 6
INT	*p*-iodonitrotetrazolium violet
LPS	Lipopolysaccharide
MBC	Minimum bactericidal concentration
MDA	Malondialdehyde
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
m/v	Mass/volume
m/z	Mass to charge ratio
NBT	Nitroblue tetrazolium
NF-kB	Nuclear factor kappa beta
ORAC	Oxygen radical absorbance capacity
PBS	Phosphate-buffered saline
PDA	Photodiode array detector

RI	Refractive index
ROS	Reactive oxygen species
RP	Reducing power
rpm	Rotations per minute
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TF	Total flavonoids
THP-1	Human monocytic cell line
TNF- α	Tumor necrosis factor α
TPA	Total phenolic acids
TPC	Total phenolic compounds
TSB	Trypic soy broth
UFLC	Ultra-fast liquid chromatography
v/v	Volume/volume

1. Introduction

1.1. Medicinal plants: history and traditional uses

Across the world, medicinal plants are used to treat several diseases. Historical references indicate that the population has used these species for over 60,000 years (Azevedo & Ferreira, 2011). Without precise medical knowledge, plants were classified according to their medicinal effects on consumers and were used to prevent and reduce symptoms of many diseases (Rico, 2018). In the last year, the World Health Organization estimated that 80% of the global population depends on plant extracts or their active components for traditional folk medicine therapies (World Health Organization, 2023). With the development of technologies and scientific research, there has been increasing interest in the identification, isolation, and bioactive study of species' chemical composition, contributing to increased knowledge of their properties and, consequently, their potential applications (Saddiqe, 2019). However, empirical data regarding the efficacy and safety of medicinal plants and their metabolites is still poorly understood.

According to the literature, the wide variety of bioactive compounds detected in medicinal plants is suggested to be primarily responsible for the diverse therapeutic effects that have been demonstrated. Properties such as antioxidant, immunomodulatory, antimicrobial, anti-inflammatory, anticoagulant, and antitumor activity, among others, have been widely described (Morguette et al., 2023; Pereira et al., 2015). In line with the promising potential that has been demonstrated, the study of these plants' biologically active phytochemical compounds could constitute fundamental advances for several industrial sectors, such as pharmaceuticals, energy, and food (Nyakudya et al., 2020).

Brazil ranks among the world's most biodiverse countries, home to more than 40,000 plant species, many of which are considered medicinal plants by ancient communities, yet remain unexplored (Santos et al., 2024). According to the Oswaldo Cruz Foundation, a database of approximately 300 species of medicinal plants has been created, gathering information on scientific data and traditional uses (Fiocruz, 2022). Furthermore, over 3,900 plant species are described in Portugal, about 500 of them are medicinal plants (Rocha et al., 2017). Overall, these studies highlight the extensive use and diversity of medicinal plants in both countries, reflecting their rich ethnobotanical heritage.

In this sense, three plant species (*Plantago major* L., *Bidens pilosa* L., and *Pistacia lentiscus* L.) were studied according to their uses in traditional medicine, in Brazil and Portugal. Studies regarding their chemical composition and bioactive properties are quite scarce.

1.2. *Plantago major* L.

1.2.1. Botanical description

P. major L. (**Figure 1**), commonly known as greater or common plantain, is an invasive and medicinal species that belongs to the genus *Plantago* and the family *Plantaginaceae* (Keivani et al., 2021). It is a perennial herbaceous plant that can reach a height of around 15 cm. The leaves of *P. major* are oval and distributed in a rosette, while the inflorescences are long, non-ramified spikes with a brownish-green color. This plant is wind-pollinated, producing a substantial quantity of small, ovate seeds (Lyu et al., 2023).

Figure 1 *P. major* L. Source: *Flora-On*.

Despite being native to Europe and Asia, *P. major* is also found in northern and southern Africa, America, Australia, and New Zealand. This species has been introduced in many parts of the world due to its high resistance to environmental stress and its capacity to withstand a wide range of temperatures, pH levels, soil types, and humidity,

as well as pollution, pests, and diseases (Skrynetska et al., 2019). In traditional medicine, *P. major* seeds are used to treat respiratory and intestinal diseases, while its leaves are used due to their wound-healing properties (Kuiper & Bos, 2012). To better understand the medicinal applications of *P. major*, it is essential to study its bioactive properties and chemical composition using scientific methodologies.

1.2.2. Chemical composition

According to the literature, *P. major* exhibits a wide variety of bioactive compounds, including phenolic compounds, terpenoids, polysaccharides, tocopherols and organic acids (Haghighi et al., 2021). Flavonoids are one of the most widely described classes of phenolic compounds, particularly luteolin and apigenin derivatives (Adom et al., 2017). Beara et al. (2009) reported the presence of luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, and rutin in the methanolic extract of the aerial parts. The presence of two phenolic acids, plantamajoside and verbacoside, was also reported in the hydroethanolic extracts of the whole plant (Skari et al., 1999).

Several terpenoids were also detected in the aerial parts of *P. major*. Lupeol, β-amyrin, β-sitosterol, and α-amyrin were identified in the methanolic extract of the leaves (Turgumbayeva et al., 2022), while isoborneol was found in the essential oils of the leaves (Haghighi et al., 2022). The polysaccharide composition of *P. major* leaves was also assessed by Lukova et al. (2017), who described arabinose, rhamnose, galactose, and galacturonic acid as the most abundant monosaccharides (37.36%, 16.96%, 46.11%, and 62.64%, respectively).

The presence of vitamin E was reported only in *P. major* seeds, in the forms of alpha and gamma tocopherols, with alpha-tocopherol being the most abundant one (between 524.53 and 754.67 μg/g) (Wang et al., 2017). Moreover, Olennikov (2005) identified malic and succinic acid in *P. major* leaves, with succinic acid being the most abundant organic acid.

The studies described in the literature are quite scarce, with the majority of data published more than a decade ago. The chemical characterization of the species using emerging chromatographic methodologies is extremely important for the comprehensive understanding of its potential.

1.2.3. Bioactive properties

P. major is a medicinal plant widely used in traditional medicine. **Table 1** summarizes the studies regarding the bioactive properties of *P. major* extracts, particularly its antioxidant, antimicrobial, anti-inflammatory, and wound healing potential.

Table 1 Summary of the research findings on *P. major* L. bioactivities.

Part used	Extract	Assay	Main results	Reference
Antioxidant				
Leaves, seeds	Water, ethanol	DPPH assay (% of inhibition)	70-90%	Mohamed et al. (2011)
Leaves	70% ethanol	DPPH assay (µg/µg)	IC_{50} = 0.85 µg/µg	Stanisavljević et al. (2008)
Aerial parts	80% methanol	DPPH, FRAP, OH^- and NO scavenger capacity, reduction of NBT, lipid peroxidation (µg/mL)	IC_{50} = 5.35 – 340 µg/mL	Beara et al. (2009)
Aerial parts	75% ethanol	TBARS (mg/mL)	IC_{50} = 3.4 mg/mL	Çoban et al. (2003)
Aerial parts	50% ethanol	DPPH assay (% of inhibition), mitochondrial ROS inhibition (% of inhibition)	40% and 55%, respectively	Mello et al. (2015)
Antimicrobial				
Leaves	95% ethanol, methanol, or hot water	Disk diffusion (*Staphylococcus aureus, Pseudomonas aeruginosa, Candida glabrata, Candida albicans*) (zone of inhibition)	10 – 20 mm	Soliman et al. (2022)
Leaves	10% ethanol	Disk diffusion (*P. aeruginosa*) (zone of inhibition)	9.93 – 22.18 mm	Monawer & Mammani (2023)
Leaves	70% ethanol	Disk diffusion (*Bacillus subtilis*, *S. aureus*, *Escherichia coli*, *P. aeruginosa*, *Saccharomyces cerevisiae*, *C. albicans*, *Aspergillus niger*) (zone of inhibition)	10.1 – 23.5 mm	Stanisavljević et al. (2008)
Anti-inflammatory				
Leaves	Water, 50% ethanol	NF-kB assay with LPS induced oral epithelial cells	Reduced activation of NF-kB pathway	Zubair et al. (2019)
Aerial parts	80% methanol	COX-1 and 12-LOX inhibition in human platelets (mg/mL)	IC_{50} = 0.65 – 1.73 mg/mL	Beara et al. (2010)
Roots, stem, and leaves	Dichloromethane, ethanol	COX-1 and COX-2 catalyzed prostaglandin biosynthesis assay (% of inhibition)	21.7 – 59%	Stenholm et al. (2012)
Leaves	60% ethanol, 60% methanol, deionized water	Sprague Dawley rats (IL-1α, IL-1β, TNF-α inhibition, % of inhibition)	12.23 – 26.74%	Hussan et al. (2015)
Wound healing				
Seeds	70% ethanol	Porcine wound healing model	Increased wound healing	Zubair et al. (2016)
Leaves	70% ethanol	Mice of Swiss line wound model	100% of wound closure	Thomé et al. (2012)
Leaves	50% ethanol	Scratch assay with oral epithelial cell line H400	100% scratch cover	Zubair et al. (2012)
Leaves	Ethanol	Wistar rats wound model	100% of wound closure	Kartini et al. (2021)

DPPH – 2,2-diphenyl-1-picrylhydrazyl; FRAP – ferric reducing antioxidant power; NBT – nitroblue tetrazolium; NO – nitric oxide; TBARS – Thiobarbituric acid reactive substances; ROS – reactive oxygen species; NF-kB – nuclear factor kappa beta; COX-1 – cyclooxygenase 1; COX-2 – cyclooxygenase 2; 12-LOX – lypoxigenase; IL-1α – interleukin 1α; IL-1β – interleukin 1β; TNF-α – tumor necrosis factor α; IC_{50} – extract concentration responsible for 50% of inhibition.

The antioxidant capacity was evaluated using several *in vitro* methodologies. The most described method is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay; however, other assays, such as ferric reducing antioxidant power (FRAP), hydroxyl and nitric oxide (NO) scavenger capacity, reactive oxygen species (ROS) inhibition, and lipid peroxidation, have been also described.

Beara et al. (2009) evaluated the antioxidant potential of an 80% methanolic extract of *P. major*, which showed significant DPPH scavenging activity (IC_{50} = 5.35 µg/mL) compared to the synthetic antioxidant butylated hydroxytoluene (BHT, IC_{50} = 8.28 µg/mL). The extract also exhibited higher superoxide (O_2) and NO scavenging activity (IC_{50} values of 25.70 µg/mL and 0.34 mg/mL, respectively) when compared to other Plantago species (i.e., *Plantago aregentea*, *Plantago holosteum*, *Plantago maritima*, and *Plantago media*). In another study, Mohamed et al. (2010) compared the antioxidant activities of 70% ethanolic and hot and cold-water extracts of *P. major* leaves with *Cyamopsis tetragonoloba* L. (guar) seeds through DPPH assay. *P. major* showed higher antioxidant activity (70-90%) than guar seeds (20-30%) and slightly higher than BHT (80%). Also, Mello et al. (2015) described a promising antioxidant activity in ethanolic extracts of aerial parts, demonstrating a cell viability improvement through the reduction of ROS generation in both rat liver mitochondria cells and human liver HepG2 cells. This plant's potential to inhibit lipid peroxidation through thiobarbituric acid reactive substances (TBARS) was studied by Çoban et al. (2003). In this case, 75% ethanolic extract of the aerial parts showed an IC_{50} of 3.4 mg/mL and a similar inhibition percentage compared to the positive control alpha-tocopherol (69% and 77% for plant and control, respectively).

The antimicrobial activity of *P. major* extracts was mainly assessed using the disk diffusion method. The authors described that these extracts have an interesting potential of inhibiting the growth of several microorganisms, including both bacteria and fungi. Soliman et al. (2022) tested *P. major* leaf extracts (95% ethanol, 95% methanol, and aqueous) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial strains and the fungi *Candida glabrata* and *Candida albicans*. The methanolic and aqueous extracts inhibited the growth of both bacteria and *C. glabrata* (zones of inhibition between 9 and 20 mm). In contrast, the ethanolic extract was only efficient against *S. aureus* (zones of inhibition between 10 and 15 mm). Similarly, Monawer et al. (2023), evaluated the ethanolic extract of the plant against *P. aeruginosa* isolated from burn infections and compared its activity with commercial antibiotics (e.g., piperacillin, ceftazidime,

cefepime, meropenem, ofloxacin, tobramycin). The observed zone of inhibition increased with increasing extract concentration, while the commercial antibiotics showed no efficacy against the studied pathogen. Stanisavljević et al. (2008) demonstrated that the 70% ethanolic extract of the leaves also inhibits the bacterial strains *Bacillus subtilis*, *S. aureus*, *Escherichia coli*, and *P. aeruginosa* and the fungi *Saccharomyces cerevisiae* and *C. albicans*; however, it did not inhibit *Aspergillus niger*.

The anti-inflammatory activity of aqueous and ethanolic extracts of *P. major* leaves, as well as their combinations, were tested through the nuclear factor kappa beta (NF-kB) assay on oral epithelial cells (H400 cell line) (Zubair et al. 2019). All solvent combinations (e.g., water, ethanol, water, and ethanol) and concentrations (between 0.01 and 1 mg/mL) exhibited the ability to inhibit the inflammatory mediator. The authors suggested that the observed activity could be related to the higher concentration of compounds such as polyphenols (Zubair et al., 2019). The capacity to inhibit the enzymes cyclooxygenase 1 (COX-1) and 2 (COX-2) was used to evaluate the anti-inflammatory activity of heptane, dichloromethane, and ethanol extracts of *P. major* leaves, roots, and stems. The authors showed that dichloromethane extracts inhibited COX-1 more effectively than heptane and ethanol extracts (56.5%, 47%, and 51.7%, respectively), while for COX-2, ethanol extracts (59%) exhibited higher inhibitory capacity than heptane and dichloromethane (21.7% and 46%, respectively) (Stenholm et al., 2013). A similar study evaluated the inhibition of COX-1 and 12-lipoxygenase (12-LOX) using human platelets, of the 85% ethanolic extract of *P. major* and *Plantago lanceolata* with aspirin and quercetin as standards for COX-1 and 12-LOX, respectively. Both extracts demonstrated an inhibitory capacity against COX-1 and 12-LOX (IC_{50} between 0.65 and 2.0 mg/mL), higher than the standards used (IC_{50} = 0.001 and 0.084 mg/mL for aspirin and quercetin, respectively) (Beara et al., 2010). Also, Hussan et al. (2015) evaluated the ethanolic and methanolic extracts of *P. major* leaves' anti-inflammatory capacity in Sprague Dawley rats induced with acetaminophen (APAP), focusing on the reduction of interleukins 1α and 1β (IL-1α and IL-1β) and tumor necrosis factor-α (TNF-α). The authors concluded that the normal group showed significantly higher concentrations of these inflammatory cytokines (2, 1.1, and 14 pg/mL of IL-1α, IL-β, and TNF-α, respectively) than the methanolic extracts (0.6, 0.5, 7 pg/mL of IL-1α, IL-β, and TNF-α, respectively).

One of the most common uses of *P. major* in traditional medicine is its wound-healing potential. Several studies have evaluated this capacity using *in vitro*, *ex vivo*, and

in vivo assays, demonstrating the plant´s interesting potential to promote wound closure (Zhakipbekov et al., 2023). Thomé et al. (2012) studied a 70% ethanolic extract of the leaves through an *in vivo* assay with Swiss mice. The animals treated with *P. major* extracts showed better wound closure and re-epithelization compared to control animals treated with collagenase ointment. Also, Kartini et al. (2021) used Wistar rats as a wound model and compared three different treatments: with the plant leaf ethanolic extract, with ursolic acid, and with oleanolic acid (all extracted from the plant). The positive control used was a Chinese commercial gel Mebo®, while a gel without active ingredients was the negative control. The results showed that treatment with the plant extract took 16 days to achieve epithelization, demonstrating an effectiveness comparable to the positive control, which takes 19 days. The fastest wound closure was achieved with the gel containing oleanolic acid, although the gel with the plant extract also reduced the inflammation in the wounded area. In turn, a study by Zubair et al. (2012) utilized an *in vitro* scratch assay with the oral epithelial cell line H400 to demonstrate that 50% ethanol extracts could promote scratch coverage. The same authors conducted another study comparing the wound-healing properties of *P. major* leaf extracts (water and ethanol) through an *ex vivo* porcine model. In this case, the ethanol-based extracts exhibit higher efficacy in wound healing than water extracts, both at a concentration of 1.0 mg/mL. The authors identified plantamajoside, verbascoside, aucubin, and ursolic acid as the main compounds responsible for the demonstrated activity (Zubair et al., 2016).

1.3. *Bidens pilosa* L.

1.3.1. Botanical description

B. pilosa L. is a small plant belonging to the Asteraceae family (**Figure 2**). This species is commonly known as *picão-preto*, *malpica*, *pica-pica*, or Spanish needles (Bartolome et al., 2013; Borges et al., 2013; Silva et al., 2011). It exhibits high reproductive potential and the ability to adapt and resist adverse environmental conditions, including different soil types and moisture levels, as well as both low and high elevations. These characteristics have contributed to its widespread distribution.

Despite being considered native to South America, *B. pilosa* can also be found in tropical and subtropical areas, such as Brazil, Peru, Colombia, Kenya, China, Uganda, Australia, and Hawaii (Arthur et al., 2012; Xuan & Khanh, 2016).

Figure 2 *B. pilosa* L. Source: Flora on.

