INVESTIGATING THE THERAPEUTIC POTENTIAL OF *ACHILLEA FRAGRANTISSIMA* FROM AL JAWF REGION, KSA: PHYTOCHEMICAL PROFILING, ANTIOXIDANT, ANTIMICROBIAL, AND PREBIOTIC ACTIVITIES

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Abstract. This study investigates the phytochemical composition, antioxidant, antimicrobial, and prebiotic potential of *Achillea fragrantissima* collected from the Al Jawf province, Kingdom of Saudi Arabia (KSA). The aerial parts were collected, dried, and extracted using 80% methanol. The methanolic extract was analyzed for phenolic and flavonoid content, revealing significant levels of chlorogenic acid (1684.48 µg/g DM) and gallic acid (435.16 µg/g DM), as well as other bioactive compounds such as caffeic acid and ellagic acid. The extract demonstrated moderate antioxidant activity, with 42.75% DPPH radical scavenging and 25.92% inhibition of linoleic acid oxidation, attributed to its rich polyphenolic content. Antimicrobial assays showed broad-spectrum activity, with inhibition zones ranging from 16.33 mm (*Bacillus subtilis*) to 20.51 mm (*Escherichia coli*), comparable to standard antibiotics. In addition, the extract exhibited potent antifungal potential, with an inhibition zone of 16.37 mm (*Aspergillus niger*) to 18.59 mm (*Candida albicans*), compared to the commercial antifungal fluconazole. Additionally, the extract promoted the growth of probiotic *Lactobacillus* strains, particularly *L. plantarum* and *L. acidophilus*, suggesting prebiotic potential. HPLC analysis identified a diverse array of phenolics and flavonoids, underscoring the plant's therapeutic value. These findings highlight *A. fragrantissima* as a promising source of natural antioxidants, antimicrobials, and prebiotics, with potential applications in functional foods and nutraceuticals.
Keywords: *Achillea fragrantissima, secondary metabolites, gut microbiota, antifungal agents, HPLC profiling*

Introduction

Achillea fragrantissima (Forssk.) Sch. Bip., commonly known as fragrant yarrow, is a perennial shrub belonging to the Asteraceae family. This aromatic plant is native to arid and semi-arid regions, predominantly found in the Middle East, North Africa, and parts of Southwest Asia, including Saudi Arabia, Egypt, Jordan, and Palestine (Abd EL-Fattah et al., 2018). It thrives in rocky and sandy soils, often growing in desert wadis and mountainous terrains, demonstrating high drought resistance (Abd-ElGawad et al., 2023). The plant is characterized by its silvery-green, dissected leaves and small yellowish flowers clustered in dense inflorescences. It typically grows to a height of 0.5 to 1 meter and releases a strong, pleasant fragrance due to its high essential oil content (Tawfik et al., 2024).

A. fragrantissima has been traditionally used in folk medicine, perfumery, and as a flavoring agent in teas and culinary preparations. In therapeutic applications, Arabic communities have used *A. fragrantissima* to treat gastrointestinal disorders, fever, inflammation, diabetes, dysmenorrhea, eye infections, smallpox, headache, and respiratory disorders (Eissa et al., 2014; Awad et al., 2017). Additionally, modern pharmacological studies have confirmed its medicinal potential, demonstrating anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and hepatoprotective properties

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(Saaty, 2021; Khalifa et al., 2023). The plant's therapeutic effects are attributed to its rich phytochemical composition that contribute to its wide range of pharmacological activities which includes essential oils (e.g. α-pinene, 2-thujene, α-thujone, β-thujone, and α-terpineol) (Tawfik et al., 2024), flavonoids (e.g. apigenin, luteolin, vitexin cirsiliol, and diosmetin) (Patocka and Navratilova, 2019), sesquiterpene lactones (e.g. 13-O-desacetyl-1-β-hydroxyafraglouclide, achilloide A, 1α, 4α-endoperoxypseudoguaia-7, and 10-diene-6β,12-olide) (Bakr et al., 2014), phenolic acids (ferulic acid, salicylic acid, ascorbic acid, and jasmonatic cid) (Elsharkawy et al., 2021), and tannins (El-Ashmawy et al., 2016).

A. fragrantissima antioxidant capacity was primarily attributed to its high phenolic and flavonoid content. Studies have shown that extracts from this plant exhibit strong free radical scavenging activity, reducing oxidative stress and preventing cellular damage as these phenolic and flavonoid play a significant role in neutralizing reactive oxygen species and inhibiting lipid peroxidation (Patocka and Navratilova, 2019; Saaty, 2021). Additionally, phenolic compounds like chlorogenic acid and caffeic acid enhance the plant's antioxidant effects by chelating metal ions and upregulating endogenous antioxidant enzymes (Afshari et al., 2018). These properties make *A. fragrantissima* a potential therapeutic agent for managing oxidative stress-related diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer (Elmann et al., 2011; Patocka and Navratilova, 2019).

