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Phytochemical, antioxidant and antibacterial profile of *Achillea millefolium* L.: a literature review

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ABSTRACT
The literature indicates the use of *A. millefolium* since ancient times, predominantly for addressing various gastrointestinal disorders. With advancements in analytical techniques and plant biotechnology, research have revealed therapeutic properties attributed to this plant, such as anti-inflammatory, antifungal, analgesic, hemostatic, cholagogue, hepatoprotective, as well as antibacterial effects. More recent studies performed on *in vitro* cell lines, also indicated the apoptotic effect of *A. millefolium* extracts on human cervical cancer (HeLa) cells. The pharmacological activity observed in *Achillea* species can likely be attributed to the diversity and intricate composition of their phytochemical constituents, mainly phenolic acids (caffeic, cinnamic and benzoic acid derivatives), flavonoids, terpenes, organic and fatty acids, volatiles and phytosterols. The primary objective of this study is to present a documented overview of the phytochemical composition of *Achillea millefolium* L. and to investigate its phytopharmaceutical effects, with a particular focus on assessing its antioxidant and antibacterial properties.

Keywords: phytochemistry, antioxidant activity, antibacterial potential, plant biotechnology, bioactive compounds

Introduction

Asteraceae plants are worldwide distributed, although they are particularly common in arid and semi-arid regions of the subtropics and cooler latitudes. The genus *Achillea*, which includes over 130 varieties of perennial and blooming plants, is primarily found in temperate regions of Asia and America as well as Europe. One significant family member is *Achillea millefolium*, an erect herbaceous perennial plant, with a maximum height of 50 cm. Numerous roots and stolons are produced by the plant's slender underground stem. The feathery leaves, which range in length from 5 to 20 cm, can be either tripinnate or bipinnate and have different levels of pubescence, or hairiness. Clustered at the ends of stems and branches, the flowers are typically white but can also be pink or pale purple [20, 3]. The plant grows vigorously in the spring and blooms from May to June [11]. *A. millefolium* is a well-known medicinal plant with various phytopharmaceutical uses [22] due to its chemical properties ensuring important therapeutic actions [20, 42]. Anti-inflammatory, hepatoprotective, antirheumatic, antispasmodic, antiseptic, sedative, astringent, digestive, and expectorant properties are just few of the clinical research documented properties of the plant [6]. According to other research, the plant has potent antibacterial [44] and antioxidant [15] effects. This plant's anti-inflammatory and antiseptic qualities are due to its abundance of polyphenolic compounds, some forms of flavonoids, terpenes, lactones, betaine, acetylene compounds, resins, tannins, phosphates, nitrates, potassium salts, and antifungals [20]. In this context, this paper's purpose is to highlight the therapeutic potential of *Achillea millefolium* by analyzing and synthesizing data from the specialized literature about its phytochemical composition, antioxidant activity, and antimicrobial properties.

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Material and method

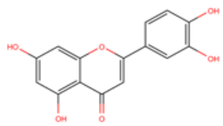
The scientific literature from the previous years was reviewed in an attempt to shape an exhaustive perspective of phytochemistry, antioxidant, and antimicrobial properties of *Achillea millefolium L*. within the broader context of medicinal plant research. The most relevant scientific databases such as Web of Science, PubMed, Scopus, or Google Scholar were consulted for this study. To identify the most relevant articles for this context, keywords such as the phytochemical components, antioxidant activity, antibacterial activity, and medicinal uses of the plant (*Achillea millefolium*) were used during the selection process.

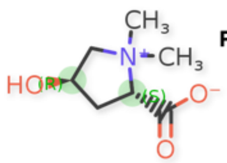
1. Phytochemical profile of Achillea millefolium L.

Achillea millefolium L. presents a complex phytochemical profile that plays an essential role in the therapeutic efficacy of the plant. The main compounds found in the plant are phenolic acids, flavonoids, terpenes and essential oils (Table 1).