B. pilosa is an erect perennial herb with a quadrangular stem and lateral branches, both green and smoothly marked with parallel lines. The leaves are opposite, with sharply serrated ovate leaflets that are slightly hairy, leading to white or yellowish flowers. The seeds, or achenes, are dark brown or black, straight and long, playing a crucial role in the wind dispersion and propagation of the plant (Bairwa et al., 2010).

All parts of *B. pilosa* have been used as food ingredients and in traditional medicine (Xuan & Khanh, 2016). This species is traditionally used to treat headaches, ear infections, kidney problems, flatulence, stomach and mouth ulcers, arterial hypertension, diabetes, diarrhea, hemorrhoids, hangovers, and malaria, as an antidote for poisoning, and for treating wounds (Abajo et al., 2004; Taylor, 2005).

1.3.2. Chemical composition

According to the literature, *B. pilosa* is rich in phenolic compounds, fatty acids, polyacetylenes, terpenoids, and alkaloids, commonly identified in its leaves, stems, and roots (Ajanaku et al., 2018; Son et al., 2022).

Regarding the phenolic composition of *B. pilosa*, a wide variety of phenolic acids and flavonoids have been described. Kusano et al. (2003) identified 3-*O*-caffeoylquinic acid (17 mg/g dw), 3,4-di-*O*-caffeoylquinic acid (21 mg/g dw), 3,5-di-*O*-caffeoylquinic (18 mg/g dw), 4,5-di-*O*-caffeoylquinic acid (17 mg/g dw), and rutin (18 mg/g dw) as the

most abundant compounds in the aerial parts. Idris et al. (2023) identified hydroxybenzoic and hydroxycinnamic acids, and flavonoids in the 70% ethanolic extract, with caffeic, ferulic, and coumaric acids as the most abundant ones. Similarly, Nguyen et al. (2023) studied the aqueous, methanolic, acetonic, and ethyl acetate leaf extracts, and the most abundant phenolic compounds were: *p*-coumaric acid (between 0.25 and 7.85 mg/g of extract), salicylic acid (between 0.48 and 6.70 mg/g of extract), and gallic acid (between 1.90 and 2.20 mg/g of extract). Also, Angelini et al. (2021) identified some phenolic compounds in *B. pilosa* extracts, among them gallic and chlorogenic acids, and epicatechin.

The composition of polyacetylenes in *B. pilosa* includes at least 37 polyacetylenes and derivatives. Most of these are aliphatic, containing triple or double bonds, along with cyclic, aromatic, and glucoside rings or heterocyclic end groups. The major polyacetylenes identified were 1,2-dihydroxytrideca-5,7,9,11-tetrayne, 1,3-dihydroxy-6(*E*)-tetradecene-8,10,12-triyne, 2-β-d-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7,9,11-triyne, 3-β-d-glucopyranosyloxy-1-hydroxy-6(*E*)-tetradecene-8,10,12-triyne, cytopiloyne, and pilosol (7-phenyl-hepta-4,6-diyne-2-ol) (Chang et al., 2013; Chang et al., 2004; Chiang et al., 2007; Wu et al., 2004).

Moreover, terpenoids have been detected in the essential oils of *B. pilosa* flowers and leaves, with β-caryophyllene (5.1% and 10.9%, respectively) and τ-cadinene (6.13% and 7.82%, respectively) being the most abundant compounds (Deba et al., 2008). Fatty acids were detected in higher abundance in the plant's roots than in the leaves (Angelini et al., 2021). However, Quaglio et al. (2020) studied the aerial parts of *B. pilosa* and reported palmitic, oleic, and linoleic acids as the major fatty acids (30%, 27%, and 24.3%, respectively).

Studies have also described the presence of alkaloids in both the whole plant ethanolic extract and various leaf extract fractions (i.e., hexane, dichloromethane, ethyl acetate, and methanol) (Ajanaku et al., 2018; Son et al., 2022).

1.3.3. Bioactive properties

Several biological properties of *B. pilosa* extracts have been studied, including antioxidant, antimicrobial, anti-inflammatory, and wound healing (Chang et al., 2007; Masako & Yoshiyuki, 2006). **Table 2** summarizes the literature regarding B. pilosa bioactive properties, and the methodologies used in those studies.

Table 2 Overview of bioactive properties of *B. pilosa* L.

Part used	Extract	Method	Main results	Reference
Antioxidant				
Whole plant	99% ethanol	DPPH, ABTS, RP, Phosphomolybdenum method (µg/mL)	EC_{50} = 139.14 – 462.09 µg/mL	Son et al. (2022)
Aerial parts	Water	Hemolysis of human erythrocytes (mg/mL)	IC_{50} = 1.19 mg/mL	Abajo et al. (2004)
Whole plant	70% ethanol	DPPH and superoxide scavenging activity (µg/mL)	IC_{50} = 11.43 – 100 µg/mL	Chiang et al. (2004)
Leaves	70% ethanol	DPPH, ABTS	77.1% and 83.24%, respectively	Falowo et al. (2017)
Leaves, flowers	Essential oil, water extract	DPPH (µg/mL), β-carotene bleaching assay	IC_{50} = 47 – 172 µg/mL	Deba et al. (2008)
Leaves, flowers	Water, methanol, acetone, ethyl acetate	DPPH and ABTS (% of inhibition)	68.07 – 83.36%	Nguyen et al. (2023)
Antimicrobial				
Roots	Water, acetone, methanol	Agar dilution method for bacteria (*S. aureus*, *Staphylococcus epidermidus*, *Bacillus cereus*, *Micrococcus kristinae*, *Streptococcus faecalis*, *E. coli*, *P. aeruginosa*, *Shigelia flexneri*, *Klebsiella pneumoniae*, *Serratia marcescens*), and fungus (*A. niger*, *Aspergillus flavus*, *Penicillium notatum*, and *C. albicans*) (mg/mL)	MIC = 5 – 10 mg/mL	Ashafa & Afolayan (2009)
Whole plant	Water, ethanol, hexane	Agar diffusion method (*S. aureus*, *P. aeruginosa*, *E. coli*, *Streptococcus β hemolytic*, *B. cereus*, and *C. albicans*) (µg/mL)	MIC = 1.8 – 47.1 µg/mL	Rojas et al. (2006)
Leaves, flowers	Steam-distillation (essential oil), water extract	Disk diffusion method (*B. subtilis*, *B. cereus*, *Bacillus pumilus*, *E. coli* and *Pseudomonas ovalis*) (mm of inhibition), agar dilution method (*Fusarium solani*, *Fusarium oxysporum* and *Corticum rolfsii*)	7.7 – 20.3 mm 33.1 – 98.0% inhibition	Deba et al. (2008)
Leaves	Hexane, ethyl acetate, dichloromethane, methanol	Agar diffusion (*Bacillus spp*., *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *C. albicans*, and *Rhizopus sp.*) (zone of inhibition)	10 – 40 mm	Ajanaku et al. (2018)
Whole plant	99% ethanol	Agar diffusion method (*Aeromonas dhakensis*, *Aeromonas hydrophyla*, *Edwardsiella ictalurid*, *Streptococcus agalactiae*, *Colletotrichum sp*., and *F. oxysporum*)	MIC = 625 – 1250 µg/mL 100% of inhibition	Son et al. (2022)
Anti-inflammatory				
Aerial parts	Water	DNP-ascaris induced Wistar rats	Suppressed IgE production	Masako & Yoshiyuki (2006)
Whole plant	70% ethanol	NO inhibition using LPS-induced RAW 264.7 cells (µg/mL)	IC_{50} = 36.2 – 250.8 µg/mL	Chiang et al. (2004)
Whole plant	80% ethanol	LPS induced macrophages	Suppressed IgE production	Yan et al. (2022)
Wound healing				
Leaves	90% ethanol	Excision wound model on Wistar albino rats	15 days for total healing	Hassan et al. (2011)
Leaves	Methanol, cyclohexane, and methylene chloride	Excision wound model on Wistar albino rats	100% inhibition of gastric ulcer	Tan et al. (2000)

DPPH – 2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); RP – reducing power; AAPH - 2,2'-Azobis(2-amidinopropane) dihydrochloride; DNP – 2,4-dinitrophenylated; LPS – lipopolysaccharide; IgE – immunoglobin E; IC_{50} – extract concentration responsible for 50% of inhibition; EC_{50} – extract concentration responsible for half of the maximum activity; MIC – Minimum inhibitory concentration; NO – Nitric oxide.

B. pilosa has demonstrated a promising antioxidant potential. Several studies have shown that different parts of the plant exhibit significant antioxidant activity, often comparable to or higher than positive controls. Son et al. (2022) and Chiang et al. (2004) evaluated the antioxidant capacity of the hydroethanolic extract from the whole plant through several *in vitro* assays, such as DPPH, ABTS, reducing power, total antioxidant capacity (TAC), and superoxide scavenging activity. These studies described a concentration-dependent increase of its antioxidant capacity. Besides, Abajo et al. (2004) demonstrated that the aqueous extracts exhibit the capacity to inhibit AAPH-induced hemolysis in erythrocytes, with an IC_{50} value of 1.19 mg/mL. In turn, Deba et al., (2008) compared the antioxidant capacity of flowers and leaves aqueous extracts and essential oils. Essential oils of both flower and leaves exhibit stronger DPPH free radical scavenging activity than the aqueous extract (IC_{50} values equal to 50, 47, 172, and 61 µg/mL, respectively). Both extracts and essential oils exhibited a strong activity in the β-carotene bleaching assay, although the results were not superior to the antioxidant activity of the positive controls (α-tocopherol and BHT). More recently, Nguyen et al. (2023) evaluated the antioxidant activity of various extracts (i.e., water, methanol, acetone, and ethyl acetate) from the flowers and leaves of *B. pilosa*. For the ABTS assay, the flower methanolic extract and the leaf acetone extract showed higher free radical inhibition (72.17% and 68.07%, respectively) when compared with the other extracts. However, in the DPPH assay, the highest activity was observed for the flowers' water, acetone, and ethyl acetate extracts, as well as the leaves methanolic and ethyl acetate extracts (activity ranging from 76.38% to 83.36%). Similarly, Falowo et al. (2017) obtained percentages of DPPH and ABTS inhibition for the leaves ethanolic extract (77.1% and 83.24%, respectively), which were also similar to the control BHT (70.6% and 85.0%). The leaves extract was able to reduce TBARS levels on fresh beef samples (1.14 and 0.85 µg MDA/g for negative control and extract, respectively), indicating higher inhibition of lipid peroxidation than the positive control BHT (0.98 µg MDA/g).

The antimicrobial properties of *B. pilosa* have also been studied, usually expressed in terms of minimum inhibitory concentration (MIC) or percentage of inhibition, using methods such as agar or disk diffusion and agar dilution. Ajanaku et al. (2018) observed that methanolic extracts from the leaves of *B. pilosa* inhibited the growth of various microorganisms, including the bacteria *Bacillus spp.*, *E. coli*, *P. aeruginosa*, *S. aureus*, *Klebsiella pneumoniae*, and the fungi *C. albicans* (MIC values between 3.125 and 6.25 mg/mL). Son et al. (2022), observed that ethanolic extracts inhibited aquatic and plant

pathogenic bacteria and fungi, with MIC values ranging from 0.625 to 2.5 mg/mL. Similarly, Ashafa & Afolayan, (2009) showed that methanolic root extract inhibits both Gram-positive and Gram-negative bacteria, with *S. aureus* and *E. coli*, demonstrating higher susceptibility (MIC = 5.0 mg/mL). The same study reported antifungal activity, with acetone, methanol, and water extracts resulting in total inhibition of *Penicillium notatum* fungi at a concentration of 0.1 mg/mL. Also, Rojas et al. (2006) described similar results, demonstrating that ethanolic extract of the whole plant inhibited *S. aureus*, *Bacillus cereus*, and *E. coli* (MICs of 36.3, 27.7, and 8.2 μg/mL, respectively).

The anti-inflammatory activity of *B. pilosa* has been evaluated through both *in vitro* and *in vivo* assays. Yan et al., (2022) demonstrated that hydroethanolic extracts significantly inhibit NO and IL-6 in LPS-stimulated murine macrophage RAW 246.7 cells (decrease of 30% to 70%, respectively). Additionally, Masako & Yoshiyuki (2006) described that aqueous extracts of *B. pilosa* administrated to Wistar rats reduced the production of immunoglobin E (IgE) and modulated the T-cells' immune responses, exhibiting immunoregulatory effects.

Wound healing properties have also been associated with *B. pilosa*. Hassan et al. (2011) studied the effects of 90% ethanol extracts from the plant's leaves on excision wounds in Wistar albino rats, showing faster wound closure and epithelialization compared to a neomycin cream control. In turn, Tan et al. (2000) studied the potential of methanol, cyclohexane, and methylene chloride extracts of *B. pilosa* in Wistar rats healing gastric lesions and observed that the methylene chloride extract at 750 mg/kg provided 100% inhibition of gastric ulceration.

1.4. *Pistacia lentiscus* L.

1.4.1. Botanical description

P. lentiscus L., commonly known as the mastic tree or Aroeira, belongs to the Anacardiaceae family and is native to the Mediterranean region (**Figure 3**) (Henning & Raab-Straube, 2016). The geographic distribution of this species includes North Africa, the western Iberian Peninsula, southern France, Turkey, and the Canary Islands (Codif Recherche & Nature, 2016). Well-adapted to Mediterranean climate conditions, *P. lentiscus* thrives in dry, rocky soils, and elevated temperatures (Barra et al., 2007).

Figure 3 *P. lentiscus* L. Source: Jardim Botânico da UTAD.

This dioecious shrub has separate male and female flowers on independent trees, pollinated by the wind. The leaves are alternate, typically consisting of three to five leaflets, while the fruit is a small round drupe that matures from red to dark brown (Rauf et al., 2017). The bark produces a resin widely used for medicinal applications and in the production of mastic gum (Pachi et al., 2020).

Traditionally, the nuts of *P. lentiscus* are consumed for their nutritional value and have been used in traditional medicine to reduce coronary heart diseases, high blood pressure, cancer, inflammation, gallstones, and diabetes (Dreher, 2012). Other parts of the plant are also used to treat conditions such as hepatitis, jaundice, gastrointestinal, urinary, respiratory, and dental diseases (Dimas et al., 2012; Ljubuncic et al., 2005; Rauf et al., 2017). Given its extensive use in traditional medicine, the exploration of the plant's chemical composition and bioactive properties is essential to better understand and validate its traditional applications.

1.4.2. Chemical composition

The main compounds identified in *P. lentiscus* are phenolic compounds and terpenes. Although fatty acids, tocopherols, and organic acids were also identified in this plant's leaves, seeds, and fruits.

Garofulić et al. (2020) identified more than 34 phenolic compounds in the leaves and fruit extracts obtained by optimized Microwave-Assisted Extraction. In the fruit extracts, myricetin glucuronide (251.44 mg/100 g), myricetin rutinoside (172.63 mg/100 g), and quercetin-3-glucoside (156.61 mg/100 g) were the most abundant, while the leaf

extracts showed higher concentrations of myricetin rhamnoside (1782.39 mg/100 g), myricetin glucuronide (750.00 mg/100 g), and digalloylquinic acid (605.88 mg/100 g). Similarly, Dragović et al. (2020) analyzed methanolic and ethanolic extracts of *P. lentiscus* leaves, identifying seven phenolic acids and five flavonol glycosides. With the phenolic acids 5-*O*-galloyl-quinic acid and monogalloyl glucose present in higher concentrations (between 28.9 and 186.5 mg/L in the methanolic extract and between 2.6 and 104.4 mg/L in the ethanolic extract). The major flavonol glycosides detected included myricetin-3-*O*-rhamnoside and myricetin-3-*O*-glucoside (between 17.9 and 97.5 mg/L in the methanolic extract and between 8.12 and 74.5 mg/L in the ethanolic extract). Both extracts exhibited higher concentrations of phenolic acids (between 17.31 and 457.2 mg/mL) than flavonol glycosides (between 34.1 and 183.7 mg/L).

Additionally, the essential oil and mastic gum of this species has been characterized by their phenolic composition. Studies have highlighted significant concentrations of triterpenoids, monoterpenes, and sesquiterpenes (Rauf et al., 2017). Mastic gum , in particular, contains high amonts of two triterpenic acids, 24Z-isomasticadienonic acid and 24Z-isomasticadienolic acid, along with their isomers (masticadienonic and masticadienolic acid), as well as oleanonic and moronic acids (Lemonakis et al., 2011).

Regarding the fatty acid composition, Harrat et al. (2018) reported alpha-linolenic and linoleic acids as the most abundant in *P. lentiscus* leaves (between 20.92 – 48.92%, and 10.94 – 16.99%, respectively). In turn, Brahmi et al. (2020) described oleic, palmitic, and linoleic acids as the most abundant in the plant's seeds and fruits (between 19.49 – 53.13%, 18.43 – 20.34%, and 18.85 – 42.5%, respectively). Vitamin E was also detected in *P. lentiscus* leaves, being alpha-tocopherol the most abundant form (58.51 – 89.17% on dw), followed by delta-tocopherol (7.59 – 27.47% on dw), and beta-tocopherol (3.24 – 14.02% on dw). Furthermore, according to Hajji-Hedfi et al. (2024), quinic acid is one of the major constituents of *P. lentiscus* leaves (5464.09 ppm).