A. fragrantissima has been shown to exhibit broad-spectrum antimicrobial activity against many pathogenic bacteria and fungi. The essential oils, particularly those rich in camphor and 1,8-cineole, disrupt microbial cell membranes, leading to cell lysis and death (Derengowski et al., 2009). Alkaloids, phenols, flavonoids and tannins contribute to *A. fragrantissima* antifungal and antibacterial effects by inhibiting microbial enzyme systems interfering with bacterial biofilm formation and reducing pathogen virulence (Abd-ElGawad et al., 2023; Tawfik et al., 2024). These properties highlight its potential as a natural alternative to synthetic antibiotics, especially regarding drug-resistant infections.

While prior research has confirmed the therapeutic potential of *A. fragrantissima*, significant knowledge gaps persist. Firstly, the phytochemical composition and bioactivity of this species are known to be highly influenced by geographic and climatic factors (edaphoclimatic conditions). A comprehensive profiling of plants from the understudied Al Jawf region of Saudi Arabia, a distinct arid environment, has not been conducted. Secondly, although its general antimicrobial and antioxidant properties are recognized, a direct comparative evaluation of its efficacy against a defined panel of foodborne and clinical pathogens alongside standard antibiotics remains limited. Most critically, despite its traditional use for gastrointestinal ailments, the specific prebiotic potential of *A. fragrantissima*, its ability to selectively stimulate the growth of beneficial probiotic lactobacilli, has never been scientifically investigated. To bridge these gaps by providing a region-specific phytochemical profile, assessment of antimicrobial and antioxidant capacities, and the first-ever evaluation of novel prebiotic effects, adding a new dimension to its validated therapeutic utility, the primary aim of this study is focused on quantifying the phenolic compounds and flavonoids present in *A. fragrantissima* collected from Al Jawf governorate, KSA, and characterizing their specific profiles using HPLC analysis. Furthermore, the study aims to investigate the antioxidant activity and the antimicrobial potential of *A. fragrantissima* against the selected pathogenic bacterial

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strains. Also, the impact of *A. fragrantissima* extracts on lactic acid bacteria; beneficial microorganisms crucial for gut health and fermentation processes, was evaluated.

Materials and methods

Collection of Achillea fragrantissima plant samples

The aerial parts of *A. fragrantissima* were collected from the Al Jawf province in the northwestern region of KSA during May 2023, with a total of 34 individual plants sampled, with specific geographical coordinates provided for the collection site (29°56'57.7"N 39°38'58.9"E). The coordinates of *A. fragrantissima* collection site can be observed in *Figure 1*. Following collection, the plant samples were promptly stored in polyethylene bags and transported to the laboratory for further investigations. Upon arrival at the laboratory, the leaves of *A. fragrantissima* were manually separated from their stems, washed thoroughly with tap water to eliminate any sand particles and impurities, and then rinsed repeatedly with distilled water. Subsequently, the cleaned plant leaves underwent a process of aerial drying within a well-ventilated room for 5 days, shielded from direct sunlight to maintain the integrity of their phytochemical components. After drying, the air-dried leaves were finely pulverized with an electric mixer and sieved through a 2 mm sieve to achieve uniform particle sizes.

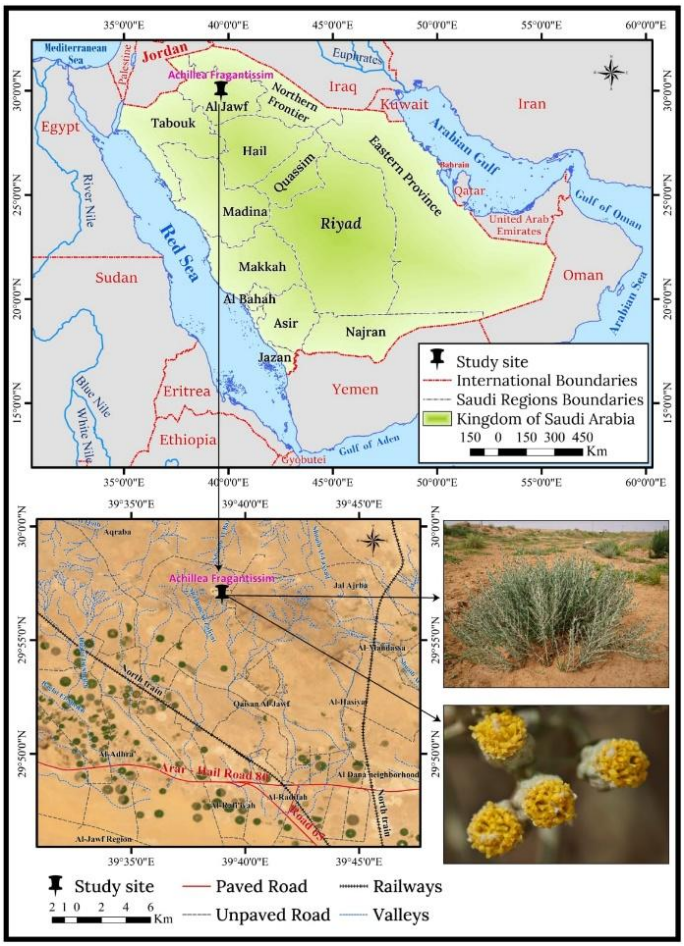

Figure 1. *The collection site (Al Jawf province, Saudi Arabia) and the study plant (Achillea fragrantissima)*