Table 1. Main bioactive compounds classes from A. millefolium organs

Class of compounds	Class specific examples	Main organ
Flavonoids	Apigenin, luteolin, quercetin, rutin	Leaves, flowers
Phenolic acids	Caffeic acid, chlorogenic acid, salicylic acid	Leaves, stems
Essential oils	Camazulene, 1,8-cineole, α- and β-pinene, borneol, eucalyptol	Flowers, leaves
Sesquiterpene lactones	Achilina, matricarina	Leaves, flowers
Tannins	Hydrolyzable and condensed tannins	Leaves, flowers
Polysaccharides	Arabinose, galactose	Stems, roots
Alkaloids	Achilles	Leaves, roots
Terpenes and sesquiterpenes	Bisabolol, α- and β-pinene, borneol, eucalyptol	Flowers, leaves
Sterols	β-sitosterol	Leaves





1.1. Phenolic compounds

Phenolic acids and derivatives compounds found in *A. millefolium* L. are choline, 1,3-dicapheoylquinic acid (DCQA), 1,4 DCQA, apigenin 4-O-glucoside and luteolin 4-O-glucoside, 3,4-DCQA, 3,5-DCQA, 1, 5-DCQA, chlorogenic acid, luteolin-7-β-D-oglucuronide, caffeic acid, p-coumaric acid and neochlorogenic acid, ferulic acid, and stachydrin, carboxylic acid, salicylic acid, pyrocatechol, adenine, mandelic acid, methyl esters of caprylic-linolenic and undecylenicacids [38, 42].

1.2. Flavonoids and derivates

Regarding the flavonoid content, the plant is distinguished by its content of aglycone flavonoids, flavonoid glycosides, flavonol, and flavones O-glycosides. Flavonoid aglycosides found in different parts of the plant are resveratrol, morin, myricetin, naringin, naringenin, quercetin, kaempferol, apigenin, apigenin, luteolin, centaureidin, casticin, artemetin, isorhamnetin, acacetin, chrysophenol-D, salvigenin, quercetagetin, centaureidin, hispidulin, cirsimarin, and nepetin [4]. The primary flavonoid glycosides found in extracts of *Achillea millefolium* L. are apigenin 7-O-glucozide, luteolin 7-O-glucozide (cynaroside), luteolin-3,7-di-O-glucozide, vicenin-2, rutin, dihydrodehydrodiconiferyl alcohol 9-O-β-D-glucopyranoside, apigenin-7-O-β-O-β-D-glucopyranoside, luteolin-7-O-β-O-β-D-glucopyranoside, luteolin-4-O-β-O-β-D-glucopyranoside, vitexin, vicenin, swertjaponin and swertisin. In terms of flavonol and flavones O-glycosides content the relevant compounds are quercetin-3-O-glycoside, quercetin-3-O-rhamnoglycoside, luteolin-7-O-glycoside, diosmetin-7-O-glycoside, and kaempferol-3-O-glycoside [4, 38].

1.3. Terpenic compounds

Monoterpenes constitute about 90% of the essential oil obtained from leaves and flowers of *A. millefolium*. The flowers are distinguished by a high concentration of borneol (16.51%), lower concentrations of 1, 8-cineole (9.80%), β-pinene (5.31%) α-pinene (4.64%) and the presence of camphor (8.37%). On the other hand, the leaves possess borneol (12.32%) and high concentrations of 1,8-cineole (10.51%), β-pinene (9.33%) and α-pinene (8.82%) [2, 4, 24]. The plant also contains a wide range of compounds from the sesquiterpenoids class, which besides the terapeutic effect contribute to the plant's distinct aromatic profile. According to Ali et al (2017) [4], sesquiterpenoids found in the plant are sesquiterpene lactone ester A and B, sesquiterpene lactone-diol, and seco-pseudo guaianolides such as paulitin, isopaulitin, psilostachyin C, desacetylmatricarin, and sintenin. Additionally, sesquiterpenoids include achimillic acids A, B, and C, isoachifolidiene, 8-acetyl egelolide, 8-angeloyl egelolide, austricin (deacetylmatricarin), millefin, 8-hydroxyachillin, and artelesin [4, 24]. Unique to the plant, another sesquiterpene lactone is miltefosine, which is a specific compound of *Achillea millefolium L.* and shows important therapeutic effects [24]. Regarding proazulenes content, only 38% of the plants contain it, and they show different concentrations depending on the growth stage. Studies have shown a directly proportional relationship between leaf width, number of nodes per plant, and proazulenes content [4, 34]. In terms of triterpene content, the plant has a low content with only four compounds identified which are α-amyrin, β-amyrin, taraxasterol, and pseudotaraxasterol. Likewise, a low content of compounds is also the case for sterols which are represented by β-sitosterol, stigmasterol, campesterol, and cholesterol [4, 34]. In addition to terpenes, lignans were identified, with the most recently discovered compound according to Liu et. al. (2017) being (+)-lyoniresinol 4-O-α-L−arabinofuranoside [25].