1.4.3. Bioactive properties

Several studies have investigated the biological properties of *P. lentiscus*, including its antioxidant, antimicrobial, anti-inflammatory, and wound healing effects (Dragović et al., 2020; Milia et al., 2021; Sehaki et al., 2023). **Table 3** provides a summary of the main investigations regarding the bioactive properties of *P. lentiscus*.

Table 3 Summary of the *P. lentiscus* bioactive properties.

Part used	Extract	Method	Main results	Reference
Antioxidant				
Leaves	Dichloromethane, ethyl acetate, ethanol, methanol	DPPH (µg/mL), FRAP (Abs), β-carotene bleaching assay (% of inhibition)	IC_{50} = 5.44 – 38.70 Abs = 0.12 – 1.20 79.66 – 90.3%	Salhi et al. (2019)
Leaves	70% ethanol	DPPH and FRAP (µg/mL)	IC_{50} = 455 EC_{50} = 15.0	Bakli et al. (2020)
Fruits, twigs, leaves	Water, hexane, ethyl acetate, methanol, ethanol	DPPH (mg/mL) and anti-radical power	IC_{50} = 0.09 – 93.98 ARP = 1.05 – 26.12	Barbouchi et al. (2020)
Fruits	Choline chloride-based deep eutectic solvent	TBARS (% of inhibition)	81.32%	Tebbi et al. (2024)
Antimicrobial				
Leaves	Dichloromethane, ethyl acetate, ethanol, methanol, water	Disk diffusion method (*Micrococcus luteus*, *Listeria innocua*, *B. subtilis*, *E. coli*, *Candida pelliculosa*, *F. oxysporum*) (zone of inhibition)	9 – 15 mm	Salhi et al. (2019)
Leaves	Water	Disk diffusion method (*S. aureus*, *E. coli*, *P. aeruginosa*, *Enterobacter faecalis*, *B. cereus*, *Citrobacter freundii*, *Proteus mirabilis*, *K. pneumonia*) (zone of inhibition)	9 – 12 mm	Ghenima et al. (2015)
Leaves	70% ethanol	Disk diffusion method (*S. aureus*, *B. cereus*, *E. faecalis*, *C. freundii*, *E. coli*, *K. pneumoniae*, *Salmonella typhi*, *Salmonella enterica, Salmonella typhimurium*, *P. aeruginosa*, *P. mirabilis*, *Vibrio cholerae*, *L. innocua*, *A. niger*, *A. flavus*, *C. albicans*) (zone of inhibition, mg/mL)	7.3 – 18.8 mm MIC = 0.5 – 5 mg/mL	Bakli et al. (2020)
Anti-inflammatory				
Leaves	Water	Ulcerative colitis induced Wistar rats (IL-6 production)	175 – 125 pg/mg	Zahouani et al. (2020)
Leaves	80% methanol	Croton oil-induced ear edema in mice, acetic acid-induced vascular permeability in mice, and carrageenan-induced pleurisy in rats (% of inhibition)	51 – 65%; 28 – 46%; 29 – 36%	Bouriche et al. (2016)
Leaves	95% ethanol	IL-1β secretion in THP-1 cells stimulated by ATP or by H_2O_2 (pg of IL-1β/supernatant)	68.02 – 93.43	Remila et al. (2015)
Wound healing				
Leaves	Methanol	Excision wound model on Wistar albino rats (% of wound closure)	88.83 – 94.67%	Elloumi et al. (2022)
Leaves	70% ethanol	Excision wound model on Wistar albino rats (% of wound closure)	100%	Hemida et al. (2022)

DPPH – 2,2-diphenyl-1-picrylhydrazyl. FRAP – ferric reducing antioxidant power. IL-6 – interleukin 6. IL-1β – interleukin 1β. ATP – adenosine triphosphate. H_2O_2 – hydrogen peroxide. IC_{50} – extract concentration responsible for 50% of inhibition; EC_{50} – extract concentration responsible for half of the maximum activity; MIC – Minimum inhibitory concentration; Abs - absorbance.

Several authors highlight this species antioxidant capacity. Salhi et al. (2019) evaluated the antioxidant activity of *P. lentiscus* leaves extracted using Soxhlet extraction with several solvents (i.e., dichloromethane, ethyl acetate, ethanol, methanol, and water). For the DPPH assay, the ethanolic extract exhibited an IC_{50} similar to the positive controls tested (i.e., butylated hydroxyanisole (BHA) and ascorbic acid) (IC_{50} values of 5.44, 5.54, and 2.82 µg/mL, respectively). Also, in the FRAP assay, the ethanolic, methanolic, and aqueous extracts exhibited reducing activities similar to the positive control ascorbic acid. The authors suggested that extracts with higher concentrations of phenolic compounds exhibit higher antioxidant activity. Similarly, Barbouchi et al. (2020) evaluate the antioxidant activity of aqueous and ethanolic extracts from *P. lentiscus* leaves and twigs through the DPPH assay and obtained IC_{50} values between 90 and 530 µg/mL for the aqueous extract and between 130 and 1550 µg/mL for the ethanolic extract. The aqueous extract showed antioxidant activity stronger than the positive control quercetin (IC_{50} = 100 µg/mL). In turn, Bakli et al., 2020 demonstrated a weaker DPPH scavenging activity for the 70% ethanolic extracts of the leaves, compared with the control BHT (IC_{50} value of 455 µg/mL and 4.31 µg/mL, respectively). However, the same authors obtained similar ferric reducing power results, with EC_{50} values of 15 and 16 µg/mL for extract and BHT, respectively. More recently, Tebbi et al. (2024) studied the fruits extracts (using choline chloride-based deep eutectic solvent) for lipid peroxidation inhibition through TBARS and obtained a high percentage of inhibition (81.32%), although no positive control was used. These results highlight the antioxidant potential of this plant, although the inconsistencies between the studies highlight the need for further investigation.

The antimicrobial activity of *P. lentiscus* extracts was assessed mainly by the agar diffusion and dilution methods against a range of microorganisms. Bakli et al. (2020) studied the antimicrobial properties of 70% ethanol leaf extract against 16 microorganisms, including bacteria, fungi, and yeasts, using a disk diffusion assay. The extract demonstrated reduced MIC values for some bacteria tested, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella typhi*, *Vibrio cholerae*, and the fungus *C. albicans* (MIC values of 0.5, 0.6, 0.3, and 0.1 mg/mL, respectively). Besides, the leaf aqueous extract was efficient against the bacteria *S. aureus*, *B. cereus*, *P. aeruginosa*, *Proteus mirabilis*, *L. monocytogenes*, *K. pneumoniae*, *E. faecalis*, *E. coli*, *Salmonella typhimurium*, *Citrobacter freundi* (inhibition zones between 9 and 12 mm) (Ghenima et al., 2015). Ethyl acetate, ethanol, methanol, and water extracts from the plant's leaves also inhibited the growth of *B. subtilis*, *Listeria innocua*, *Micrococcus*

luteus, and *E. coli* (between 9 and 15 mm of inhibition), though only ethyl acetate and ethanolic extracts inhibited the growth of the fungi *Candida pelliculosa* (12 and 13 mm of inhibition, respectively) (Salhi et al., 2019).

The anti-inflammatory activity of *P. lentiscus* has been studied using different extracts and methodologies. Remila et al., (2015) showed that ethanolic leaf extract significantly reduces IL-1β levels in THP-1 cells, with the cells stimulated with ATP showing higher IL-1β concentrations (121.87 pg/supernatant) than leaf extracts (IL-1β between 68.02 and 93.43 pg/supernatant). Zahouani et al. (2020) tested aqueous leaf extracts on Wistar albino rats induced with ulcerative colitis and verified a reduction of secreted IL-6 from the control (225 pg/mg of IL-6) compared to the extract (125 pg/mg of IL-6).

Studies have used croton oil-induced ear edema, acetic acid-induced vascular permeability, and carrageenan-induced pleurisy assays to assess the anti-inflammatory effects of methanol and aqueous leaf extracts in Wistar and Swiss albino rats. *P. lentiscus* extracts significantly reduced ear edema, inhibiting ear thickness by 51 to 65%, compared to 54% inhibition demonstrated by the control indomethacin. The extracts also inhibited vascular permeability by 28 to 46%, while the control inhibited 44%. Furthermore, the pleurisy development was also reduced, inhibiting exudation by 29 to 38%, compared to the positive control λ-carrageenan. These results suggest that *P. lentiscus* extracts effectively inhibit different stages of inflammation (Bouriche et al., 2016).

Finally, *P. lentiscus* extracts have shown promising wound-healing potential. Elloumi et al. (2022) used an excision wound model on Wistar albino rats to study methanolic leaf extract at two concentrations (5 and 20 mg/mL) and compared them with negative and positive controls (physiological serum and Centasia cream, respectively) and with isolated quercetin and myricetin. After 14 days, myricitrin achieved the highest wound closure rate (94.67%), followed by Centasia cream (91.67%) and the plant extract at 20 mg/mL (88.83%). The extract promoted re-epithelization by stimulating collagen fiber and fibroblast organization, as well as reducing inflammation and swelling at the wound site. Similarly, Hemida et al. (2022) used the same animal wound model to evaluate the activity of 70% ethanol leaf extract in different concentrations (10% and 30%). The 10% plant extract resulted in the fastest re-epithelization (10 days) when compared with the groups treated with 30% extract and with both positive (Cicatrylbio®), and negative controls. Both tested extract concentrations achieved 100% wound contraction at day 24, whereas the control groups reached 100% only at day 28. The

histological study indicated that the extracts promoted fibroblast proliferation and neovascularization. In general, *P. lentiscus* shows promising wound healing potential, which is closely related to its anti-inflammatory, antimicrobial, and antioxidant activities (Elloumi et al., 2022; Hemida et al., 2022).

2. Objectives

The main objective of this study is to increase the scientific knowledge of *P. major*, *B. pilosa*, and *P. lentiscus*. These species are broadly used in traditional medicine in countries such as Portugal and Brazil due to their therapeutic potential. In this sense, the study aims to characterize the chemical composition of these species using appropriate chromatographic methodologies, as well as to evaluate different bioactive properties. A schematic representation of the main objectives of this study is shown in **Figure 4**.

Figure 4 Schematic representation of the main objectives of the present study.

The chemical composition of each plant was determined through the identification and quantification of fatty acids, tocopherols, free sugars, and organic acids. In addition, the hydroethanolic extracts of the plants was characterized regarding their composition in phenolic compounds, using high-performance liquid chromatography coupled to a diode array detector and electrospray ionization-mass spectrometry (HPLC-DAD-ESI/MS).

The bioactive properties of the hydroethanolic extracts were assessed, namely through their antioxidant and antimicrobial activities. The antioxidant activity was

determined through the inhibition of thiobarbituric acid reactive substances (TBARS). Antimicrobial activity, including antibacterial and antifungal properties, was determined using the colorimetric microdilution method with *p*-iodonitrotetrazolium violet (INT) as an indicator.

This study expected to provide valuable scientific validation of the medicinal properties of these species. These findings will contribute to the scientific community's knowledge and could potentially support the identification of new molecules of interest derived from these medicinal plants.

3. Materials and methods

3.1. Plant material

P. major, *B. pilosa*, and *P. lentiscus* (**Figure 5**) were commercially obtained from *Chás do Mundo*, located in Leiria, Portugal. The leaves of *P. major* and *P. lentiscus* leaves, as well as the aerial parts of *B. pilosa*, were the vegetable tissues studied due to their application in traditional medicine. The plant materials were reduced to a fine powder (~20 mesh) and stored protected from light until further analysis.

Figure 5 *P. major* (a), *B. pilosa* (b), and *P. lentiscus* (c) commercially obtained.

3.2. Chemical constituents' characterization

3.2.1. Fatty acids

The lipidic fraction of the samples was extracted with petroleum ether in a Soxhlet extraction system at 120 °C for approximately 7 hours. Subsequently, the obtained lipidic fraction was subjected to transesterification, following the procedure described by Liberal et al. (2024). Briefly, the samples were extracted with 5 mL of reagent A, consisting of MeOH, H_2SO_4, and C_7H_8, in a ratio of 2:1:1, under agitation for 12 hours at 50 °C. Subsequently, 3 mL of distilled water and 3 mL of diethyl ether were added to the mixture, with vortexing between each addition. Once phase separation was achieved, the supernatant was collected with a Pasteur pipette and transferred to a vial containing anhydrous sodium sulfate. The mixture was then filtered through a 0.22 μm nylon syringe filter to vials for analysis.

The fatty acid methyl esters (FAME) profile was assessed by gas-liquid chromatography using a YOUNG IN Chomass 6500 GC system (Gyeonggi, South Korea)

equipped with a flame ionization detector (FID) set at 260 °C, a split/splitless injector at 250 °C and a split ratio of 1:80, and a Zebron-Fame column (30 m × 0.25 mm i.d. × 0.20 µm df; Phenomenex, Lisbon, Portugal).

The identification and quantification of the individual fatty acids were achieved by comparing the relative retention times of FAME peaks with commercial standards (standard mixture 47,885-U, Sigma, St Louis, MO, USA). Data analysis was conducted using Clarity DataApex 4.0 software (Prague, Czech Republic), and the results were expressed as relative percentages of the identified fatty acids.

3.2.2. Tocopherols

The tocopherol composition of the samples was determined following the procedure described by Barros et al. (2013). To 500 mg of sample mass, it was added in the following order: BHT solution in *n*-hexane (100 µL, 10 mg/mL), an internal standard of tocol solution in *n*-hexane (400 µL, 50 µg/mL), and methanol (4 mL). The mixture was homogenized in a vortex, and then n-hexane (4 mL) and saturated NaCl solution (2 mL) were added. After that, the mixture was homogenized in a vortex (1 min), centrifuged (Centurion K24OR- 2003) at 4000 rpm for 5 min, and the supernatant was transferred to a vial, previously wrapped in aluminum foil. The extraction with *n*-hexane was repeated twice. The combined extracts were then dehydrated with anhydrous sodium sulfate and taken to dryness under a nitrogen steam, redissolved in *n*-hexane (2 mL), filtered through a 0.22 µm nylon filter, transferred into an amber vial for subsequent analysis by HPLC.

The HPLC system consisted of a quaternary pump (Smartline 1000, Knauer), a degasser (Smartline 5000), an automatic sampler (AS-2057 2500), and a fluorescence detector (Jasco) with a normal phase Polyamide II column (250 x4.6 mm, 5 µm, YMC from Waters) at 35°C (oven 7971 R Grace). The system was programmed for excitation at a wavelength of 290 nm and emission at 330 nm. The mobile phase consisted of a mixture of n-hexane and ethyl acetate (70:30 *v/v*) at a flow rate of 1 mL/min.

Data were analyzed using Clarity 2.4 Software (DataApex). Compounds were identified by comparing retention times with authentic standards, and the quantification was based on the fluorescence signal using chromatographic comparison with the internal standard. Results were expressed in mg per 100 of dry weight (dw).

3.2.3. Free sugars

The free sugar content was determined following the procedure previously described by Barros et al. (2013). Briefly, 1 g of the plant material was extracted with a solution of EtOH:H_2O (80:20, *v/v*, 40 mL) and with the internal standard melezitose (1 mL, 25 mg/mL). The extraction was carried out in a bath at 80 °C for 90 minutes, and the solution was agitated every 15 minutes. After extraction, the mixture was centrifuged at 3500 rpm for 5 minutes and filtered through Whatman N°4 filter paper. The ethanol was then evaporated under reduced pressure using a rotary evaporator (Hei-VAP Core, Heidolph Instruments) at 40°C. The obtained solution was transferred to a separating funnel and washed three times with diethyl ether (10 mL). The lower phase was collected into a volumetric flask and adjusted to a final volume of 5 mL with water. The mixture was then filtered through a 0.22 µm nylon filter for analysis.

Free sugars determination was performed using a high-performance liquid chromatography system (Knauer, Smartline system) coupled with a refractive index detector (Knauer Smartline 2300) (HPLC-RI) and with the column 100-5 NH_2 Eurospher (4.6 x 250 mm, 5 µm, Knauer). The mobile phase consisted of acetonitrile:deionized water (70:30, *v/v*) with a flow rate of 1 mL/min, at 35°C (oven 7971 R Grace).

The identification of free sugars was performed by comparing the peak retention times with those of commercial standard. Data analysis was carried out using Clarity 2.4 (DataApex), and quantification was carried out using the internal standard method. The results were expressed in g per 100 g of dry weight (dw).

3.2.4. Organic acids

A sample mass of 1.5 g was extracted with metaphosphoric acid (25 mL, 4.5%) at 25°C, under agitation (150 rpm) for about 20 minutes; then filtered through a Whatman No. 4 paper filter and transferred to a syringe from which it was filtered through a 0.22 µm nylon filter.

The analysis was performed by a Shimadzu 20A series UFLC system (Shimadzu Corporation). Separation was performed on a reverse phase C_{18} column (5 µm, 250 x 4.6 mm, Phenomenex), thermostated at 35°C. The elution was carried out with sulphuric acid (3.6 mM). A photodiode array detector (PDA) was used to perform the detection of compounds, at wavelengths of 215 and 245 nm.

The identification and quantification of organic acids was achieved by comparing retention times and area of the peaks with commercial standards and their respective calibration curves. The results were expressed in mg per 100 g of dry weight (dw).