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Preparation of Achillea fragrantissima methanolic extract

For the methanolic extract preparation, 10 g of leaf powder was immersed in 90 ml of 80% methanol and left at room temperature for 72 h on an orbital shaker (Heidolph Titramax 1000, Schwabach, Germany) set at 120 rpm. The resulting mixture was then filtered through dual layers of filter paper. This extraction process was repeated twice, and the filtrates were combined and subjected to evaporation under reduced pressure utilizing a rotary evaporator (RV 10DS93, IKA, Tokyo, Japan) set at 40°C until dryness was achieved. The resultant residues were combined and stored in an airtight container in a refrigerator at 4°C until analysis time. Upon utilization, the concentrated dry extracts were redissolved in distilled water to create aqueous solutions with a concentration of 100 mg/mL. This prepared solution was employed for subsequent analyses involving phytochemical assessments of phenols and flavonoids, evaluation of antimicrobial activity, as well as the identification of active constituents through the HPLC technique.

Quantification of total phenols

The total phenolic content of *A. fragrantissima* methanolic extract was determined using the Folin-Ciocalteu method as described by Shiban et al. (2012). In summary, 0.5 mL of diluted extract from *A. fragrantissima* was combined with 5 ml of Folin-Ciocalteu reagent (0.2 N) in a test tube. After 8 min, 2 mL of Na_2CO_3 (15%) was added, and the reaction mixture was incubated at 50 °C for 15 min. The absorbance of the mixtures at 760 nm was measured against a blank using a JENWAY spectrophotometer (model 7305, UK). The total phenolic content of the extract was calculated using standard gallic acid solutions (0–0.1 mg/mL) and expressed as mg gallic acid equivalents (GAE)/g DM.

Quantification of total flavonoids

The total flavonoid content of *A. fragrantissima* methanolic extract was determined using a colorimetric assay based on the method described by Brighente et al. (2007). A volume of 0.5 mL of 2% $AlCl_3$ in methanol was mixed with 0.5 mL of the methanolic plant extract. After the incubation for 1h at room temperature, the absorbance was measured at 415 nm. Total flavonoid content was quantified using a standard curve prepared with rutin and expressed in mg/g DM.

Determination of DPPH free-radical scavenging activity

The efficacy of the prepared plant extract in scavenging the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed following the technique delineated by Bondet et al. (1997), with minor modifications. Specifically, 0.5 mL of the test sample was introduced to 3 mL of DPPH solution (0.67%) in methanol. Subsequently, the reaction mixture was allowed to incubate at room temperature within a dark environment for 30 min. The reduction in the intense violet color of DPPH was quantified by measuring the absorbance at 517 nm. The percentage of DPPH scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = [(A\ control\ -\ A\ sample)/(A\ control\ )] \times 100 \quad \text{(Eq.1)}$$

where, A control = control absorbance, and A sample = sample absorbance.

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Antioxidant activity determination in linoleic acid system

The antioxidant activity of *Achillea fragrantissima* methanolic extract was assessed by monitoring linoleic acid oxidation. A mixture containing 5 mg of the extract, 0.13 mL linoleic acid, 10 mL ethanol (99.8%), and 10 mL sodium phosphate buffer (0.2 M, pH 7) was diluted to 25 mL with distilled water and incubated at 40°C for 7 days. Oxidation was quantified using the thiocyanate method. Briefly, 10 mL ethanol (75% v/v), 0.2 mL ammonium thiocyanate (30% w/v), 0.2 mL sample, and 0.2 mL $FeCl_2$ solution (20 mM in 3.5% HCl) were mixed, and absorbance was measured at 500 nm after 3 minutes (Ozsoy et al., 2008). A control without the extract and ascorbic acid as a positive control were included. Percent inhibition of oxidation was calculated from the formula:

$$\text{Inhibition of linoleic acid peroxidation} = 100 - [(\Delta A \text{ sample} / \Delta A \text{ control}) \times 100] \quad \text{(Eq.2)}$$

Determination of the antimicrobial activity of A. fragrantissima methanolic extract

Eight human pathogenic microbial strains, including *Candida albicans* (ATCC 10231), *Aspergillus niger* (NRRL-3), *Penicillium chrysogenum* (NRRL 824), *Bacillus subtilis* (NRRL-B-4219), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (NRRL B23 27853), and *Klebsiella pneumoniae* (ATCC 27736), were obtained from the Department of Microbiology at King Abdul-Aziz University, Saudi Arabia. Bacterial strains were maintained on nutrient agar, while fungal strains were preserved on potato dextrose agar (PDA) at 4°C. Before testing, strains were sub-cultured in nutrient broth and incubated at 37°C for 24 h to ensure microbial activation.

The antimicrobial activity of *A. fragrantissima* extract was evaluated using the agar well diffusion method. Microbial suspensions (1.0×10^7 CFU/mL) were spread onto nutrient agar (bacteria) or PDA (fungi), and 8 mm wells were created. A 30 µL aliquot of the extract was added to each well. Plates were incubated at 37°C for 24 h (bacteria) or 30°C for 48 h (fungi). Neomycin (30 µg/mL) and fluconazole (30 µg/mL) served as antibacterial and antifungal controls, respectively. Inhibition zone diameters were measured in millimeters (Gani et al., 2023).