Methods for extraction of the bioactive compounds

The most effective methods for obtaining essential oils, which do not differ significantly from each other, are HD (hydrodistillation), SMDE (microwave-assisted small-scale distillation) and MADH (microwave-assisted hydrodistillation). With these methods, it is possible to obtain pure essential oils without contamination with organic solvents. Additionally, SMDE (microwave-assisted small-scale distillation) is a method that allows the obtaining of essential oils from a small amount of plant material [25, 43]. For flavonoids, according to studies, among the various solvents tested for extraction, the hydroalcoholic solvent demonstrated the higher efficiency, making it the most suitable—choice for obtaining flavonoid-rich extracts [4, 43]. For the extraction of polyphenols from *A. millefolium* the most commonly used solvents are water, 30% ethanol-aqueous, 50% ethanol-aqueous, and 70% ethanol-aqueous. Among these, the most efficient solvent was found to be 50% ethanol-aqueous [12, 43]. For the extraction of terpenes and lignans the most efficient solvent was found to be hydroalcoholic, 80% methanol, and 20% water. For this type of analysis, purification involving partitioning with petroleum ether, ethyl acetate, and n-BuOH is necessary. Regarding the identification of the compounds of interest, the most used chromatographic methods are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas-chromatography [25].

The impact of environmental factors on the phytochemical composition of Achillea millefolium L.

It is recognized that abiotic factors influence both the growth and development of the plant as well as the content of bioactive compounds. In the case of *A. millefolum*, the most influenced by abiotic factors compound in the plant is the essential oil. Foroozeh, et al (2019) [17], analyzed by GC/MS (Gas Chromatography-Mass Chromatography) an aqueous extract of *A. millefolium* derived from three different habitats, summer habitat (Chaharbagh), sub-summer habitat (Tuskestan-Chaharbagh distance), winter habitat (Kechik Basin). The relationship between abiotic factors and essential oil was tested by Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (DCA) [17]. The analysis showed that the most important factors that are responsible for the variation in essential oil composition are temperature, elevation, total nitrogen volume (TNV), silt, and moisture. Although the plant has an adaptive character, and grows in varied habitats, to obtain high quality essential oil (in terms of valuable components) it is important to take these factors into account [17]. Changes were also observed in some monoterpenes and sesquiterpenes along with changes in the composition of essential oils. α- and β-thujone were not present in the oils from the fields at high altitudes (1020m and 1100m) which positively influences the oil composition due to the toxicity and legal regulation of these compounds. Moreover, another important compound influenced by environmental factors is chamazulene present in the leaves, which was absent in the controlled field culture but present in the wild-grown plants. The compound has an important role in essential oils used in herbal or phytotherapeutic sectors [18].

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2. *Antibacterial activity of Achillea millefolium L.*

In recent years, the interest in finding alternatives to antibiotics has increased considerably, due to the resistance that pathogenic microorganisms have acquired over time against antibiotics. This is why scientists have turned their attention to medicinal plant species as a solution against pathogenic microorganisms [18]. Aromatic plants possess antimicrobial activity due to the fact that they are a rich source of bioactive compounds. Extracts and essential oils obtained from aromatic plants have a similar activity as antibiotics [45]. *Achillea millefolium L.* extracts have been demonstrated to possess antifungal and anti-aflatoxigenic qualities. Because they inhibit fungal growth and reduce aflatoxin generation, these extracts can be utilized as natural preservatives in food products to suppress fungal development and aflatoxin production [1].

2.1. Target bacteria and spectrum of antimicrobial action

Achillea millefolium has been tested against a wide range of bacteria according to the literature. Table 2 summarizes the most common bacterial species on witch extracts of *A. millefolium* were succesfully tested.