3.3. Extraction procedure

The samples studied (*P. major*, *B. pilosa*, and *P. lentiscus*) were extracted through a hydroethanolic maceration. Briefly, 1.5 g of each studied species was mixed with a solution of EtOH:H_2O (80:20, *v/v*, 30 mL), and stirred for 1 h at room temperature. The mixture was filtered through a paper filter, and the retained residue was re-extracted under the same conditions. The obtained solutions were concentrated through reduced pressure at 40ºC using a rotary evaporator (Hei-VAP Core, Heidolph Instruments) (**Figure 6**). The aqueous phase was frozen at -20 ºC and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). The obtained extract was stored and protected from light until further analysis.

Figure 6 Obtention of the hydroethanolic extracts.

3.4. Phenolic compounds analysis

The obtained hydroethanolic extracts were re-dissolved in EtOH:H_2O (20:80, *v/v*) at a final concentration of 10 mg/mL and filtered through a 0.22 µm nylon syringe filter. The phenolic composition of the studied species was analyzed by high-performance liquid chromatography coupled to a diode array detector and electrospray ionization–mass spectrometry (HPLC-DAD-ESI/MS), according to a procedure previously described (Bessada et al., 2016). The separation was performed using a Waters Spherisorb

S3 ODS-2 C_{18} column (3 µm, 150 × 4.6 mm, Waters, Milford, MA, USA), operated at 35 ºC. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The elution gradient was as follows: 15% B (5 min), 15% B to 20% B (5 min), 20-25% B (10 min), 25-35% B (10 min), 35-50% B (10 min), followed by column re-equilibration (10 min) using a flow rate of 0.5 mL/min.

Mass detection was carried out using a Linear Ion Trap Mass Spectrometer LTQ XL (Thermo Finnigan, San Jose, CA, USA) with an electrospray ionization (ESI) source and nitrogen (50 psi) as the carrier gas; the voltage used was 5 kV for spray, with an initial temperature of 325 ºC and a capillary voltage of -20 V; the tube lens offset voltage was maintained at -66 V and using 280 and 370 nm as selected wavelengths. Spectra were obtained in negative ion mode between 100 and 1500 *m/z*. The collision energy was 35 arbitrary units.

The chromatographic data were collected and analyzed in the program Xcalibur® (Thermo Finnigan, San Jose, CA, USA). The tentative identification of phenolic compounds was performed by comparison of the chromatographic data, such as retention times, UV–Vis, and mass spectra, with the available standard compounds and the literature information. The quantification of the individual compounds was performed through peak area determination and chromatographic comparison with the calibration curves. The results were expressed in mg per g of extract.

3.5. Bioactive properties evaluation

3.5.1. Antioxidant activity

The antioxidant capacity of the studied species was evaluated using two cell-based methodologies: the thiobarbituric acid reactive substances (TBARS) formation inhibition.

For the TBARS assay, the obtained extracts were re-dissolved in water and successively diluted to obtain a range of concentrations to be tested (0.0097 – 1.25 mg/mL). A porcine brain cell solution (1:2, *w/v*; 100 µL) was incubated with the extract solutions (200 µL), ascorbic acid (0.1 mM; 100 µL), and iron sulfate (10 µM; 100 µL) at 37.5 ºC for 1 hour. After the incubation period, trichloroacetic (28%, *w/v*, 500 µL) and thiobarbituric acid (TBA, 2% *w/v*, 380 µL) acids were added and the mixture was heated at 80 ºC for 20 minutes. Finally, the obtained solution was centrifuged at 3000 rpm for 5

minutes and the malondialdehyde - thiobarbituric acid (MDA-TBA) complex was measured at 532 nm. The commercial Trolox was used as a positive control. The obtained results were expressed as the extract concentration responsible for 50% TBARS formation inhibition (IC_{50} values, μg/mL).

3.5.2. Antimicrobial activity

The antimicrobial activity was assessed against ATCC strains (Liofilchem, Italy). Five Gram-negative bacteria (*Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica subsp. enterica serovar Enteritidis* (ATCC 13076) and *Yersinia enterocolitica* (ATCC 8610)), and four Gram-positive strains (*Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19111), and *Staphylococcus aureus* (ATCC 25923)) were used. Besides, two fungal strains (*Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404), Liofilchem, Italy), were used to assess the antifungal activity.

The antimicrobial assays were performed using the microdilution method, following a procedure previously described (Petropoulos et al., 2017). Briefly, the hydroethanolic extracts of *P. major*, *B. pilosa*, and *P. lentiscus* were redissolved in water to obtain a concentration of 10 mg/mL that was further successively diluted (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 mg/mL). For the antibacterial activity, the bacterial inoculum was added to all wells (10 μL), and after 24 hours of incubation, *p*-iodonitrotetrazolium violet (INT) solution (50 μL, 0.2 mg/mL) was added, and the plates were incubated for 30 minutes. Viable bacteria change the color of the INT solution from yellowish to pink. For the antifungal activity, a fungal suspension was prepared through the dilution of spores in phosphate-buffered saline (PBS) solution (10^6 spores). The plates were incubated at 25°C for 72 hours, and the antifungal activity was determined by visualizing the presence or absence of spores in the plates.

The obtained results were expressed as minimum inhibitory (MIC), bactericidal (MBC), and fungicidal (MFC) concentrations. The commercial streptomycin (1 mg/mL), methicillin (1 mg/mL), ampicillin (1 mg/mL), and ketoconazole (1 mg/mL) were used as positive control.

3.6. Statistical analysis

All assays were performed in triplicate. The results were presented as the mean value ± standard deviation (except for antimicrobial activity). The differences between the obtained results were analyzed through a two-tailed paired Student's t-test at a 5% significance level, using the SPSS Statistics software (IBM SPSS Statistics for Mac OS, Version 26.0; IBM Corp., Armonk, NY, USA). Samples with significant differences were considered when the *p*-value was lower than 0.05. For the comparison between two samples, a two-tailed paired Student's t-test was applied to assess the statistical differences ($\alpha = 0.05$).

4. Results and discussion

4.1. Chemical composition

4.1.1. Fatty acids

The lipidic content and fatty acid composition of *P. major*, *B. pilosa*, and *P. lentiscus* are described in **Table 4**. The content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) is also presented.

Table 4 The lipidic fraction content and the fatty acid composition of *P. major*, *B. pilosa*, and *P. lentiscus*.

	P. major	***B. pilosa***	***P. lentiscus***
Lipidic Fraction (g per 100 g dw)			
	1.705 ± 0.003[c]	0.6 ± 0.1[a]	1.2 ± 0.1[b]
Fatty Acids (relative percentage, %)			
C6:0	0.45 ± 0.01[a]	0.66 ± 0.04[c]	0.614 ± 0.004[b]
C8:0	0.38 ± 0.01[a]	0.79 ± 0.05[c]	0.730 ± 0.005[b]
C10:0	0.29 ± 0.01[b]	0.48 ± 0.02[c]	0.669 ± 0.004[a]
C11:0	n.d.	n.d.	0.251 ± 0.004
C12:0	0.326 ± 0.002[b]	0.379 ± 0.005[b]	0.93 ± 0.06[a]
C13:0	n.d.	n.d.	0.070 ± 0.001
C14:0	2.49 ± 0.05[c]	1.35 ± 0.02[b]	5.2 ± 0.1[a]
C14:1	0.157 ± 0.001[b]	0.299 ± 0.025[c]	0.47 ± 0.02[a]
C15:0	0.79 ± 0.03[b]	1.10 ± 0.01[c]	1.30 ± 0.01[a]
C16:0	44.1 ± 0.1[c]	37.8 ± 0.1[a]	39.2 ± 0.1[b]
C16:1	3.1 ± 0.2[b]	1.42 ± 0.05[a]	2.087 ± 0.001[b]
C17:0	0.915 ± 0.004[b]	1.46 ± 0.04[c]	1.75 ± 0.04[a]
C18:0	6.69 ± 0.04[b]	7.65 ± 0.04[c]	10.1 ± 0.2[a]
C18:1n9c	4.84 ± 0.01[c]	10.590 ± 0.005[b]	19.6 ± 0.4[a]
C18:2n6c	29.3 ± 0.3[b]	26.67 ± 0.02[a]	6.9 ± 0.1[c]
C20:0	1.8 ± 0.1[b]	1.80 ± 0.02[b]	2.4 ± 0.1[a]
C20:1	0.170 ± 0.002	n.d.	n.d.
C20:2	0.37 ± 0.02	n.d.	n.d.
C21:0	n.d.	n.d.	1.15 ± 0.04
C22:0	2.899 ± 0.003[a]	4.8 ± 0.3[b]	5.01 ± 0.15[a]
C23:0	0.88 ± 0,04[a]	2.8 ± 0.1[c]	1.585 ± 0.004[b]
SFA	62.0 ± 0.1[c]	61.02 ± 0.01[b]	70.8 ± 0.4[a]
MUFA	8.3 ± 0.2[b]	12.31 ± 0.03[c]	22.2 ± 0.4[a]
PUFA	29.3 ± 0.3[b]	26.67 ± 0.02[a]	7.0 ± 0.1[c]

Results are presented as mean values ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.05$). Fatty acids are expressed as relative percentages of each fatty acid. C6:0 – caproic acid; C8:0 – caprylic acid; C10:0 – capric acid; C11:0 – undecanoic acid; C12:0 – lauric acid; C13:0 – tridecanoic acid; C14:0 – myristic acid; C14:1 – tetradecanoic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C16:1 – palmitoleic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C18:1n9c – oleic acid; C18:2n6c – linoleic acid; C20:0 – arachidic acid; C20:1 – gondoic acid; C20:2 –eicosadienoic acid; C21:0 – heneicosanoic acid; C22:0 – behenic acid; C23:0 – tricosanoic acid; n.d. – not detected; dw – dry weight; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

P. major exhibited the highest lipidic fraction (1.705 g per 100 g dw), followed by *P. lentiscus* and *B. pilosa* (1.2 and 0.6 g/100g of dw, respectively). A total of 21 fatty acids were identified in the medicinal plants studied. *P. lentiscus* was the species that exhibited the highest variety of fatty acids, with a total of 19 identified, followed by *P. major* with 18 fatty acids and *B. pilosa* with 16. Palmitic acid (C16:0 – between 37.8% and 44.1%) and linoleic acid (C18:1n9c – between 6.9% and 29.3%) were the fatty acids present in the highest relative abundance in all species. In turn, the fatty acids detected in the lowest relative abundance were myristic acid (C14:1) in both *P. major* and *B. pilosa* (0.157% and 0.299%, respectively) and tridecanoic acid (C13:0) in *P. lentiscus* (0.07%).

In this study, saturated fatty acids (SFA) were the most abundant class of fatty acids in all species studied (with relative abundances between 61.02 and 70.8%), while monounsaturated fatty acids (MUFA) were the least abundant in *P. major* and *B. pilosa* (8.3 and 12.31%, respectively). In contrast, polyunsaturated fatty acids (PUFA) were the class of fatty acids with the lowest abundance in *P. lentiscus* (7.0%).

According to the literature, the fatty acid composition of these three species has not yet been significantly studied. Similar to our findings, palmitic acid has been reported as the most abundant fatty acid in the leaves and aerial parts of *P. major* (12.68% and 15.18%, respectively), even though in lower relative abundances than our results (Haghighi et al., 2021; Turgumbayeva et al., 2022). Differently from our results, Harrat et al. (2018) reported a higher relative abundance of linoleic acid in *P. lentiscus* leaves (between 10.94 and 16.99%), but lower abundance of palmitic and oleic acids (between 5.31 – 9.03%, and 3.42 – 4.85% respectively). However, this plant's seeds and fruits contained oleic acid as the most abundant (19.49 – 53.13%), followed by palmitic (18.43 – 20.34%) and linoleic acids (18.85 – 42.5%) (Brahmi et al., 2020). For *B. pilosa*, palmitic, oleic, and linoleic acids were reported as the major fatty acids in the aerial parts (30%, 27%, and 24.3%, respectively) (Quaglio et al., 2020). Therefore, the most abundant fatty acids identified in our study were consistent with those previously described in the literature.

Researchers have described higher contents in the plant seeds, fruits, roots, and stems of MUFA and PUFA contents, than in leaves and aerial parts (Angelini et al., 2021; Rauf et al., 2017; Wang et al., 2017). Trabelsi et al. (2012) described higher MUFA and PUFA contents than SFA content in *P. lentiscus* fruits (between 22.08 – 55.4% of MUFA, 19.81 – 56.31% of PUFA, and 20.9 – 26.8% of SFA). Similarly, Ait Mohand et a. (2020)

described that *P. lentiscus* seeds also contained more MUFA and PUFA, but less SFA than the plant's leaves (53.67%, 22.27%, and 22.25%, respectively).

4.1.2. Tocopherols

The obtained results regarding the studied species' tocopherol composition are presented in **Table 5**.

Table 5 Tocopherols composition of *P. major*, *B. pilosa*, and *P. lentiscus*.

Tocopherols (mg per 100 g dw)			
	P. major	*B. pilosa*	*P. lentiscus*
Alpha-tocopherol	35.5 ± 0.3[a]	0.08 ± 0.01[c]	1.7 ± 0.1[b]
Gamma-tocopherol	4.0 ± 0.5[a]	0.09 ± 0.01[b]	n.d.
Total tocopherols	39.6 ± 0.2[a]	0.1664 ± 0.0005[c]	1.7 ± 0.1[b]

Results are presented as mean values ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.05$). n.d. – not detected; dw – dry weight.

Alpha and gamma tocopherols were identified and quantified in the medicinal plants studied. Alpha-tocopherol was detected in a higher concentration in *P. major* and *P. lentiscus* (35.5 and 1.7 mg/100 g). Regarding *B. pilosa*, the presence of alpha and gamma isoforms is very similar, displaying equivalent concentrations (0.08 and 0.09 mg/100 g, respectively). The higher content of tocopherols within the plants studied was in *P. major* (39.6 mg/100 g), followed by *P. lentiscus* (1.7 mg/100 g), and *B. pilosa* (0.1664 mg/100 g).

According to the literature, alpha-tocopherol was identified in *P. major* leaves (Morales et al., 2021), while in seed oil, alpha and gamma isoforms were detected, with alpha-tocopherol as the most abundant (between 524.53 and 754.67 µg/g) (Wang et al., 2017). Alpha isoform was the most abundant tocopherol in *P. lentiscus* leaves (Harrat et al., 2018; Kıvçak & Akay, 2005) and seed oil (Wissal et al., 2013). In agreement with our results, authors have described low contents of alpha and gamma tocopherols in *B. pilosa* aerial parts (Gowele et al., 2019; Shen et al., 2018).

4.1.3. Free sugars

The content of the free sugars detected in the medicinal species under study are presented in **Table 6**.

Table 6 Free sugars identified in *P. major*, *B. pilosa*, and *P. lentiscus*.

Free Sugars (g per 100 g dw)			
	P. major	*B. pilosa*	*P. lentiscus*
Fructose	0.27 ± 0.01[b]	3.4 ± 0.5[a]	1.03 ± 0.05[c]
Glucose	0.51 ± 0.04[b]	1.12 ± 0.05[a]	0.47 ± 0.02[b]
Sucrose	n.d.	n.d.	1.76 ± 0.03
Total free sugars	0.8 ± 0.1[b]	4.5 ± 0.5[a]	3.260 ± 0.001[c]

Results are presented as mean values ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.05$). n.d. – not detected; dw – dry weight.

The free sugar content of the three plant species studied exhibited differences, both in composition and content. *B. pilosa* showed the highest total free sugar content (4.5 g per 100 g dw), followed by *P. lentiscus* (3.26 g per 100 g dw) and *P. major* (0.8 g per 100 g dw). Among the individual sugars, fructose, glucose, and sucrose were the free sugars detected, with sucrose being detected only in *P. lentiscus*, where it was present at the highest concentration (1.76 g per 100 g dw). In turn, *B. pilosa* exhibits the highest fructose content (3.4 g/100 g dw), while *P. major* showed a higher glucose content (0.51 g per 100 g dw).

According to the existing literature, studies evaluating the free sugar composition of these species are relatively limited, typically focusing only on quantifying the total sugar content. Rahman et al. (2020) described a total sugar content of *P. major* leaves between 2.89 and 6.78 mg/g dw. Furthermore, Bouta et al. (2024) studied the *P. lentiscus* leaves and reported a total sugar content of 14.84%. According to Bartolome et al., (2013), *B. pilosa* contains a sugar content between 6 and 8.4 g/100 g, slightly higher than the results otained in our study. In this turn, Ruiz-Reyes et al. (2022) described a low presence of sugars in *B. pilosa* leaves, and Muhamad et al. (2019) reported a soluble sugar content between 0.2% and 0.7%.

4.1.3. Organic acids

The quantitative and qualitative composition of organic acids composition of the studied medicinal plants are presented in **Table 7**.

Table 7 Organic acids of *P. major*, *B. pilosa*, and *P. lentiscus*.

Organic Acids (g per 100 g dw)			
	P. major	*B. pilosa*	*P. lentiscus*
Quinic	n.d.	0.76 ± 0.05*	0.46 ± 0.04*
Malic	0.22 ± 0.02*	0.25 ± 0.1*	n.d.
Succinic	1.62 ± 0.04**	n.d.	0.29 ± 0.01**
Total organic acids	1.8 ± 0.1[a]	1.02 ± 0.04[b]	0.75 ± 0.02[c]

Results are presented as mean values ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.05$). *No statistically significant differences were found (p-value >0.05). **Means statistical differences obtained by a *t*-student test (p-value <0.05). n.d. – not detected; dw – dry weight.