Effects of A. fragrantissima methanolic extract on the growth of lactic acid bacteria

Five dairy strains of lactic acid bacteria (LAB); *Lactobacillus bulgaricus, L. salivarius, L. plantarum, L. acidophilus,* and *L. rhamnosus*, were obtained from the culture bank at King Abdul-Aziz University. These strains, previously isolated from various sources and preserved, were cultured by inoculating 15 mL of overnight starter cultures into 150 mL of MRS broth medium and incubated at 37°C for 20 h. Bacterial concentration, adjusted to approximately 1×10^6 CFU/mL, was measured spectrophotometrically at 600 nm. The cultures were centrifuged at 10,000 × g for 5 min. at 4°C and the resulting pellets were resuspended in 0.1 M PBS (pH 7.0) before use.

To assess the effect of *A. fragrantissima* extract on LAB growth, 100 µL of the methanolic solution of the extract was added to 200 µL of pre-cultured bacterial biomass (1×10^5 CFU/mL) in 5 mL of MRS broth. The mixtures were incubated statically in the dark at 37°C for 24, 48, 72, and 96 h. LAB growth was monitored by measuring optical density (OD) at 600 nm, with concentrations determined by using standard curves

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correlating OD values to colony-forming units (CFU/mL) for each strain. Controls without the extract were included for comparison (Sezer et al., 2013).

HPLC analysis of polyphenolic and flavonoid compounds

Polyphenolic and flavonoid constituents in the methanolic extract of *A. fragrantissima* were characterized using high-performance liquid chromatography (HPLC) by an Agilent 1260 series system (Agilent Technologies, Santa Clara, CA, USA). Separation was conducted on a Zorbax Eclipse Plus C8 column (4.6 mm × 250 mm, 5 µm) with a mobile phase consisting of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B), delivered at a flow rate of 0.9 mL/min. A linear gradient elution program was applied as follows: 0–1 min (82% A), 1–11 min (75% A), 11–18 min (60% A), 18–22 min (82% A), and 22–24 min (82% A). Detection was carried out at 280 nm using a multi-wavelength detector, with an injection volume of 5 µL. The column temperature was maintained at 40°C throughout the analysis (Pyrzynska and Sentkowska, 2019).

Statistical analysis

All experiments were performed in triplicate, with results expressed as mean values ± standard deviation (SD). Statistical estimation was conducted using one-way analysis of variance (ANOVA) by the CoStat software (CoHort, Version 6.311), with mean comparisons assessed at a 5% significance level ($p < 0.05$). For pairwise comparisons of means following ANOVA, Duncan's multiple range test was employed at $p < 0.05$ level.

Results and discussion

Phenolic content of Achillea fragrantissima methanolic extract

Phenolic compounds, a prominent class of plant secondary metabolites, are extensively studied for their multifaceted biological activities, which contribute significantly to their therapeutic and pharmacological relevance. In the present investigation, the methanolic leaf extract of *Achillea fragrantissima* collected from Al Jawf province, KSA exhibited a notable total phenolic content of 32.3 mg/g dry matter (DM), as illustrated in *Figure 2*. This measured phenol content in *A. fragrantissima* is higher than the 25.57 mg/g DW previously reported for this species by El-Ashmawy et al. (2016) in Gouf and Qassim Districts, KSA. The high concentration of these bioactive constituents in *A. fragrantissima* points out its potential as a source of natural compounds with significant health benefits. Prior studies have consistently documented the high phenolic content in *A. fragrantissima* extracts and established a correlation between their biological activities and phenolic composition (Patocka and Navratilova, 2019; Elsharkawy et al., 2021; Khalifa et al., 2023).

The antioxidant capacity of phenolic compounds is particularly important, as it enables plants to mitigate oxidative stress and adapt to environmental harsh conditions (Zhou et al., 2024). In human health, these antioxidant properties are linked to a range of protective effects, including the reduction of oxidative damage, modulation of inflammatory responses, and decreased risk of chronic diseases (Jantan et al., 2021; Rudrapal et al., 2022). Beyond their antioxidant role, phenolic compounds exhibit antimicrobial activity (Soleimani et al., 2022), which not only promotes plant defense mechanisms but also holds promise for applications in pharmaceuticals and food preservation, where natural antimicrobial agents are increasingly valued. Furthermore, these compounds demonstrate

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anti-inflammatory, antidiabetic, and anticancer potential, with certain phenolics exhibiting cytotoxic effects against malignant cells, positioning them as promising candidates for cancer prevention and treatment (Tatipamula and Kukavica, 2021). So, exploring the phenolic profile of *A. fragrantissima* might result in the development of innovative therapeutic agents and functional foods designed to promote health and prevent disease.