Table 2. Most common bacterial species on witch A. millefolium was succesfully tested

Bacteria	Class	Gram type	Effectiveness	Method used for testing	Active compounds responsible
Staphylococcus aureus	*Cocci*	+	Strong inhibition	Disc diffusion, MIC tests	Flavonoids, sesquiterpenes, tannins
Escherichia coli	*Bacilli*	-	Moderate inhibition	Disc diffusion	Essential oils (e.g., cineole, camphor)
Pseudomonas aeruginosa	*Bacilli*	-	Moderate inhibition	MIC, zone of inhibition	Polyphenols, essential oils
Bacillus subtilis	*Bacilli*	+	Strong inhibition	Disc diffusion, MIC tests	Terpenes, tannins
Klebsiella pneumoniae	*Bacilli*	-	Moderate inhibition	Zone of inhibition	Essential oils

According to Yildirim et al. 2023 [45], *Achillea millefolium* presented an antibacterial activity against *Staphylococcus aureus* ATCC 12600, *Bacillus subtilis* ATCC 6051, *Pseudomonas aeruginosa* ATCC 10145, *Enterecoccus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 25241, *Escherichia coli* ATCC 11775. According to the study, the 6 strains of bacteria were tested using the disc diffustion method with a 6 mm filter paper disc. *A millefolium* showed to have an antibacterial effect agains these bacteria: *Staphylococcus aureus* showed to be sensitive against the essential oil of *A. millefollium* with a 8-10 mm diameter, *Bacillus subtilis* was sensitive with a ring diameter of 10-12 mm, *Pseudomonas aeruginosa* was also sensitive with a ring diameter of 10-12mm, *Enterecoccus faecalis* showed to have a diameter of 12-14 mm which is also in the sensitive category, *Salmonella typhimurium* had a 8-10 mm in the sensitive category, *Escherichia coli* was reported as insensitive with a diameter of 8 mm [45]. They also compared antibiotics with *Achillea millefolium* and demonstrated the efficacy of both antibiotics (ofloxacin and ampicillin) and *A millefolium* essential oil. Ofloxacin had an inhibition zone of 24-26 mm, ampicillin 20-22 mm, and *A. millefollium* essential oil of 8-10 mm against *S. aureus*, ofloxacin had an inhibition zone of 24-26 mm, ampicillin 25 mm, and *A. millefollium* 10-12 mm against *B. subtillis*, ofloxacin had 26-28 mm, ampicillin 22-23 mm, and *A. millefolium* 10-12 mm against *P. aeruginosa*; ofloxacin had 20-22 mm, ampicillin 22-24 mm, and *A. millefolium* 12-14 mm against *E. faecalis*, ofloxacin 24-26 mm, ampicillin 22-24 mm, and *A. millefolium* with 8-10 mm against *S. typhimurium*; ofloxacin had 26-28 mm, ampicillin with 26-28 mm, and *A. millefolium* with 8-10 mm against *E. coli* [45].

2.2. Antibacterial activity testing methods

Agar well diffusion method

The agar well diffusion method measures the inhibition halo created against the tested microorganism and enables quick identification with susceptibility [10]. In this method, the microorganisms that is tested is suspended onto the surface of the best medium for your bacteria. 6 mm wells are created in the nutrient media by cutting the medium. The essential oil (100 µl) is delivered into the wells. After the incubation stage, each well is examined for zones of growth inhibition, the inhibition zones are measured in millimetres [2]. Mazandarani et al. 2013 [28] determined the antibacterial activity of *A. millefolium* using the well diffusion method, they tested the antibacterial activity on the following species of microorganisms: *Staphylococcus aureus* (31.4 mm inhibition zone), *Staphylococcus epidermidis* (33.6 mm inhibition zone), *Bacillus cereus* (22.1 mm inhibition zone), *Entrococcus faecalis* (14.2 mm inhibition zone), *Escherichia coli* (16.5 mm inhibition zone), *Pseudomonas aeruginosa* (12.2 mm inhibition zone); *Klebsiella pneumonia* (13.5 mm inhibition zone), *Salmonella typhymorium* (12.3 mm inhibition zone) and *Shigella dysentria* (12.6 mm inhibition zone) [28].