Quinic, malic, and succinic acids were the organic acids identified and quantified in the medicinal plants studied. Quinic acid was the organic acid detected in higher concentrations in *B. pilosa* and *P. lentiscus* (0.76 and 0.46 g per 100 g, respectively), while succinic acid was the most abundant organic acid detected in *P. major* leaves (1.62 g per 100 g). Among the medicinal plants studied, *P. major* exhibits a higher content of organic acids (1.8 g per 100 g), followed by *B. pilosa* and *P. lentiscus* (1.02 and 0.75 g per 100 g, respectively).

Olennikov et al. (2005) identified malic and succinic acid in *P. major* leaves with succinic acid present in higher relative abundance (0.25%). Moreover, Nxumalo et al. (2023) detected the presence of quinic acid in *B. pilosa* and described a lower content of organic acids than the obtained in our study. Quinic acid was also detected in *B. pilosa* and *P. lentiscus* aerial parts and whole plant, respectively (Anagnostou et al., 2023; Idris et al., 2023; Scognamiglio et al., 2019). In contrast, Hajji-Hedfi et al. (2024) detected quinic acid as the most abundant organic acid in *P. lentiscus*. Besides, malic and succinic acids were detected with higher concentrations in other Plantago species, namely *Plantago algarbiensis* and *Plantago almogravensis* (Martins et al., 2013).

4.2. Phenolic compounds

The phenolic compounds composition of the hydroethanolic extracts of the studied medicinal plants is presented in **Tables 8-10**. The analytical data, such as the retention time (Rt), the wavelength of maximum absorption (λ_{max}), pseudomolecular ion ($[M-H]^-$), and fragmentation pattern (MS^2) of each detected compound are presented. The tentative identification was based on the comparison between the obtained analytical data with the available standard and the literature information.

4.2.1. *Plantago major*

The phenolic compounds tentatively identified in the hydroethanolic extract of *P. major* is presented in **Table 8**.

Table 8 Retention time (Rt), wavelengths of maximum absorption (λ_{max}) in the UV-Vis region, mass spectral data, and tentative identification of the phenolic compounds detected in the hydroethanolic extracts of *P. major*.

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Quantification (mg/g extract)
1	5.92	323	353	191,179,135	3-*O*-Caffeoylquinic acid	0.12 ± 0.02
2	8.16		337	163	*p*-Coumaroylquinic acid	tr
3	8.53	324	353	191,179,173,135	5-*O*-Caffeoylquinic acid	0.23 ± 0.03
4	11.74		639	621	β-Hydroxyverbascoside	0.044 ± 0.003
5	14.88	327	639	477	β-Hydroxyverbascoside isomer	0.28 ± 0.04
6	15.16	330	755	593	Verbascoside-*O*-pentoside	0.025 ± 0.002
7	16.03	329	755	593,623	Verbascoside-*O*-pentoside	0.31 ± 0.04
8	16.72	330	623	461	Verbascoside	8 ± 1
9	17.73		609	301	Rutin	0.11 ± 0.01
10	19.45	327	623	461	Verbascoside isomer	0.27 ± 0.04
11	20.12	331	1111	755	Verbascoside derivative	0.65 ± 0.11
12	21.86		623	315	Isorhamnetin-*O*-rutinoside	0.12 ± 0.01
13	22.67		461	285	Luteolin-*O*-glucuronide	0.134 ± 0.004
					Total phenolic acids	9.7 ± 1.5
					Total flavonoids	0.36 ± 0.02
					Total phenolic compounds	10.1 ± 1.5

Results are expressed as mean ± standard deviation. tr - traces.

A total of thirteen phenolic compounds were tentatively identified, corresponding to ten phenolic acids (peaks **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **10**, and **11**) and three flavonoids (peaks **9**, **12**, and **13**).

Most of the identification was based on the comparison of the chromatographic patterns with the available standards and literature information. The 3-*O*-caffeoylquinic (peak **1**) and 5-*O*-caffeoylquinic acids (peak **3**) were previously identified in *P. major* (Meinhart et al., 2017). Rutin (peak **9**) was previously identified in plant flowers and leaves, methanolic and ethanolic extracts (Ganeshpurkar & Saluja, 2017; Salem et al., 2023; Soliman et al., 2022), and whole plant (Zbuchea et al., 2016) extracts. Phenolic acids derived from verbascoside (**Figure 7**) stand out as the most abundant, corresponding to seven of the 13 compounds tentatively identified. This compound exhibits a concentration comprised between 0.025 and 8 mg/g extract. Verbascoside derivatives were previously identified in *P. major* extracts by several authors (Gonçalves et al., 2019; Laanet et al., 2024; Mazzutti et al., 2017; Vendruscolo et al., 2024; Zubair et al., 2011), with some studies highlighting the presence of verbascoside and rutin derivatives as responsible for the *P. major* bioactive properties. Rutin exhibits antimicrobial activity, particularly against *E. coli* (Araruna et al., 2012). Moreover, its combination with other flavonoids, such as kaempferol, has been shown to enhance antibacterial effects against *B. cereus* and *S. enteritidis* (Arima et al., 2002). In turn, Xiao et al. (2022) it relates verbascoside with properties such as antioxidant, anti-inflammatory, and antimicrobial. However, further studies to better understand the biological mechanisms associated with this compound would be of great interest.

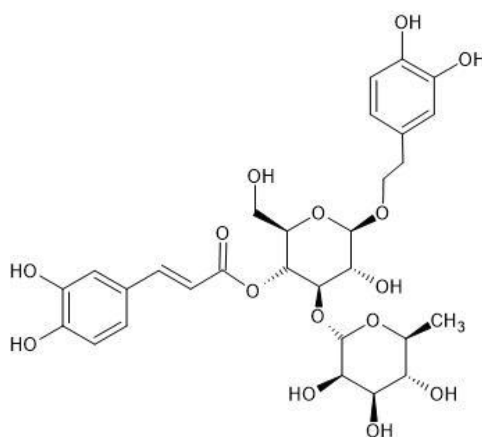

Figure 7 Chemical structure of verbascoside.

4.2.2. Bidens pilosa

The polyphenolic composition of the hydroethanolic extract of *B. pilosa* is presented in **Table 9**.

Table 9 Retention time (Rt), wavelengths of maximum absorption (λ_{max}) in the UV-Vis region, mass spectral data, and tentative identification of the phenolic compounds detected in the hydroethanolic extracts of *B. pilosa*.

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Quantification (mg/g extract)
1	5.82	261,294	153	109	Protocatechuic acid	0.125 ± 0.030
2	5.89		353	191,179,135	3-*O*-Caffeoylquinic acid	0.15 ± 0.02
3	6.76		315	153,109	Protocatechuic acid hexoside	tr
4	8.47	326	353	191	5-*O*-Caffeoylquinic acid	0.73 ± 0.15
5	10.38	325	179	135	Caffeic acid	0.06 ± 0.02
6	14.91		839	677	1,3,4,5-*O*-Tetracaffeoylquinic acid	0.11 ± 0.01
7	14.91		367	191,173	5-*O*-Feruloylquinic acid	0.040 ± 0.002
8	17.65		609	301	Rutin	0.15 ± 0.03
9	18.52		463	301	Quercetin-*O*-hexoside	0.11 ± 0.01
10	18.88		623	315,300	Isorhamnetin-*O*-neohesperidoside	0.24 ± 0.04
11	19.96		523	477	Isorhamnetin-*O*-hexoside	0.086 ± 0.015
12	22.13	254	477	301	Quercetin-*O*-glucuronide	0.8 ± 0.2
13	22.13	337	515	353	3,4-*O*-Dicaffeoylquinic acid	0.6 ± 0.1
14	24.37		637	329	Quercetin-dimethyl ether rutinoside	0.13 ± 0.03
15	25.15	254,354	637	329,299	Quercetin-dimethyl ether rutinoside	2.5 ± 0.5
16	27.24		491	329	Quercetin-dimethyl ether hexoside	0.35 ± 0.06
17	30.37		505	329	Quercetin-dimethyl ether glucuronide	0.27 ± 0.05
18	35.77		547	329	Quercetin-dimethyl ether derivative	0.10 ± 0.01
19	38.95		547	329,487,411	Quercetin-dimethyl ether acetyl glucuronide	0.068 ± 0.004
20	39.9		329	314	Quercetin-dimethyl ether	0.10 ± 0.01
					Total phenolic acids	1.83 ± 0.35
					Total flavonoids	5 ± 1
					Total phenolic compounds	7 ± 1

Results are expressed as mean ± standard deviation. tr - traces.

Regarding *B. pilosa* hydroethanolic extract, a total of twenty phenolic compounds were tentatively identified, from which eight phenolic acids (peaks **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **13**) and twelve flavonoids (peaks **8**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **19**, and **20**).

Protocatechuic (peak **1**) and caffeic acids (peak **5**) were previously detected in *B. pilosa* leaves, stems, and roots aqueous extracts (Deba et al., 2007), 3-*O*-caffeoylquinic (peak **2**) and rutin (peak **8**) were previously reported in leaves methanolic extracts (Moyo et al., 2020), 5-*O*-caffeoylquinic (peak **4**) and 5-*O*-feruloylquinic acid (peak **7**) were

detected in the methanolic extracts (Ramabulana et al., 2020), and 3,4-*O*-dicaffeoylquinic acid (peak **13**) in the whole plant butanol extract (Chiang et al., 2004).

B. pilosa exhibits a higher variety of quercetin derivatives, quercetin-*O*-hexoside (peak **9**) and quercetin-*O*-glucuronide (peak **12**) were previously detected in the ethanolic extract of the plant aerial parts (Idris et al., 2023). Also, Liang & Xu (2016) studied the phenolic composition of *B. pilosa* hydroethanolic extract, and besides caffeic, rutin, and luteolin, the authors also detected a high variety of quercetin derivatives, such as quercetin-*O*-glucuronide (peak **12**), quercetin-dimethyl ether rutinoside (peak **14** and **15**), and quercetin-dimethyl ether hexoside (peak **16**). Quercetin-dimethyl ether rutinoside (peak **15**, **Figure 8**) was the compound detected in higher concentration in *B. pilosa* hydroethanolic extract (2.5 mg/g extract), followed by chlorogenic acid (0.73 mg/g extract).

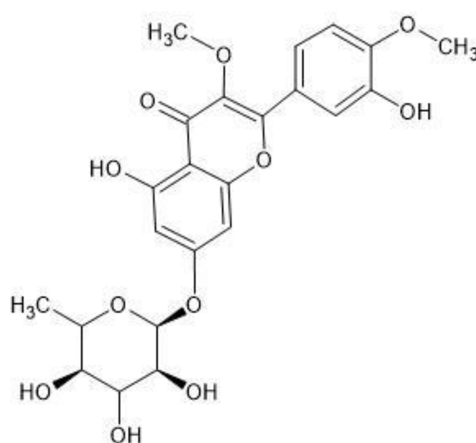

Figure 8 Chemical structure of quercetin-dimethyl ether rutinoside.

4.2.3. Pistacia lentiscus

The phenolic compound composition of the hydroethanolic extract of *P. lentiscus* is presented in **Table 10**.

Table 10 Retention time (Rt), wavelengths of maximum absorption (λ_{max}) in the UV-Vis region, mass spectral data, and tentative identification of the phenolic compounds detected in the hydroethanolic extracts of *P. lentiscus*.

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Quantification (mg/g extract)
1	4.15	273	343	161,169	Galloyl quinic acid	15 ± 4
2	5.55	280	483	271,331,313,169,465	Digalloylglucose	tr
3	5.78	261,294	153	109	Protocatechuic acid	0.21 ± 0.05
4	6.1	275	495	343	Digalloyl quinic acid isomer I	8.5 ± 2.0
5	6.83	276	495	343,325	Digalloyl quinic acid isomer II	0.90 ± 0.03
6	10.97	276	647	495,479,629	Trigalloyl quinic acid	0.13 ± 0.01
7	10.97	276	647	161	Galloyl hexoside	0.13 ± 0.01
8	12.23	275	647	495	Trigalloyl quinic acid isomer	0.6 ± 0.2
9	14.03	272	647	343	Trigalloyl quinic acid isomer	0.128 ± 0.001
10	14.63	264,299,357	625	316,317	Myricetin-*O*-hexoside-deoxyhexoside	1.0 ± 0.3
11	15.11	264,299,357	479	317,316	Myricetin-*O*-hexoside	0.6 ± 0.1
12	17.12		615	463	Quercetin-*O*-galloyl-hexoside	0.10 ± 0.01
13	17.7	262,350	463	316,317	Myricetin-*O*-deoxyhesoxide	4 ± 1
14	17.7		449	316,317	Myricetin-*O*-pentoside	4 ± 1
15	18.61	261,351	493	317	Myricetin-*O*-glucoronide	0.5 ± 0.1
16	19.16	264,353	463	301	Quercetin-3-*O*-glucoside	0.11 ± 0.01
17	19.34	276	469	393,769,599,169	Pentagalloyl glucoside	0.299 ± 0.001
18	19.84	267,357	761	301,609	Quercetin-*O*-galloyl-rutinoside	0.17 ± 0.04
19	20.88		593	285	Kaempferol-3-*O*-rutinoside	tr
20	21.39		433	300,301	Quercetin-*O*-pentoside	0.16 ± 0.03
21	22.51	256,349	447	300,301	Quercetin-*O*-rhamnoside	0.9 ± 0.2
22	24.46		585	301	Quercetin-*O*-acetyl-malonyl-hexoside	0.11 ± 0.02
23	25.61		615	317,463	Myricetin-*O*-galloyl-deoxyhexoside	tr
24	27.09		431	285	Kaempferol-*O*-deoxyhexoside	0.11 ± 0.02
					Total phenolic acids	0.21 ± 0.05
					Total flavonoids	12 ± 3
					Total gallotannins	26 ± 6
					Total phenolic compounds	38 ± 9

Results are expressed as mean ± standard deviation. tr - traces.

A total of twenty-four phenolic compounds were tentatively identified in *P. lentiscus* hydroethanolic extract, corresponding to one phenolic acid (peak **3**), fourteen flavonoids (peaks **10**, **11**, **12**, **13**, **14**, **15**, **16**, **18**, **19**, **20**, **21**, **22**, **23**, and **24**), and nine gallotanins (peaks **1**, **2**, **4**, **5**, **6**, **7**, **8**, **9**, and **17**).

The compounds galloyl quinic acid (peak **1**), trigalloyl quinic acid (peak **6**), myricetin-*O*-glucuronide (peak **15**), quercetin-3-*O*-glucoside (peak **16**), kaempferol-3-*O*-rutinoside (peak **19**), quercetin-*O*-pentoside (peak **20**), quercetin-*O*-rhamnoside (peak **21**) were previously detected in *P. lentiscus* leaves and fruit ethanolic extract (Garofulić et al., 2020). In turn, Anagnostou et al. (2023) described the presence of digalloyl quinic

acid (peaks **4** and **5**), trigalloyl quinic acid (peak **6**), myricetin-*O*-deoxyhesoxide (peak **13**), and myricetin-*O*-glucuronide (peak **15**) in leaves dichloromethane, methanol, and water extracts. The presence of the phenolic acid protocatechuic acid (peak **3**) was also described (Aissat et al., 2022; Brahmi et al., 2020; Daoued et al., 2016).

Unlike the other plants studied, *P. lentiscus* exhibits a significant presence of gallotannins, corresponding to a concentration of 26 mg/g extract, followed by flavonoids (12 mg/g extract) and phenolic acids (0.21 mg/g extract). Galloyl quinic acid (peak **1**, **Figure 9**) and digalloyl quinic acid isomer I (peak **4**) were the tentatively identified compounds present in higher abundance (15 and 8.5 mg/g extracts, respectively).

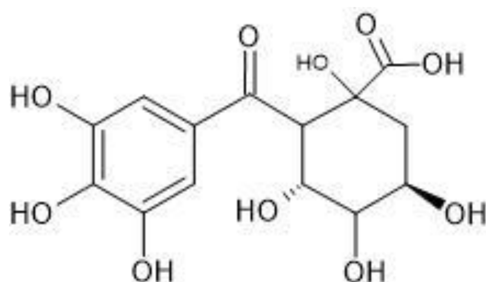

Figure 9 Chemical structure of galloyl quinic acid.

4.3. Bioactive properties

4.3.1. Antioxidant activity

The antioxidant activity of the studied medicinal plants was evaluated through TBARS assay. The obtained results are presented in **Table 11** and were expressed as IC_{50} values.

Table 11 Antioxidant activity of the hydroethanolic extracts of *P. major*, *B. pilosa*, and *P. lentiscus*.

Antioxidant Activity				
	P. major	*B. pilosa*	*P. lentiscus*	Positive Control
TBARS (IC_{50} values, μg/mL)	151 ± 11[a]	98 ± 2[b]	1.7 ± 0.1[c]	9.1 ± 0.3

Results are presented as mean values ± standard deviation. Different letters in the same row are significantly different according to Tukey's HSD test (p = 0.05). n.d. – not detected; dw – dry weight.

All medicinal plants studied exhibit the capacity to inhibit lipid peroxidation. Lower IC_{50} values (i.e., highest antioxidant activity) were obtained for *P. lentiscus* (1.7 μg/mL), followed by the hydroethanolic extracts of *B. pilosa* and *P. major* (IC_{50} values = 98 and 151 μg/mL, respectively). Nevertheless, *P. lentiscus* exhibited lower IC_{50} values than those of the positive control (Trolox, IC_{50} = 9.1 μg/mL), highlighting the promising antioxidant potential of this species.