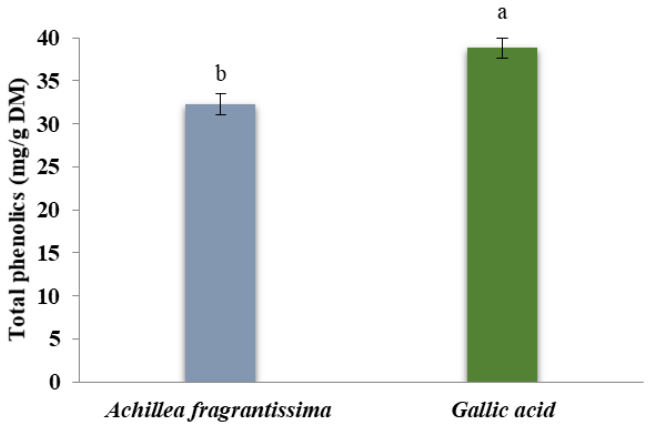

Figure 2. *Total phenolic content in the methanol extract of A. fragrantissima leaf powder. Data are presented as mean ± SD (n=3). Different superscript letters display a significant difference at the 0.05 level following Duncan's multiple range test ($p < 0.05$)*

Flavonoids content of Achillea fragrantissima methanolic extract

Flavonoids, a prominent subclass of polyphenolic compounds, are widely recognized for their extensive biological activities and health-promoting properties, rendering them a critical focus of research in phytochemical studies. In the present investigation, the methanolic extracts derived from *Achillea fragrantissima* leaf powder, collected from Al Jawf province in Saudi Arabia, demonstrated a substantial total flavonoid content of 3.56 mg/g DM, as depicted in *Figure 3*. This result highlights the phytochemical value of *A. fragrantissima* and emphasizes its potential as a source of bioactive flavonoids with considerable therapeutic applications.

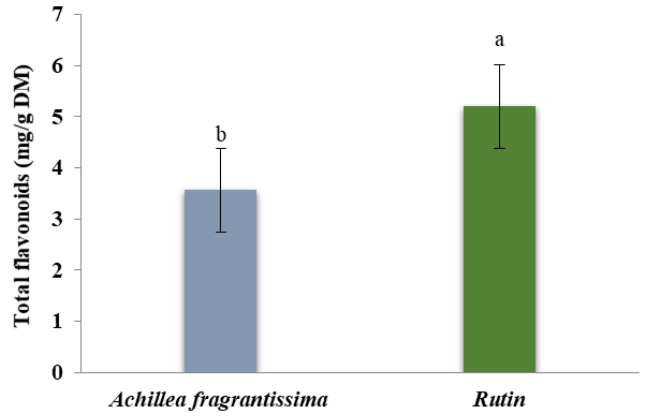

Figure 3. *Total flavonoid content in the methanol extract of A. fragrantissima leaf powder. Data are presented as mean ± SD (n=3). Different superscript letters display a significant difference at the 0.05 level following Duncan's multiple range test ($p < 0.05$)*

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The existence of a large flavonoid content in the methanolic extracts of *A. fragrantissima* supports some previous studies (El-Ashmawy et al., 2016; Awad et al., 2020; Khalifa et al., 2023) highlighting the role of flavonoids as key contributors to the biological activities of medicinal plants. Flavonoids are known for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, which are attributed to their ability to modulate cellular signaling pathways and scavenge free radicals (Kumar and Pandey, 2013; Azeem et al., 2023). The significant flavonoid content of *A. fragrantissima* suggests that environmental factors, such as soil composition, climate, and geographical location, may influence the biosynthesis and accumulation of these compounds (Alara et al., 2021). This finding supports the studies demonstrating that plants grown in arid or semi-arid regions often exhibit enhanced production of secondary metabolites, including flavonoids, as an adaptive response to environmental stress (Zhou et al., 2021; Yeshi et al., 2022).

Radical scavenging activity of Achillea fragrantissima methanolic extract

The evaluation of antioxidant capacity using the DPPH radical scavenging assay offers significant insights into the health-promoting potential of plant-derived extracts. In this study, the methanolic extracts of *Achillea fragrantissima* demonstrated moderate DPPH radical scavenging activity, achieving 42.75%, compared to the reference antioxidant ascorbic acid, which exhibited 81.96% scavenging activity (*Figure 4*). The observed antioxidant activity in *A. fragrantissima* extract can be attributed to the presence of bioactive phytochemicals, such as phenolic compounds and flavonoids, which are known for their potent free radical-neutralizing properties. These findings align with the established role of polyphenolic compounds in conferring antioxidant effects, further underscoring the therapeutic potential of *A. fragrantissima* as a natural source of antioxidants.

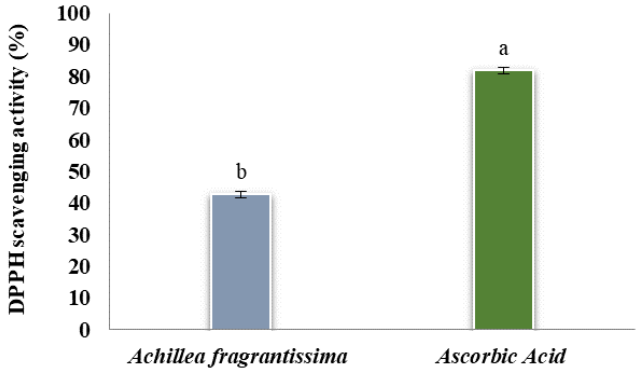

Figure 4. *DPPH scavenging activity of the methanol extract of A. fragrantissima leaf powder. Data are presented as mean ± SD (n=3). Different superscript letters display a significant difference at the 0.05 level following Duncan's multiple range test ($p < 0.05$)*