Agar disk diffusion method

Antibiogram disks of 6 mm are used in this method, the disks are placed on an empty Petri plate and 30 µL of plant extract is placed on each disk until it is absorbed. The disks are taken carefully and placed on another petri plate with the interest bacteria culture. In the final step, the plates are incubated for 24 hours at 37°C for bacteria and 30°C for yeast, in this step the test microorganism's germination and growth are typically inhibited by an antimicrobial substance that diffuses into the agar, and the diameters of the inhibitory growth zones are then determined and analysed [8, 19]. In the study of Candan, F. et al. [9] the disc diffusion method showed that the essential oil of *Achillea millefolium* do not have an effect on *Moraxella catarrhalis*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Against *Staphylococcus aureus* the diameter of inhibition was 8 mm, for *Enterobacter aerogenes* it showed a 7 mm diameter, for *Klebsiella pneumoniae* it showed to have a 9 mm diameter, for *Bacillus cereus it was* of 10mm, for both *Clostridium perfringens* and *Mycobacterium smegmatis* a 12mm diameter ring, for *Acinetobacter lwoffii* it was a 15mm diameter, for *Candida krusei* it showed to have a diameter of 16 mm and for *Candida albicans* it showed the biggest inhibition diameter of 21mm [9].

Determination of Minimum Inhibition Concentration (MIC)

MIC is the minimum inhibitory concentration (measured in mg/L, or µg/mL) of an antibacterial agent that, in carefully regulated in vitro circumstances, completely inhibits the organism's test strain from developing visible [1, 23]. *Achillea millefolium* essential oil tested on fungal strains indicated for *Aspergillus niger* has a value of 4.7 mg/mL, for *Colletotrichum gloeosporioides* 3.4 mg/mL, for *Botrytis cinerea* a value of 3.6 mg/mL, for *Rhizopus stolonifera* a value of 1.6 mg/ml, for *Verticillium dahlia* indicted a value of 3.1mg/mL. All these microorganisms were shown to be inhibited by *A. millefolium* with *R. stolonifera* being the most sensitive fungal strain [13].

Essential oil of *A. millefollium* tested on *Candida albicans* indicated a MIC value of 10 µg/mL, on *Candida tropicalis* a value of 5 µg/mL, on *Candida parapsilosis* a value of 1.25 µg/mL, on *Saccharomyces cerevisiae* a value of 2.5 µg/mL [7]. For *Staphylococcus aureus* it indicated a value of 4.5 mg/mL, for *Salmonella interitidise* a value of 7.2 mg/mL, for *Escherichia coli* with a value of 7.2 mg/mL, for *Penecilum glocum* a value of 0.45 mg/mL and for *Sacaromycec servizie* a value of 0.45 mg/mL [7].

3. The antioxidant activity and potential therapeutic applications of Achillea millefolium L.

Pharmacological studies worldwide have identified and proven the therapeutic effects of *A. millefolium* on various inflammatory and infectious processes. Research in recent years has also confirmed the plant's antimicrobial, antiviral, and antitumor properties. Beyond these, of great importance is its antioxidant activity, mainly it's capacity of counteracting oxidative stress associated processes [46]. In 1985, Helmut Sies defined the concept of oxidative stress as the result of an imbalance between pro-oxidant and antioxidant species, favoring the former, thus potentially causing damage [41]. Over time, this concept has evolved, gaining more complex definitions, such as *"the imbalance between the formation of reactive oxygen species (ROS) or nitrogen species (RNS) and the organism's ability to counteract their action through protective antioxidant systems"* [32]. Under physiological conditions, reactive oxygen and/or nitrogen species are produced in moderate concentrations and play an essential role in regulating cellular cycles (cell signaling and apoptosis) and interacting with transcription factors for gene expression. During phagocytosis, cells specialized for this action can also generate reactive oxygen species for use against pathogens, combating possible infections [26, 33]. Under pathological conditions, mitochondrial oxidative stress may arise due to the excessive production of ROS within these cells, correlating this activity with the functional failure of the body's antioxidant mechanisms [29]. Thus, oxidative stress occurs from the excessive production of ROS and RNS or when the organism's antioxidant protection is insufficient. A series of degenerative processes induced by free radical formation can contribute to the onset of pathologies such as cancer, inflammatory diseases, cardiovascular diseases, or neurodegenerative disorders [27, 30]. The way that plants act as potential antioxidant agents has been and continues to be highly studied. These antioxidant properties that plants possess and manifest are mainly due to the compounds with biological activity that they synthesize: various bioactive compounds that neutralize reactive oxygen species (ROS) and free radicals. These include phenolic compounds, flavonoids, carotenoids and vitamins, which donate electrons to stabilize free radicals and prevent oxidative damage [16, 38]. Phenolic compounds are antioxidants with redox properties that allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [31]. They also have metal chelating properties [37].