Several researchers have previously assessed the antioxidant potential of these plants through different methodologies and described promising results. Among the various bioactivities studied, the antioxidant activity of these medicinal species is one of the most explored, mainly through the DPPH assay.

Falowo et al. (2019) studied the incorporation of *B. pilosa* essential oil in ground pork meat and described that the essential oils exhibit a higher capacity to protect meat than positive control (BHT). Similarly, Tebbi et al. (2024) analyzed the lipid peroxidation inhibition of *P. lentiscus* fruit extract (using a choline chloride-based deep eutectic solvent) and described an inhibition of 81.32%. Çoban et al. (2003) studied ethanolic extract of *P. major* to protect rat liver homogenates from lipid peroxidation and reported a IC_{50} value of 3.4 mg/mL.

Furthermore, the antioxidant activity of *P. major* extracts was previously demonstrated by several authors through the DPPH assay. The comparison between its leaf and seeds hydroethanolic extracts and the controls (quercetin and BHT) described a

more promising result for the leaves, as they exhibit a higher percentage of DPPH radical scavenging activity (between 70 – 90%) in comparison with seeds (20 – 90%), and synthetic antioxidant BHT (80%), while quercetin exhibit the highest activity (95%) (Mohamed et al., 2010). A similar study was conducted by Mello et al. (2015), that reported a lower DPPH scavenging activity for the leaf hydroethanolic extract (40%), compared with the control quercetin (90%).

Regarding *B. pilosa* ethanolic extract, Son et al. (2022) reported a lower DPPH scavenging activity for the plant extract than the positive controls ascorbic and gallic acids (EC_{50} of 455.78, 2.86, and 3.92 µg/mL, respectively). Similarly, Nguyen et al. (2023) reported a DPPH scavenging activity of around 80% for the flower and leaves extracts (500 µg/mL). Moreover, Salhi et al. (2019) studied *P. lentiscus* ethanolic extract and described a DPPH scavenging activity similar to BHA and ascorbic acid (IC_{50} of 5.34, 5.55, and 2.82 µg/mL, respectively).

Several authors highlight the direct correlation between phenolic compounds composition and their antioxidant capacity. In our study, among the species studied, *P. lentiscus* exhibited the highest levels of total phenolic compounds and flavonoids (38.12 and 12.29 mg/g, respectively), along with the greatest antioxidant activity. This fact suggests that the observed activity could be positively correlated with these compounds. However, *B. pilosa* demonstrated the lowest content of phenolic compounds and was the species with the second most interesting antioxidant activity. This difference may be related to the individual compounds detected in each species studied. *B. pilosa* exhibits the presence of several phenolic acids, such as protocatechuic, chlorogenic, and caffeic acids, known for their scavenging capacity (Silva et al., 2010), and these were not detected in *P. major*.

4.3.2. Antimicrobial activity

Among the traditional uses of *P. major*, *B. pilosa*, and *P. lentiscus*, one of the most reported is the use of extracts to treat infectious diseases and wounds. Therefore, studying the antimicrobial activity of these plants contributes to the scientific knowledge of traditional use. In this study, the antimicrobial activity was evaluated through the microdilution method. The results obtained for the antibacterial and antifungal activities of these species are presented in **Table 12**.

Table 12 Antimicrobial activity of *P. major*, *B. pilosa*, and *P. lentiscus* hydroethanolic extracts.

		P. major		***B. pilosa***		***P. lentiscus***		**Positive Control**					
	Antibacterial activity - Food bacteria (MIC and MBC, mg/mL)												
		MIC	MBC	MIC	MBC	MIC	MBC	Ampicillin		Streptomycin		Methicillin	
Gram-negative	*Enterobacter cloacae*	5	>10	>10	>10	>10	>10	0.15	0.15	0.007	0.007	n.t	n.t
	Escherichia coli	5	>10	10	>10	>10	>10	0.15	0.15	0.01	0.01	n.t	n.t
	Pseudomonas aeruginosa	5	>10	>10	>10	>10	>10	0.63	0.63	0.06	0.06	n.t	n.t
	Salmonella enterica	5	>10	10	>10	10	>10	0.15	0.15	0.007	0.007	n.t	n.t
	Yersinia enterocolitica	5	>10	>10	>10	2.5	>10	0.15	0.15	0.007	0.007	n.t	n.t
Gram-positive	*Bacillus cereus*	5	>10	>10	>10	2.5	>10	n.t	n.t	0.007	0.007	n.t	n.t
	Listeria monocytogenes	5	>10	>10	>10	2.5	>10	0.15	0.15	0.007	0.007	n.t	n.t
	Staphylococcus aureus	5	>10	>10	>10	2.5	>10	0.15	0.15	0.007	0.007	0.007	0.007
	Antifungal activity (MIC and MFC, mg/mL)												
		MIC	MFC	MIC	MFC	MIC	MFC	Ketoconazole					
	Aspergillus brasiliensis	>10	>10	>10	>10	>10	>10	0.06	0.125				
	Aspergillus fumigatus	10	>10	10	>10	>10	>10	0.5	1				

MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration; MFC – Minimum fungicidal concentration; n.t. – not tested.

The hydroethanolic extract of *P. major* was the only species studied that exhibited antibacterial activity against all tested bacteria strains (MIC = 5 mg/mL). In contrast, *P. lentiscus* demonstrated effectiveness only against *S. enterica*, *Y. enterocolitica,* and the Gram-positive bacteria (i.e., *B. cereus*, *L. monocytogenes*, and *S. aureus*), with MIC values between 2.5 and 10 mg/mL. Meanwhile, the extract of *B. pilosa* showed antibacterial activity only against *E. coli* and *S. enterica* (**Table 12**).

The results obtained are in agreement with the existing literature information. Metiner et al. (2012) described that *P. major* ethanolic and acetone extracts exhibit the capacity to inhibit *E. coli* and *B. cereus* bacteria, with MIC values between 3.562 and 42.5 mg/mL, without exhibiting bactericidal activity. Monawer & Mammani et al. (2023) and Soliman et al. (2022) also studied the antibacterial activity of *P. major* using the disk diffusion method and described inhibition zones between 9.93 and 23.18 mm. In contrast, *P. lentiscus* fruit extracts showed promising activity against *S. enterica*, *S. aureus*, *K. pneumonia*, and *B. subtilis* (34.3 – 44.3 mm of inhibition) (Tebbi et al., 2024). Besides, flavonoids extracted from *P. lentiscus* leaves exhibit antibacterial activity against *S. aureus*, *S. typhi*, *P. aeruginosa*, and *V. chloreae* (Bakli et al., 2020). Several studies have also reported significant antibacterial activity of *B. pilosa* methanolic extracts, which effectively inhibited the growth of several bacteria, including *S. aureus*, *E. coli*, *B. cereus*, and *P. aeruginosa*, exhibiting MIC values between 0.031 and 0.198 mg/mL (Ajanaku et al., 2018; Angelini et al., 2021). This species was also tested against *S. typhimurium* and aquatic pathogens such as *A. dhakensis*, *A. hydrophila*, *E. ictaluri*, and *S. agalactiae*, demonstrating effectiveness against all tested microorganisms (MIC values between 0.65 to 1.25 mg/mL) (Son et al. 2022).

Regarding the antifungal activity (**Table 12**), the extracts *P. major* and *B. pilosa* demonstrated the capacity to inhibit the fungus *A. fumigatus* (MIC = 10 mg/mL). However, no activity was observed against *A. brasiliensis*. Additionally, the extract of *P. lentiscus* showed no antifungal activity against the fungi tested. According to the literature, the antifungal activity of these species was scarcely studied. Son et al. (2022) described that *B. pilosa* ethanolic extracts inhibit *Colletotrichum* sp. and *Fusarium oxysporum* (MIC values of 1250 and 2000 μg/mL, respectively). Moderate antifungal activity for the acetone, methanol, and water extracts was also described against *A. niger* and *P. notatum* (between 36.39 and 100% of growth inhibition). The authors observed that the antifungal effect is dependent on the extract concentration (Ashafa & Afolayan, 2009; Son et al., 2022). Moreover, *P. lentiscus* leaf extracts and their flavonoids were

effective against *F. oxysporum*, *C. pelliculosa,* and *C. albicans* (between 12 and 15 mm of inhibition), wihout the capacity to inhibit *Aspergillus flavus* and *A. niger* (Bakli et al., 2020; Salhi et al., 2019). Additionally, *P. major* leaves ethanolic extracts at 20 mg/mL showed moderate antifungal activity against *C. albicans* and *A. niger* (10 – 17.6 mm of inhibition). However, a better antifungal activity of the plant's ethanolic extract has only been detected at higher concentrations (>30 mg/mL) (Edalatpanah et al., 2020; Zhakipbekov et al., 2023). Edalatpanah et al. (2020) suggested that the antifungal activity of *P. major* is related to the presence of the phenolic acid verbascoside.

6. Conclusion and future perspectives

The present study focused on the chemical characterization of *P. major* and *P. lentiscus* leaves, as well as the aerial parts of *B. pilosa*, and the evaluation of the bioactive properties of their hydroethanolic extracts.

All studied species exhibit varied compositions in fatty acids. *P. lentiscus* was the species that exhibited the highest contents in SFA and MUFA, while *P. major* exhibit higher PUFA content. Alpha and gamma tocopherols were the detected tocopherol isoforms in *P. major* and *B. pilosa*; in turn, in *P. lentiscus,* only the alpha-tocopherol was detected. Moreover, *P. major* exhibits higher tocopherol content than *P. lentiscus* and *B. pilosa*. *P. lentiscus* exhibits the presence of the free sugars' fructose, glucose, and sucrose, while *B. pilosa* and *P. major* contain only fructose and glucose. For the organic acids' composition, quinic, malic, and succinic acids were identified; quinic acid was the major one in *B. pilosa* and *P. lentiscus*, while succinic acid was the most abundant in *P. major*.

It was possible to tentatively identify several phenolic compounds within the plants studied, with *P. lentiscus* containing the higher total phenolic content, followed by *P. major* and *B. pilosa*. Verbascoside, quercetin-dimethyl ether rutinoside, and galloylquinic acid were the major phenolic compounds in *P. major*, *B. pilosa*, and *P. lentiscus*, respectively. Regarding the bioactive properties, all plants' extracts exhibited an antibacterial potential. However, only *P. major* and *B. pilosa* showed antifungal activity. In turn, the most promising antioxidant activity was observed in *P. lentiscus* extracts, followed by *B. pilosa* and *P. major*.

Our study elucidated the chemical composition, antioxidant, and antimicrobial activities of these medicinal plants. This knowledge contributes to the validation of their traditional uses and enables further investigation into their extracts, not only for medical purposes but also for nutritional and pharmacological applications.

As a result of the varied potential demonstrated, it would be interesting to study bioactive properties such as anti-inflammatory and wound-healing activity. Additionally, the correlation between the chemical composition and bioactive properties of each plant could be performed, in order to identify the compounds responsible for the demonstrated bioactive properties. Finally, and considering these species' properties and their extensive use in traditional medicine, it would be interesting to explore their use in pharmaceutical and cosmetic applications.

7. Bibliographic references

Abajo, C., Boffill, M. Á., Campo, J. del, Méndez, M. A., González, Y., Mitjans, M., & Vinardell, M. P. (2004). *In vitro* study of the antioxidant and immunomodulatory activity of aqueous infusion of *Bidens pilosa*. *Journal of Ethnopharmacology*, *93*(2), 319–323. https://doi.org/10.1016/j.jep.2004.03.050

Adom, M. B., Taher, M., Mutalabisin, M. F., Amri, M. S., Kudos, M. B., Sulaiman, M. W. A. W., Sengupta, P., & Susanti, D. (2017). Chemical constituents and medical benefits of *Plantago major*. *Biomedicine and Pharmacotherapy*, *96*, 348–360. https://doi.org/10.1016/j.biopha.2017.09.152

Aissat, A. K., Chaher-Bazizi, N., Richard, T., Kilani-Atmani, D., Pedrot, E., Renouf, E., Atmani, D., & Valls Fonayet, J. (2022). Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in *Pistacia lentiscus* L. fruits during ripening. *Journal of Food Composition and Analysis*, *106*, 104286. https://doi.org/10.1016/j.jfca.2021.104286

Ajanaku, O., Echeme, J., Mordi, R., Bolade, O., Okoye, S., Jonathan, H., & Ejilude, O. (2018). *In vitro* antibacterial, phytochemical, antimycobacterial activities and GC-MS analyses of *Bidens pilosa* leaf extract. *Journal of Microbiology, Biotechnology and Food Sciences*, *8*, 721–725. https://doi.org/10.15414/jmbfs.2018.8.1.721-725

Alcántara, H. D., Cabanillas-Chirinos, L. A., De La Cruz-Noriega, M., Benites, S. M., Diaz del Aguila, K., García, D. C., & Muñoz Ganoza., E. (2023). Antimicrobial potential of the ethanolic extract of Cadillo (*Bidens pilosa* Linneo.) against *Salmonella typhimurium* causing gastrointestinal infections. https://laccei.org/LACCEI2024-CostaRica/meta/FP775.html

Anagnostou, C., Beteinakis, S., Papachristodoulou, A., Pachi, V. K., Dionysopoulou, M., Dimou, S., Diallinas, G., Skaltsounis, L. A., & Halabalaki, M. (2023). Phytochemical investigation of *Pistacia lentiscus* L. var. *Chia* leaves: A byproduct with antimicrobial potential. *Fitoterapia*, *170*, 105648. https://doi.org/10.1016/j.fitote.2023.105648

Angelini, P., Matei, F., Flores, G. A., Pellegrino, R. M., Vuguziga, L., Venanzoni, R., Tirillini, B., Emiliani, C., Orlando, G., Menghini, L., & Ferrante, C. (2021). Metabolomic profiling, antioxidant and antimicrobial activity of *Bidens pilosa*. *Processes*, *9*(6), Article 6. https://doi.org/10.3390/pr9060903

Araruna, M. K. A., Brito, S. A., Morais-Braga, M. F. B., Santos, K. K. A., Souza, T. M., Leite, T. R., Costa, J. G. M., & Coutinho, H. D. M. (2012). Evaluation of antibiotic

& antibiotic modifying activity of pilocarpine & rutin. *Indian Journal of Medical Research*, *135*(2), 252.

Arima, H., Ashida, H., & Danno, G. (2002). Rutin-enhanced antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. *Bioscience, Biotechnology, and Biochemistry*, *66*(5), 1009–1014. https://doi.org/10.1271/bbb.66.1009

Arthur, G. D., Naidoo, K. K., & Coopoosamy, R. M. (2012). *Bidens pilosa* L.: Agricultural and pharmaceutical importance. *Journal of Medicinal Plants Research*, *6*(17). https://doi.org/10.5897/JMPR12.195

Ashafa, T., & Afolayan, A. (2009). Screening the root extracts from *Bidens pilosa* L. var. Radiata (Asteraceae) for antimicrobial potentials. *Journal of Medicinal Plants Research*, *3*, 568–572.

Azevedo, A., & Ferreira, M. F. (2011). Produção de plantas aromáticas e medicinais. Universidade do Minho.

Bairwa, K., Kumar, R., Sharma, R. J., & Roy, R. K. (2010). An updated review on *Bidens pilosa* L. *Der Pharma Chemica*, *2*, 325–337.