The moderate DPPH radical scavenging activity suggests that *A. fragrantissima* contains bioactive compounds capable of mitigating oxidative stress. Phenolic compounds and flavonoids, which are abundant in this plant, are widely recognized for their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals and reducing oxidative damage (Munteanu and Apetrei, 2021; Abd-ElGawad et al., 2023). The presence of these compounds in *A. fragrantissima* aligns with previous studies

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demonstrating the antioxidant potential of polyphenol-rich plant extracts (Diab et al., 2021; Khalifa et al., 2023). Furthermore, the moderate antioxidant activity of *A. fragrantissima* extracts suggests its potential application in the development of functional foods, nutraceuticals, and natural preservatives.

Inhibition of linoleic acid oxidation by Achillea fragrantissima methanolic extract

The inhibition of unsaturated fatty acid oxidation, particularly linoleic acid, serves as a robust indicator of the antioxidant efficacy of plant-derived extracts. In this study, the methanolic extract of *Achillea fragrantissima* leaf powder demonstrated a 25.92% inhibition of linoleic acid oxidation, compared to the reference antioxidant ascorbic acid, which exhibited a 33.48% inhibition rate (*Figure 5*). These findings suggest that *A. fragrantissima* contains bioactive compounds capable of mitigating lipid peroxidation under oxidative damage conditions. The observed activity, though moderate, underscores the potential of *A. fragrantissima* as a source of natural antioxidants, likely attributable to its rich content of phenolic compounds and flavonoids, which are known to inhibit lipid oxidation through free radical scavenging and metal chelation mechanism (Tumilaar et al., 2024; Zhang et al., 2024).

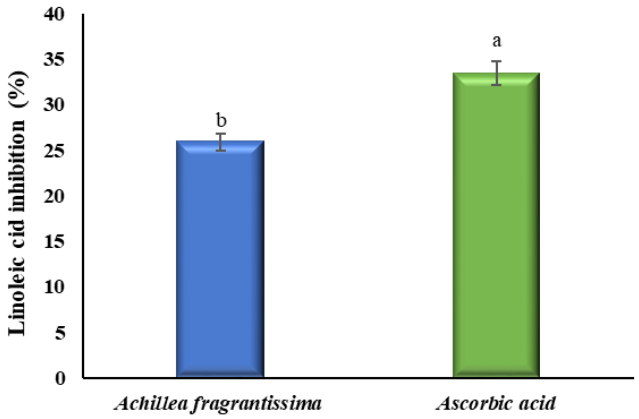

Figure 5. *Inhibition of linoleic acid oxidation by the methanolic extract of A. fragrantissima leaf powder. Data are presented as mean ± SD (n=3). Different superscript letters display a significant difference at the 0.05 level following Duncan's multiple range test ($p < 0.05$)*

Lipid peroxidation, driven by free radicals, is a critical factor in the development of oxidative stress-related disorders, including cardiovascular diseases, neurodegenerative disorders, and aging (Ali et al., 2022; Sadiq, 2023). The antioxidant activity of *A. fragrantissima* can be attributed to its phenolic and flavonoid content, which are known to disrupt the chain reactions of lipid peroxidation by neutralizing reactive oxygen species (ROS) and chelating pro-oxidant metal ions (Andrés et al., 2023).

Antimicrobial potential of Achillea fragrantissima methanolic extract

The antimicrobial potential of the methanolic extract obtained from *Achillea fragrantissima* leaf powder, collected from the Al Jawf region in Saudi Arabia, is elucidated in *Table 1*. The data reveals that the extract exhibits significant antimicrobial efficacy against both bacterial and fungal strains. The inhibition zones ranged from 16.33 mm for *Bacillus subtilis* to 20.51 mm for *Escherichia coli*, indicating robust

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antibacterial activity. Notably, the extract's performance was comparable to the standard antibiotic neomycin, which demonstrated inhibition zones ranging from 18.75 to 24.56 mm. Additionally, the extract displayed pronounced antifungal activity, with inhibition zones ranging from 16.37 mm for *Aspergillus niger* to 18.59 mm for *Candida albicans*. This antifungal efficacy is relatively comparable to that of the commercial antifungal agent fluconazole, which exhibited inhibition zones between 14.08 and 22.48 mm. These findings underscore the potential of *A. fragrantissima* as a source of natural antimicrobial agents with broad-spectrum activity.