For plants, the antioxidant function is ensured by the action of some antioxidant phyto-enzymes such as superoxide dismutase (SOD) and catalase (CAT), enzymes that have the power to break down ROS, thus reducing cellular damage [38]. In the case of *Achillea millefolium*, Muhammad Akram (2013) [3] pointed out its important antioxidant activity, primarily attributed to a high concentration of phenolic compounds, flavonoids, and essential oils. These active principles exert a radical scavenging effect that results in neutralizing free radicals and hence reducing the oxidative stress that would favor the damage of cellular components. The study of Lina Raudone et al., 2024 [35] emphasizes the potential of *A. millefolium* as a source of antioxidants and highlights the variability in its phytochemical composition depending on plant parts and morphotypes. This variability can be leveraged for targeted uses in pharmaceuticals and nutraceuticals. Regarding the antioxidant activity, the leaves of the plant exhibite the highest antioxidant activity, followed by inflorescences and stems. The ABTS assay results ranged between 500–900 μmol TE/g DW (Trolox equivalents per gram of dry weight), depending on the plant part and morphotype and the FRAP assay results were from 300–700 μmol TE/g DW, showing a similar trend to ABTS, with leaves exhibiting the highest activity, followed by inflorescences and stems. This high antioxidant activity was highly correlated with the content of caffeoylquinic acids such as chlorogenic acid, which was abundant in the leaves and inflorescences [35]. The antioxidant capacity of A*. millefolium* and its phenolic-rich extracts, linked to oxidative stress damage prevention, were also reported by Lysane Solomon et al. in 2021 [38]. With an IC_{50} value of 17.0 ± 0.26 μg/mL, the ethyl acetate fraction of *A. millefolium* indicated an effective capacity for radical scavenging [38]. Because of its essential oils, sesquiterpenes, and phenolic compounds, *Achillea millefolium* possesses hepatoprotective, antioxidant, antibacterial, antifungal, anti-inflammatory, analgesic, and antispasmodic properties [20]. In primary dysmenorrhea, tea prepared from the flowers of *A. millefolium* considerably lessens the severity of pain and relieves symptoms such as diarrhea, bloating, and chest pain [21]. *A. millefolium* methanolic extract exhibits cytotoxic effects on breast cancer cell lines and works in concert with chemotherapeutic drugs like bleomycin to treat prostate cancer [5, 39]. *A. millefolium's* antispasmodic and antibacterial qualities accelerates the healing process for gastrointestinal conditions such diarrhea and gastritis [20]. *A. millefolium* is a viable supplemental treatment for diabetes control because of its blood sugar-lowering and antioxidant properties. The findings of the study by Rezaei et. al. 2020 [36] showed that administration of *A. millefolium* extract at doses of 25 mg/ kg/d and 100 mg/kg/d in streptozocin-induced diabetic rats resulted in a reduction in blood sugar, lipids, and liver enzymes in diabetic rats. The effects above were more pronounced at the 100 mg/kg dose than the 25 mg/kg dose [36]. Scientific evidence suggests that diabetes increases oxidative stress factors and decreases antioxidant levels, NADPH levels, and glutathione levels [20, 40]. Additionally, there is a link between phenolic compounds and antioxidant action. The antioxidant properties of essential oil may be due to relatively high quantities of the phenolic compounds carvacrol and thymol. Numerous pharmacological properties, including analgesic, anti-inflammatory, antidiabetic, cholagogue, spasmolytic, anticancer, antioxidant, antifungal, and antiseptic, are linked to the phenolic compounds found in the plant [14].

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