Bakli, S., Daoud, H., Amina, Z., Nouari, S., Asma, B., Soufiane, G., & Oumaima, N. (2020). Antimicrobial and antioxidant activities of flavonoids extracted from *Pistacia lentiscus* L., leaves. *Journal of Drug Delivery and Therapeutics*, *10*(1-s), Article 1-s. https://doi.org/10.22270/jddt.v10i1-s.3895

Barra, A., Coroneo, V., Dessi, S., Cabras, P., & Angioni, A. (2007). Characterization of the volatile constituents in the essential oil of *Pistacia lentiscus* L. from different origins and its antifungal and antioxidant activity. *Journal of Agricultural and Food Chemistry*, *55*(17), 7093–7098. https://doi.org/10.1021/jf071129w

Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of *Chenopodium ambrosioides* L. *Journal of Functional Foods*, *5*(4), 1732–1740. https://doi.org/10.1016/j.jff.2013.07.019

Bartolome, A. P., Villaseñor, I. M., & Yang, W.-C. (2013). *Bidens pilosa* L. (Asteraceae): Botanical properties, traditional uses, phytochemistry, and pharmacology. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–51. https://doi.org/10.1155/2013/340215

Beara, I. N., Orčić, D. Z., Lesjak, M. M., Mimica-Dukić, N. M., Peković, B. A., & Popović, M. R. (2010). Liquid chromatography/tandem mass spectrometry study of

anti-inflammatory activity of Plantain (*Plantago* L.) species. *Journal of Pharmaceutical and Biomedical Analysis*, *52*(5), 701–706. https://doi.org/10.1016/j.jpba.2010.02.014

Bessada, S. M. F., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2016). Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: An underexploited and highly disseminated species. *Industrial Crops and Products*, *89*, 45–51. https://doi.org/10.1016/j.indcrop.2016.04.065

Borges, C. C., Matos, T. F., Moreira, J., Rossato, A. E., Zanette, V. C., & Amaral, P. A. (2013). *Bidens pilosa* L. (Asteraceae): Traditional use in a community of southern Brazil. *Revista Brasileira de Plantas Medicinais*, *15*, 34–40. https://doi.org/10.1590/S1516-05722013000100004

Bouriche, H., Saidi, A., Ferradji, A., Belambri, S. A., & Senator, A. (2016). Anti-inflammatory and immunomodulatory properties of *Pistacia lentiscus* extracts. *Journal of Applied Pharmaceutical Science*, *6,*(7), 140–146. https://doi.org/10.7324/JAPS.2016.60721

Brahmi, F., Haddad, S., Bouamara, K., Yalaoui-Guellal, D., Prost-Camus, E., de Barros, J.-P. P., Prost, M., Atanasov, A. G., Madani, K., Boulekbache-Makhlouf, L., & Lizard, G. (2020). Comparison of chemical composition and biological activities of Algerian seed oils of *Pistacia lentiscus* L*., Opuntia ficus indica* (L.) mill. And *Argania spinosa* L. Skeels. *Industrial Crops and Products*, *151*, 112456. https://doi.org/10.1016/j.indcrop.2020.112456

Chang, M. H., Wang, G. J., Kuo, Y. H., & Lee, C. K. (2013). The low polar constituents from *Bidens pilosa* L. var. Minor (Blume) Sherff. https://onlinelibrary.wiley.com/doi/abs/10.1002/jccs.200000152

Chang, S. L., Chang, C. L.-T., Chiang, Y.-M., Hsieh, R.-H., Tzeng, C.-R., Wu, T.-K., Sytwu, H.-K., Shyur, L.-F., & Yang, W.-C. (2004). Polyacetylenic compounds and butanol fraction from *Bidens pilosa* can modulate the differentiation of helper T cells and prevent autoimmune diabetes in non-obese diabetic mice. *Planta Medica*, *70*(11), 1045–1051. https://doi.org/10.1055/s-2004-832645

Chang, S.-L., Chiang, Y.-M., Chang, C. L.-T., Yeh, H.-H., Shyur, L.-F., Kuo, Y.-H., Wu, T.-K., & Yang, W.-C. (2007). Flavonoids, centaurein and centaureidin, from *Bidens pilosa*, stimulate IFN-γ expression. *Journal of Ethnopharmacology*, *112*(2), 232–236. https://doi.org/10.1016/j.jep.2007.03.001

Chiang, Y.-M., Chang, C. L.-T., Chang, S.-L., Yang, W.-C., & Shyur, L.-F. (2007). Cytopiloyne, a novel polyacetylenic glucoside from *Bidens pilosa*, functions as a

T helper cell modulator. *Journal of Ethnopharmacology*, *110*(3), 532–538. https://doi.org/10.1016/j.jep.2006.10.007

Chiang, Y.-M., Chuang, D.-Y., Wang, S.-Y., Kuo, Y.-H., Tsai, P.-W., & Shyur, L.-F. (2004). Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. *Journal of Ethnopharmacology*, *95*(2), 409–419. https://doi.org/10.1016/j.jep.2004.08.010

Çoban, T., Çitoğlu, G. S., Sever, B., & İşcan, M. (2003). Antioxidant activities of plants used in traditional medicine in Turkey. *Pharmaceutical Biology*, *41*(8), 608–613. https://doi.org/10.1080/13880200390501974

Codif Recherche & Nature. (2016). Botanical Sheet *Pistacia lentiscus*. https://www.codif-tn.com/wp-content/uploads/2016/02/LAKESIS-FICHE-BOTANIQUE-GB.pdf

Cogo, L. L., Monteiro, C. L. B., Miguel, M. D., Miguel, O. G., Cunico, M. M., Ribeiro, M. L., Camargo, E. R. de, Kussen, G. M. B., Nogueira, K. da S., & Costa, L. M. D. (2010). Anti-*Helicobacter pylori* activity of plant extracts traditionally used for the treatment of gastrointestinal disorders. *Brazilian Journal of Microbiology*, *41*, 304–309. https://doi.org/10.1590/S1517-83822010000200007

Daoued, K. B., Chouaibi, M., Gaout, N., Haj, O. B., & Hamdi, S. (2016). Chemical composition and antioxidant activities of cold pressed lentisc (*Pistacia lentiscus* L.) seed oil. *Rivista Italiana Delle Sostanze Grasse*, *93*(1), 31–38. Scopus.

Deba, F., Xuan, T. D., Yasuda, M., & Tawata, S. (2007). Herbicidal and fungicidal activities and identification of potential phytotoxins from *Bidens pilosa* L. var. Radiata Scherff: Research paper. *Weed Biology and Management*, *7*(2), 77–83. Scopus. https://doi.org/10.1111/j.1445-6664.2007.00239.x

Deba, F., Xuan, T. D., Yasuda, M., & Tawata, S. (2008). Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var. Radiata. *Food Control*, *19*(4), 346–352. https://doi.org/10.1016/j.foodcont.2007.04.011

Dimas, K. S., Pantazis, P., & Ramanujam, R. (2012). Chios Mastic Gum: A plant-produced resin exhibiting numerous diverse pharmaceutical and biomedical properties. *In Vivo*, *26*(5), 777–785.

Dragović, S., Dragović-Uzelac, V., Pedisić, S., Čošić, Z., Friščić, M., Elez Garofulić, I., & Zorić, Z. (2020). The Mastic Tree (*Pistacia lentiscus* L.) leaves as source of BACs: Effect of growing location, phenological stage and extraction solvent on

phenolic content. *Food Technology and Biotechnology*, *58*(3), 303–313. https://doi.org/10.17113/ftb.58.03.20.6662

Dreher, M. L. (2012). Pistachio nuts: Composition and potential health benefits. *Nutrition Reviews*, *70*(4), 234–240. https://doi.org/10.1111/j.1753-4887.2011.00467.x

Edalatpanah, Y., Rostampur, S., Pouladi, I., & Rajaeenejad, S. (2020). Evaluation of antifungal effects of *Prangos ferulace* and *Plantago major* L. plants against fluconazole-resistant *Candida albicans* species in extracorporeal conditions. *Navid No*, *23*(74), 44–52. https://doi.org/10.22038/nnj.2020.46408.1201

Elloumi, W., Mahmoudi, A., Ortiz, S., Boutefnouchet, S., Chamkha, M., & Sayadi, S. (2022). Wound healing potential of quercetin-3-O-rhamnoside and myricetin-3-O-rhamnoside isolated from *Pistacia lentiscus* distilled leaves in rats model. *Biomedicine & Pharmacotherapy*, *146*, 112574. https://doi.org/10.1016/j.biopha.2021.112574

Falowo, A. B., Muchenje, V., Hugo, A., Aiyegoro, O. A., & Fayemi, P. O. (2017). Antioxidant activities of *Moringa oleifera* L. and *Bidens pilosa* L. leaf extracts and their effects on oxidative stability of ground raw beef during refrigeration storage. *CyTA - Journal of Food*, *15*(2), 249–256. https://doi.org/10.1080/19476337.2016.1243587

Fiocruz. (2022, December 14). *Fiocruz disponibiliza banco de dados sobre plantas medicinais*. Agência Fiocruz de Notícias. https://agencia.fiocruz.br/fiocruz-disponibiliza-banco-de-dados-sobre-plantas-medicinais

Ganeshpurkar, A., & Saluja, A. K. (2017). The pharmacological potential of rutin. *Saudi Pharmaceutical Journal : SPJ*, *25*(2), 149–164. https://doi.org/10.1016/j.jsps.2016.04.025

Garofulić, I., Kruk, V., Martić, A., Martić, I., Zorić, Z., Pedisić, S., Dragović, S., & Dragović-Uzelac, V. (2020). Evaluation of polyphenolic profile and antioxidant activity of *Pistacia lentiscus* L. leaves and fruit extract obtained by optimized microwave-assisted extraction. *Foods*, *9*(11), 1556. https://doi.org/10.3390/foods9111556

Ghenima, A. I., Idir, M., Nadjet, M. G., Samia, M. A., Mihoub, Z. M., & Karim, H. (2015). *In vitro* evaluation of biological activities of *Pistacia lentiscus* aqueous extract. *7*, 133–139.

Gonçalves, S., Moreira, E., Andrade, P. B., Valentão, P., & Romano, A. (2019). Effect of in vitro gastrointestinal digestion on the total phenolic contents and antioxidant activity of wild Mediterranean edible plant extracts. *European Food Research and Technology*, *245*(3), 753–762. Scopus. https://doi.org/10.1007/s00217-018-3197-y

Gowele, V. F., Kinabo, J., Jumbe, T., Kirschmann, C., Frank, J., & Stuetz, W. (2019). Provitamin A carotenoids, tocopherols, ascorbic acid and minerals in indigenous leafy vegetables from Tanzania. *Foods*, *8*(1), Article 1. https://doi.org/10.3390/foods8010035

Haghighi, S. R., Bagheri, K., Danafar, H., & Sharafi, A. (2021). Anti-proliferative properties, biocompatibility, and chemical composition of different extracts of *Plantago major* medicinal plant. *Iranian Biomedical Journal*, *25*(2), 106–116. https://doi.org/10.29252/ibj.25.2.106

Haghighi, S. R., Yazdinezhad, A., Bagheri, K., & Sharafi, A. (2022). Volatile constituents and toxicity of essential oils extracted from aerial parts of *Plantago lanceolata* and *Plantago major* growing in Iran. *Pharmaceutical and Biomedical Research*. https://doi.org/10.18502/pbr.v8i3.11035

Harrat, M., Benalia, M., Gourine, N., & Yousfi, M. (2018). Variability of the chemical compositions of fatty acids, tocopherols and lipids antioxidant activities, obtained from the leaves of *Pistacia lentiscus* L. growing in Algeria. *Mediterranean Journal of Nutrition and Metabolism*, *11*(2), 199–215. https://doi.org/10.3233/MNM-18198

Hassan, K., Francis, O., Engeu, O., Deogratius, O., & Nyafuono, J. (2011). Wound healing potential of the ethanolic extracts of *Bidens pilosa* and *Ocimum suave*. *African Journal of Pharmacy and Pharmacology*, *5*(2), 132–136.

Hemida, H., Doukani, K., Zitouni, A., Miloud, B., Beggar, H., & Bouhenni, H. (2022). Assessment of wound healing activity of ethanolic extracts of *Pistacia lentiscus* L. leaves and *Quercus ilex* L. bark in full thickness skin excision in rats. *Advances in Traditional Medicine*, *22*(3), 589–597. https://doi.org/10.1007/s13596-021-00557-8

Henning, T., & Raab-Straube, E. von. (2016). *The Euro+Med Plantbase Project*. https://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameCache=Pistacia%20lentiscus&PTRefFk=7100000

Hussan, F., Mansor, A. S., Hassan, S. N., Kamaruddin, Tg. N. T. T. N. E., Budin, S. B., & Othman, F. (2015). Anti-inflammatory property of *Plantago major* leaf extract reduces the inflammatory reaction in experimental Acetaminophen-induced liver injury. *Evidence-Based Complementary and Alternative Medicine*, *2015*, e347861. https://doi.org/10.1155/2015/347861

Idris, O. A., Kerebba, N., Horn, S., Maboeta, M. S., & Pieters, R. (2023). Phytochemical-based evidence of the health benefits of *Bidens pilosa* extracts and

cytotoxicity. *Chemistry Africa*, *6*(4), 1767–1788. https://doi.org/10.1007/s42250-023-00626-2

Kartini, K., Wati, N., Gustav, R., Wahyuni, R., Anggada, Y. F., Hidayani, R., Raharjo, A., Islamie, R., & Putra, S. E. D. (2021). Wound healing effects of *Plantago major* extract and its chemical compounds in hyperglycemic rats. *Food Bioscience*, *41*, 100937. https://doi.org/10.1016/j.fbio.2021.100937

Keivani, M., Mehregan, I., & Albach, D. C. (2021). Evaluating morphological diversity among *Plantago major* L. populations and influence of ecological variables. *Biologia*, *76*(4), 1127–1139. https://doi.org/10.1007/s11756-021-00711-2

Kıvçak, B., & Akay, S. (2005). Quantitative determination of α-tocopherol in *Pistacia lentiscus*, *Pistacia lentiscus* var. *Chia*, and *Pistacia terebinthus* by TLC-densitometry and colorimetry. *Fitoterapia*, *76*(1), 62–66. https://doi.org/10.1016/j.fitote.2004.09.021

Kuiper, P. J. C., & Bos, M. (2012). *Plantago: A Multidisciplinary Study*. Springer Science & Business Media.

Laanet, P.-R., Bragina, O., Jõul, P., & Vaher, M. (2024). *Plantago major* and *Plantago lanceolata* exhibit antioxidant and *Borrelia burgdorferi* inhibiting activities. *International Journal of Molecular Sciences*, *25*(13), Article 13. https://doi.org/10.3390/ijms25137112

Lemonakis, N., Magiatis, P., Kostomitsopoulos, N., Skaltsounis, A.-L., & Tamvakopoulos, C. (2011). Oral administration of Chios Mastic Gum or extracts in mice: Quantification of triterpenic acids by Liquid Chromatography-Tandem Mass Spectrometry. *Planta Medica*, *77*(17), 1916–1923. https://doi.org/10.1055/s-0031-1279996

Liang, X., & Xu, Q. (2016). Separation and identification of phenolic compounds in *Bidens pilosa* L. by ultra high performance liquid chromatography with quadrupole time-of-flight mass spectrometry. *Journal of Separation Science*, *39*(10), 1853–1862. https://doi.org/10.1002/jssc.201600017

Liberal, A., Fernandes, A., Ferreira, I. C. F. R., Vivar-Quintana, A. M., & Barros, L. (2024). Effect of different physical pre-treatments on physicochemical and techno-functional properties, and on the antinutritional factors of lentils (*Lens culinaris* spp). https://www.sciencedirect.com/science/article/pii/S0308814624009427?via%3Dihub

Ljubuncic, P., Song, H., Cogan, U., Azaizeh, H., & Bomzon, A. (2005). The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental

liver disease. *Journal of Ethnopharmacology*, *100*(1), 198–204. https://doi.org/10.1016/j.jep.2005.03.006

Long, C., Moulis, C., Stanislas, E., & Fouraste, I. (1995). Aucuboside and catapol in *Plantago lanceolata* L., *Plantago major* L., *Plantago media* L. leaves. *Journal de Pharmacie de Belgique*, *50*(6), 484–488. Scopus.

Lukova, P. K., Karcheva-Bahchevanska, D. P., Nikolova, M. M., Iliev, I. N., & Mladenov, R. D. (2017). Comparison of structure and antioxidant activity of polysaccharides extracted from the leaves of *Plantago major* L., *P. media* L. and *P. lanceolata* L. *Bulgarian Chemical Communications*, *49*, 282–288.

Lyu, S., Mei, Q., Liu, H., Wang, B., Wang, J., Lambers, H., Wang, Z., Dong, B., Liu, Z., & Deng, S. (2023). Genome assembly of the pioneer species *Plantago major* L. (Plantaginaceae) provides insight into its global distribution and adaptation to metal-contaminated soil. *DNA Research*, *30*(4), 1–14. https://doi.org/10.1093/dnares/dsad013

Martins, N., Gonçalves, S., Andrade, P. B., Valentão, P., & Romano, A. (2013). Changes on organic acid secretion and accumulation in *Plantago almogravensis* Franco and *Plantago algarbiensis* Samp. Under aluminum stress. *Plant Science*, *198*, 1–6. https://doi.org/10.1016/j.plantsci.2012.09.001

Masako, H., & Yoshiyuki, S. (2006). Antiinflammatory and antiallergic activity of *Bidens pilosa* L. var. Radiata Scherff. *Journal of Health Science*, *52*(6), 711–717. https://doi.org/10.1248/jhs.52.711

Mazzutti, S., Salvador Ferreira, S. R., Herrero, M., & Ibañez, E. (2017). Intensified aqueous-based processes to obtain bioactive extracts from *Plantago major* and *Plantago lanceolata*. *The Journal of Supercritical Fluids*, *119*, 64–71. https://doi.org/10.1016/j.supflu.2016.09.008

Meinhart, A. D., Damin, F. M., Caldeirão, L., da Silveira, T. F. F., Filho, J. T., & Godoy, H. T. (2017). Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. *Food Research International*, *99*, 522–530. https://doi.org/10.1016/j.foodres.2017.06.017

Mello, J. C., Gonzalez, M. V. D., Moraes, V. W. R., Prieto, T., Nascimento, O. R., & Rodrigues, T. (2015). Protective effect of *Plantago major* extract against t-BOOH-induced mitochondrial oxidative damage and cytotoxicity. *Molecules*, *20*(10), Article 10. https://doi.org/10.3390/molecules201017747

Metiner, K., Ozkan, O., & Ak, S. (2012). Antibacterial effects of ethanol and acetone extract of *Plantago major* L. on Gram Positive and Gram Negative bacteria. *Kafkas Universitesi Veteriner Fakultesi Dergisi*. https://doi.org/10.9775/kvfd.2011.5824

Milia, E., Bullitta, S. M., Mastandrea, G., Szotáková, B., Schoubben, A., Langhansová, L., Quartu, M., Bortone, A., & Eick, S. (2021). Leaves and fruits preparations of *Pistacia lentiscus* l.: A review on the ethnopharmacological uses and implications in inflammation and infection. *Antibiotics*, *10*(4). https://doi.org/10.3390/antibiotics10040425

Mohamed, I. K., Osama, M. A.-F., El-Salam, S. M. A., & Mohamed, Z. E.-O. M. (2010). Biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L. *International Journal of Biodiversity and Conservation*, *3*, 83–91.