Table 1. *The antimicrobial activity of the methanolic extract of A. fragrantissima leaf powder*

Bacterial strains	***A. fragrantissima* extract**	***Neomycin***
Bacillus subtilits	16.33±0.29^{c}	24.56±0.27^{a}
Staphylococcus aureus	18.43±0.61^{b}	18.75±0.18^{d}
Escherichia coli	20.51±1.02^{a}	22.24±0.10^{b}
Pseudomonas aeruginosa	18.92±0.98^{b}	20.08±0.24^{c}
Klebsiella pneumoniae	16.69±0.37^{c}	14.36±0.11^{e}
Fungal strains	***A. fragrantissima* extract**	**Fluconazole**
Candida albicans	18.59±0.59^{a}	18.61±0.26^{b}
Aspergillus niger	16.37±0.32^{c}	14.08±0.61^{c}
Penicillium chrysogenum	18.21±0.18^{b}	22.48±0.71^{a}

Inhibition zones (mm) are presented as mean ± SD (n=3). Statistical differences were determined by one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). Different letters within the same column denote statistically significant differences

The antimicrobial activity of *A. fragrantissima* methanolic extract highlights its potential as a natural alternative to synthetic antibiotics and antifungals. The observed inhibition zones suggest that *A. fragrantissima* contains bioactive compounds capable of disrupting bacterial cell walls or interfering with essential metabolic pathways (Abd-ElGawad et al., 2023; Tawfik et al., 2024). Similarly, the extract's antifungal activity, particularly against *Candida albicans*, indicates its potential for treating mycotic diseases. The superior performance of the extract against *A. niger* compared to fluconazole underscores the presence of potent antifungal agents, possibly flavonoids, sesquiterpenes, and essential oils, which are known for their antimicrobial properties (Abd-ElGawad et al., 2023; Tawfik et al., 2024).

Effects of A. fragrantissima methanolic extract on lactic acid bacteria

The data in *Figure 6* illustrates the growth dynamics of various *Lactobacillus* strains (*L. plantarum, L. acidophilus, L. rhamnosus, L. bulgaricus,* and *L. salivarius*) in the presence and absence of *Achillea fragrantissima* methanolic extract over a 96-h incubation period. The data revealed distinct growth patterns, with *A. fragrantissima* extract prompting the growth of some of these probiotic bacteria. Notably, the addition of the extract to the growth medium enhanced the growth of certain strains, such as *L. plantarum* and *L. acidophilus*, as indicated by the higher growth curves compared to their controls. This suggests that the extract may contain bioactive compounds that promote the growth of beneficial gut microbiota, potentially through prebiotic effects or by providing essential nutrients.

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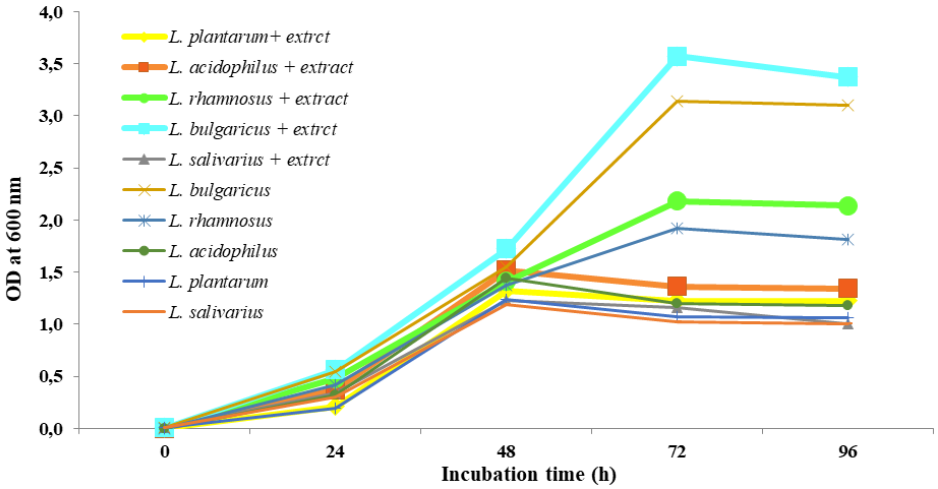

Figure 6. *The growth dynamics of five gut microbiota Lactobacillus strains over a 96-h incubation period as affected by A. fragrantissima methanolic extract*

Conversely, some strains, such as *L. bulgaricus* and *L. rhamnosus*, showed minimal or no significant growth enhancement in the presence of *A. fragrantissima* extract, indicating strain-specific responses. The observed growth promotion in some of the investigated *Lactobacillus* strains supports the previous studies demonstrating the prebiotic potential of plant extracts rich in polyphenols and polysaccharides (Mirzadeh et al., 2021; Plamada and Vodnar, 2022). These findings suggest that *A. fragrantissima* can be added to the functional foods and nutraceuticals for modulating the beneficial gut microbiota for promoting gut health and preventing dysbiosis-related disorders.

HPLC analysis of phenol and flavonoid compositions of Achellia fragrantissima methanolic extract

Table 2 and *Figure 7* provide a detailed chromatographic profile of the phenolic and flavonoid composition of the methanolic extract of *A. fragrantissima* collected from the Al Jawf region, KSA. The data revealed a diverse array of bioactive compounds, with chlorogenic acid (1684.48 µg/g DM) and gallic acid (435.16 µg/g DM) being the most abundant. Both compounds are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties (Le Sage et al., 2017; Singh et al., 2023).

The presence of significant amounts of caffeic acid (513.58 µg/g DM) and ellagic acid (269.59 µg/g DM) further highlights the extract's potential health benefits. These compounds are known for their roles in scavenging free radicals and modulating cellular signaling pathways, contributing to the prevention of oxidative stress-related diseases (Khanduja et al., 2006; Baeeri et al., 2017; Alam et al., 2022). Additionally, the detection of flavonoids such as rutin, naringenin, and quercetin in lower concentrations highlights the anticancer and cardioprotective effects of *A. fragrantissima* extract, as these compounds are recognized for their ability to inhibit cancer cell proliferation and improve vascular health (Syahputra et al., 2022; Pravin et al., 2024).

The chromatographic profile also indicated the presence of less abundant compounds like rosmarinic acid and ferulic acid, which are known for their neuroprotective and anti-inflammatory properties (Alara et al., 2021). However, the absence of kaempferol and the minimal abundance of cinnamic acid suggest that the bioactivity of *A. fragrantissima*

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extract is predominantly driven by the more abundant phenolic acids and flavonoids. These findings help in understanding the therapeutic potential of *A. fragrantissima* and its applications in functional foods and nutraceuticals.

Table 2. *HPLC analysis of phenols and flavonoids in Achillea fragrantissima methanolic extract*

Peak No.	Area %	Conc. (µg/g DM)	Compound name
1	11.9763	435.16	Gallic acid
2	31.6022	1684.48	Chlorogenic acid
3	1.8006	159.58	Catechin
4	3.2624	67.53	Methyl gallate
5	16.1562	513.58	Coffeic acid
6	2.7207	81.74	Syringic acid
7	0.5946	35.21	Pyro catechol
8	0.8935	54.14	Rutin
9	6.5699	269.59	Ellagic acid
10	9.0554	132.38	Coumaric acid
11	3.7084	56.61	Vanillin
12	1.2185	29.08	Ferulic acid
13	1.8283	68.65	Naringenin
14	0.5882	25.91	Rosmarinic acid
15	1.4518	33.45	Daidzein
16	0.3434	19.04	Quercetin
17	0.0880	0.65	Cinnamic acid
18	0.000	0.00	Kaempferol
19	6.1415	124.04	Hesperetin

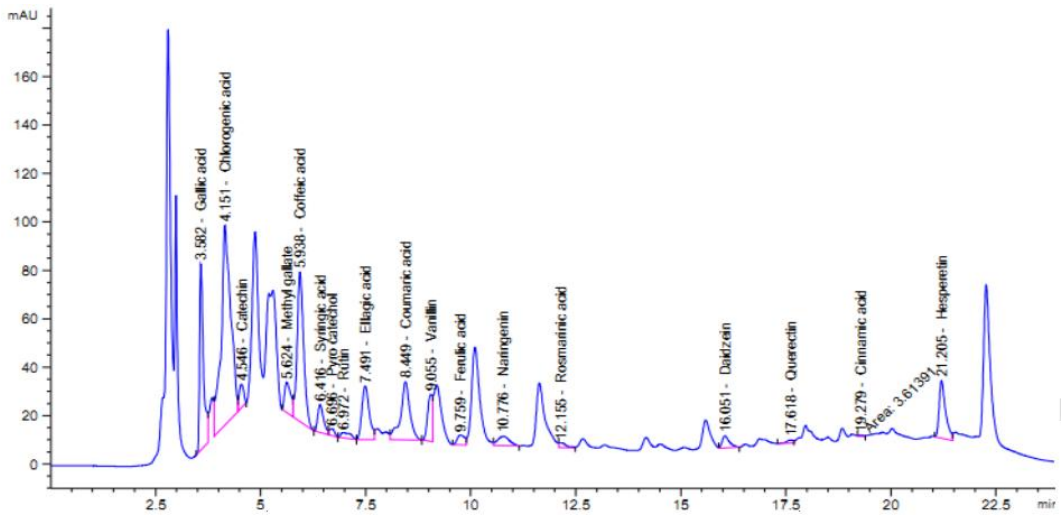

Figure 7. *HPLC chromatogram of phenols and flavonoids in Achillea fragrantissima methanolic extract*

Conclusions

The findings of this study underscore the phytochemical composition and therapeutic potential of *Achillea fragrantissima* collected from the Al Jawf region, KSA. The methanolic extract exhibited a high concentration of phenolic compounds and flavonoids, with chlorogenic acid and gallic acid being the most abundant. These bioactive

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constituents contributed to the extract's notable antioxidant activity, as evidenced by its DPPH radical scavenging and inhibition of linoleic acid oxidation. Furthermore, the extract demonstrated broad-spectrum antimicrobial activity against both bacterial and fungal strains, highlighting its potential as a natural alternative to synthetic antimicrobial agents. The prebiotic effects observed in the growth promotion of beneficial *Lactobacillus* strains further emphasize the extract's potential in modulating gut microbiota and promoting gut health. HPLC analysis revealed the presence of various phenolic and flavonoid compounds, unlocking the basis of *A. fragrantissima's* medicinal uses. Overall, this study suggests *A. fragrantissima* as a valuable source of natural antioxidants, antimicrobials, and prebiotics, with promising applications in functional foods, nutraceuticals, and pharmaceuticals. Future research should focus on the identification and purification of the bioactive compounds and conducting *in vivo* studies to validate these findings and explore their therapeutic applications further.

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