Monawer, A. T., & Mammani, I. M. A. (2023). Antibacterial activity of ethanolic extracts of *Plantago major* leaves against *Pseudomonas aeruginosa* from burn infections. *The Journal of Infection in Developing Countries*, *17*(02), Article 02. https://doi.org/10.3855/jidc.17576

Morales, M., Pasques, O., & Munné-Bosch, S. (2021). English plantain deploys stress tolerance mechanisms at various organization levels across an altitudinal gradient in the Pyrenees. *Physiologia Plantarum*, *173*(4), 2350–2360. https://doi.org/10.1111/ppl.13586

Morguette, A. E. B., Bartolomeu-Gonçalves, G., Andriani, G. M., Bertoncini, G. E. S., Castro, I. M. de, Spoladori, L. F. de A., Bertão, A. M. S., Tavares, E. R., Yamauchi, L. M., & Yamada-Ogatta, S. F. (2023). The antibacterial and wound healing properties of natural products: A review on plant species with therapeutic potential against *Staphylococcus aureus* wound infections. *Plants*, *12*(11). Scopus. https://doi.org/10.3390/plants12112147

Moyo, S. M., Serem, J. C., Bester, M. J., Mavumengwana, V., & Kayitesi, E. (2020). Influence of boiling and subsequent phases of digestion on the phenolic content, bioaccessibility, and bioactivity of *Bidens pilosa* (Blackjack) leafy vegetable. *Food Chemistry*, *311*, 126023. https://doi.org/10.1016/j.foodchem.2019.126023

Nyakudya, T. T., Tshabalala, T., Dangarembizi, R., Erlwanger, K. H., & Ndhlala, A. R. (2020). The potential therapeutic value of medicinal plants in the management of metabolic disorders. *Molecules*, *25*(11), 2669. https://doi.org/10.3390/molecules25112669

Pachi, V. K., Mikropoulou, E. V., Gkiouvetidis, P., Siafakas, K., Argyropoulou, A., Angelis, A., Mitakou, S., & Halabalaki, M. (2020). Traditional uses, phytochemistry and pharmacology of Chios mastic gum (*Pistacia lentiscus* var. Chia, Anacardiaceae): A review. *Journal of Ethnopharmacology*, *254*. https://doi.org/10.1016/j.jep.2019.112485

Pereira, C., Barros, L., & Ferreira, I. C. F. R. (2015). A comparison of the nutritional contribution of thirty-nine aromatic plants used as condiments and/or herbal infusions. *Plant Foods for Human Nutrition*, *70*(2), 176–183. https://doi.org/10.1007/s11130-015-0476-7

Petropoulos, S., Fernandes, Â., Barros, L., Ciric, A., Sokovic, M., & Ferreira, I. C. F. R. (2017). The chemical composition, nutritional value and antimicrobial properties of *Abelmoschus esculentus* seeds. *Food & Function*, *8*(12), 4733–4743. https://doi.org/10.1039/C7FO01446E

Quaglio, A. E. V., Cruz, V. M., Almeida-Junior, L. D., Costa, C. A. R. A., & Di Stasi, L. C. (2020). *Bidens pilosa* (Black Jack) standardized extract ameliorates acute TNBS-induced intestinal inflammation in rats. *Planta Medica*, *86*(5), 319–330. Scopus. https://doi.org/10.1055/a-1089-8342

Ramabulana, A.-T., Steenkamp, P., Madala, N., & Dubery, I. A. (2020). Profiling of chlorogenic acids from *Bidens pilosa* and differentiation of closely related positional isomers with the aid of UHPLC-QTOF-MS/MS-based in-source collision-induced dissociation. *Metabolites*, *10*(178). https://doi.org/10.3390/metabo10050178

Rauf, A., Patel, S., Uddin, G., Siddiqui, B. S., Ahmad, B., Muhammad, N., Mabkhot, Y. N., & Hadda, T. B. (2017). Phytochemical, ethnomedicinal uses and pharmacological profile of genus Pistacia. *Biomedicine & Pharmacotherapy*, *86*, 393–404. https://doi.org/10.1016/j.biopha.2016.12.017

Remila, S., Atmani-Kilani, D., Delemasure, S., Connat, J.-L., Azib, L., Richard, T., & Atmani, D. (2015). Antioxidant, cytoprotective, anti-inflammatory and anticancer activities of *Pistacia lentiscus* (Anacardiaceae) leaf and fruit extracts. *European Journal of Integrative Medicine*, *7*(3), 274–286. https://doi.org/10.1016/j.eujim.2015.03.009

Rico, J. M. T. (2018). Plantas medicinais: Passado, presente e futuro. *45*, 363–377. https://doi.org/10.58164/cm71-st70

Ringbom, T., Segura, L., Noreen, Y., Perera, P., & Bohlin, L. (1998). Ursolic acid from *Plantago major*, a selective inhibitor of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. *Journal of Natural Products*, *61*(10), 1212–1215. https://doi.org/10.1021/np980088i

Rocha, F. C., Gaspar, C., Lopes, V. R., & Barata, A. M. (2017). Medicinal and Aromatic Plants collecting missions in Portugal. *Arabian Journal of Medicinal & Aromatic Plants*, *3*(1). https://www.semanticscholar.org/paper/Medicinal-and-Aromatic-Plants-collecting-missions-Rocha/6001d59201fda09833bf76e46ae222d49a5a53f6

Saddiqe, Z. (2019). *Medicinal Plants Biotechnology*. https://research.ebsco.com/linkprocessor/plink?id=e426f3e8-af0f-3b0b-b7ef-70d95c737b40

Salem, O., Szwajkowska-Michałek, L., Przybylska-Balcerek, A., Szablewski, T., Cegielska-Radziejewska, R., Świerk, D., & Stuper-Szablewska, K. (2023). New Insights into Bioactive Compounds of Wild-Growing Medicinal Plants. *Applied Sciences*, *13*(24), Article 24. https://doi.org/10.3390/app132413196

Salhi, A., Bellaouchi, R., Barkany, S. E., Rokni, Y., Bouyanzer, A., Asehraou, A., Zarrouk, A., & Hammouti, B. (2019). Total phenolic content, antioxidant and antimicrobial activities of extracts from *Pistacia lentiscus* leaves. *Caspian Journal of Environmental Sciences*, *17*(3).

Sanna, F., Piluzza, G., Campesi, G., Molinu, M. G., Re, G. A., & Sulas, L. (2022). Antioxidant contents in a Mediterranean population of *Plantago lanceolata* L. exploited for quarry reclamation interventions. *Plants*, *11*(6), Article 6. https://doi.org/10.3390/plants11060791

Santos, M. B. V. dos, Oliveira, A. B. de, & Mourão, R. H. V. (2024). Brazilian plants with antimalarial activity: A review of the period from 2011 to 2022. *Journal of Ethnopharmacology*, *322*, 117595. https://doi.org/10.1016/j.jep.2023.117595

Scognamiglio, M., Graziani, V., Tsafantakis, N., Esposito, A., Fiorentino, A., & D’Abrosca, B. (2019). NMR-based metabolomics and bioassays to study phytotoxic extracts and putative phytotoxins from Mediterranean plant species. *Phytochemical Analysis*, *30*(5), 512–523. Scopus. https://doi.org/10.1002/pca.2842

Sehaki, C., Jullian, N., Ayati, F., Fernane, F., & Gontier, E. (2023). A review of *Pistacia lentiscus* polyphenols: Chemical diversity and pharmacological activities. *Plants*, *12*(2). https://doi.org/10.3390/plants12020279

Shen, Y., Sun, Z., Shi, P., Wang, G., Wu, Y., Li, S., Zheng, Y., Huang, L., Lin, L., Lin, X., & Yao, H. (2018). Anticancer effect of petroleum ether extract from *Bidens pilosa* L and its constituent’s analysis by GC-MS. *Journal of Ethnopharmacology*, *217*, 126–133. https://doi.org/10.1016/j.jep.2018.02.019

Silva, F. L., Fischer, D. C. H., Tavares, J. F., Silva, M. S., Athayde-Filho, P. F. de, & Barbosa-Filho, J. M. (2011). Compilation of secondary metabolites from *Bidens pilosa* L. *Molecules*, *16*(2), 1070–1102. https://doi.org/10.3390/molecules16021070

Silva, M. L. C., Costa, R. S., Santana, A. S., & Koblitz, M. G. B. (2010). *Compostos fenólicos, carotenóides e atividade antioxidante em produtos vegetais*. https://www.researchgate.net/publication/276228318_Compostos_fenolicos_carotenoides_e_atividade_antioxidante_em_produtos_vegetais_Phenolic_compounds_carotenoids_and_antioxidant_activity_in_plant_products

Skari, K. P., Malterud, K. E., & Haugli, T. (1999). Radical scavengers and inhibitors of enzimatic lipid peroxidation from *Plantago major*, a medicinal plant. In *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease* (240th ed., pp. 200–202). Elsevier. https://encurtador.com.br/OWlEG

Skrynetska, I., Karcz, J., Barczyk, G., Kandziora-Ciupa, M., Ciepał, R., & Nadgórska-Socha, A. (2019). Using *Plantago major* and *Plantago lanceolata* in environmental pollution research in an urban area of Southern Poland. *Environmental Science and Pollution Research*, *26*(23), 23359–23371. https://doi.org/10.1007/s11356-019-05535-x

Soliman, M. A., Galal, T. M., Naeim, M. A., & Khalafallah, A. A. (2022). Seasonal variation in the secondary metabolites and antimicrobial activity of *Plantago major* L. from Egyptian heterogenic habitats. *Egyptian Journal of Botany*. https://doi.org/10.21608/ejbo.2021.94145.1778

Son, N. H., Tuan, N. T., & Tran, T. M. (2022). Investigation of chemical composition and evaluation of antioxidant, antibacterial and antifungal activities of ethanol extract from *Bidens pilosa* L. *Food Science and Technology*, *42*, e22722. https://doi.org/10.1590/fst.22722

Stenholm, Å., Göransson, U., & Bohlin, L. (2013). Bioassay-guided Supercritical Fluid extraction of Cyclooxygenase-2 inhibiting substances in *Plantago major* L. *Phytochemical Analysis*, *24*(2), 176–183. https://doi.org/10.1002/pca.2398

Tan, P. V., Dimo, T., & Dongo, E. (2000). Effects of methanol, cyclohexane and methylene chloride extracts of *Bidens pilosa* on various gastric ulcer models in rats. *Journal of Ethnopharmacology*, *73*(3), 415–421. https://doi.org/10.1016/S0378-8741(00)00290-7

Taylor. (2005). *Picao Preto—*Bidens pilosa *Database file in the Tropical Plant Database of herbal remedies*. https://rain-tree.com/picaopreto.htm

Tebbi, S. O., Trapali, M., & Letsiou, S. (2024). Exploring the anti-diabetic, antioxidant and antimicrobial properties of *Clematis flammula* L. leaves and *Pistacia lentiscus* L. fruits using choline chloride-based deep eutectic solvent. *Waste and Biomass Valorization*, *15*(5), 2869–2879. Scopus. https://doi.org/10.1007/s12649-023-02360-9

Thomé, R. G., Santos, H. B. D., Santos, F. V. D., Oliveira, R. J. D. S., De Camargos, L. F., Pereira, M. N., Longatti, T. R., Souto, C. M., Franco, C. S., De Oliveira Aquino Schüffner, R., & Ribeiro, R. I. M. A. (2012). Evaluation of healing wound and genotoxicity potentials from extracts hydroalcoholic of *Plantago major* and *Siparuna guianensis*. *Experimental Biology and Medicine*, *237*(12), 1379–1386. https://doi.org/10.1258/ebm.2012.012139

Tian, X.-Y., Li, M.-X., Lin, T., Qiu, Y., Zhu, Y.-T., Li, X.-L., Tao, W.-D., Wang, P., Ren, X.-X., & Chen, L.-P. (2021). A review on the structure and pharmacological activity of phenylethanoid glycosides. *European Journal of Medicinal Chemistry*, *209*, 112563. https://doi.org/10.1016/j.ejmech.2020.112563

Turgumbayeva, A., Zhakipbekov, K., Shimirova, Z., Akhelova, S., Amirkhanova, A., Koilybayeva, M., Seitimova, G., & Abdambayev, D. (2022). The study of phytochemical compounds of plantain leaves grown in Kazakhstan. *Pharmacia*, *69*(4), 1019–1026. https://doi.org/10.3897/pharmacia.69.e96526

Vendruscolo, M. H., Koetz, M., von Poser, G. L., & Henriques, A. T. (2024). Development and validation of an UPLC method for quality control of *Plantago major*. *Revista Brasileira de Farmacognosia*, *34*(4), 871–879. Scopus. https://doi.org/10.1007/s43450-024-00528-5

Wang, Y., Niu, Y., Zhao, X., Wang, B., Jiang, Q., Liu, J., & Sheng, Y. (2017). Fatty acid and phytochemical compositions of Plantago seed oils and their functionalities. *Journal of the American Oil Chemists' Society*, *94*(7), 905–912. https://doi.org/10.1007/s11746-017-3003-1

Wissal, D., Nahida, J., Emna, C., Maroua, B., Saloua, F., Semia, O., & Wissem, M. (2013). Chemical composition of lentisk (*Pistacia lentiscus* L.) seed oil. *African Journal of Agricultural Research*, *8*(16), 1395–1400. https://doi.org/10.5897/AJAR11.1837

World Health Organization. (2023). *Integrating Traditional Medicine in Health Care*. https://www.who.int/southeastasia/news/feature-stories/detail/integrating-traditional-medicine

Wu, L.-W., Chiang, Y.-M., Chuang, H.-C., Wang, S.-Y., Yang, G.-W., Chen, Y.-H., Lai, L.-Y., & Shyur, L.-F. (2004). Polyacetylenes function as anti-angiogenic agents. *Pharmaceutical Research*, *21*(11), 2112–2119. https://doi.org/10.1023/B:PHAM.0000048204.08865.41

Xiao, Y., Ren, Q., & Wu, L. (2022). The pharmacokinetic property and pharmacological activity of acteoside: A review. *Biomedicine & Pharmacotherapy*, *153*, 113296. https://doi.org/10.1016/j.biopha.2022.113296

Xuan, T. D., & Khanh, T. D. (2016). Chemistry and pharmacology of *Bidens pilosa*: An overview. *Journal of Pharmaceutical Investigation*, *46*(2), 91–132. https://doi.org/10.1007/s40005-016-0231-6

Yan, Z., Chen, Z., Zhang, L., Wang, X., Zhang, Y., & Tian, Z. (2022). Bioactive polyacetylenes from *Bidens pilosa* L. and their anti-inflammatory activity. *Natural Product Research*, *36*(24), 6353–6358. https://doi.org/10.1080/14786419.2022.2029432

Zahouani, Y., Ben Rhouma, K., Kacem, K., Sebai, H., & Sakly, M. (2021). Aqueous leaf extract of *Pistacia lentiscus* improves acute acetic acid-induced colitis in rats by reducing inflammation and oxidative stress. *Journal of Medicinal Food*, *24*(7), 697–708. https://doi.org/10.1089/jmf.2020.0020

Zbuchea, A., Lungu, L., Popa, C.-V., Tecuceanu, V., Alexandru, V., & Tatia, R. (2016). An innovative ointment made of natural ingredients with increased wound healing activity. *Romanian Biotechnological Letters*, *21*(2).

Zhakipbekov, K., Turgumbayeva, A., Issayeva, R., Kipchakbayeva, A., Kadyrbayeva, G., Tleubayeva, M., Akhayeva, T., Tastambek, K., Sainova, G., Serikbayeva, E., Tolenova, K., Makhatova, B., Anarbayeva, R., Shimirova, Z., & Tileuberdi, Y. (2023). Antimicrobial and other biomedical properties of extracts from *Plantago major*, Plantaginaceae. *Pharmaceuticals*, *16*(8). https://doi.org/10.3390/ph16081092

Zubair, M., Ekholm, A., Nybom, H., Renvert, S., Widen, C., & Rumpunen, K. (2012). Effects of *Plantago major* L. leaf extracts on oral epithelial cells in a scratch assay. *Journal of Ethnopharmacology*, *141*(3), 825–830. https://doi.org/10.1016/j.jep.2012.03.016

Zubair, M., Nybom, H., Lindholm, C., Brandner, J. M., & Rumpunen, K. (2016). Promotion of wound healing by *Plantago major* L. leaf extracts – *ex-vivo* experiments confirm experiences from traditional medicine. *Natural Product Research*, *30*(5), 622–624. https://doi.org/10.1080/14786419.2015.1034714

Zubair, M., Nybom, H., Lindholm, C., & Rumpunen, K. (2011). Major polyphenols in aerial organs of greater plantain (*Plantago major* L.), and effects of drying temperature on polyphenol contents in the leaves. *Scientia Horticulturae*, *128*(4), 523–529. https://doi.org/10.1016/j.scienta.2011.03.001

Zubair, M., Widén, C., Renvert, S., & Rumpunen, K. (2019). Water and ethanol extracts of *Plantago major* leaves show anti-inflammatory activity on oral epithelial cells. *Journal of Traditional and Complementary Medicine*, *9*(3), 169–171. https://doi.org/10.1016/j.jtcme.2017.09.002