https://doi.org/10.31925/farmacia.2026.5.5 **ORIGINAL ARTICLE**

LC-MS/MS PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTIOXIDANT AND CHOLINESTERASE INHIBITORY ACTIVITIES OF THE METHANOLIC EXTRACT OF *ACACIA ARABICA*

TEBIB ARBIA [1]*, BOUHAFSOUN AICHA [1], ÇIFTÇI BEGÜM HAZAR [2], KELLAL HASSIBA [1], TAŞ KÜÇÜKAYDIN MELTEM [2], KÜÇÜKAYDIN SELÇUK [2], CHERIFI FADILA [1], MEHTOUGUI AMEL [1], DURU MEHMET EMIN [2], ERENLER RAMAZAN [3], DJABEUR ABDERREZAK [1]

[1]*Laboratory of production and valorization of plants and microorganisms (LP2VM), Department of Biotechnology, Faculty of Natural and Life Sciences, University of Science and Technology of Oran - Mohamed Boudiaf USTO-MB, B.P. 1505, El M'naouer, Oran 31000, Algeria*
[2]*Mugla Sitki Kocman University Faculty of Science Department of Chemistry 48121 Muğla, Turkey*
[3]*Department of Chemistry, Faculty of Arts and Sciences, Gaziosmanpaşa University, 60240 Tokat, Türkiye*

**corresponding author: arbia.souad@univ-usto.dz*

Manuscript received: July 2026

Abstract

This study aimed to analyse the phytochemical composition, antioxidant activity, and anticholinesterase potential of methanolic extracts from branches and leaves of *Acacia arabica* from Western Algeria. Total phenolic and flavonoid contents were determined using colorimetric methods. Antioxidant activities were evaluated *via* five assays (DPPH, ABTS, β-carotene bleaching, CUPRAC, and ferrous ion chelation). Anticholinesterase inhibitory activity and LC-MS/MS analysis were performed to identify phenolic compounds and their organ-specific distributions. Results showed that leaves contained higher total phenolic and flavonoid contents, whereas branch extracts exhibited stronger antioxidant properties. Regarding anticholinesterase activity, both extracts showed moderate AChE inhibition, with IC_{50} values of 152.7 ± 0.85 µg/mL for leaves and 99.55 ± 0.79 µg/mL for branches, while BChE inhibition was more pronounced in the branch extract (IC_{50} = 40.17 ± 0.93 µg/mL), with activity statistically comparable to that of galantamine (42.20 ± 0.35 µg/mL). LC-MS/MS analysis revealed that branches contained catechin, epigallocatechin, gallic acid, gentisic acid, and vanillin, while rutin, salicylic acid, and trans-cinnamic acid were detected in the leaves. Epigallocatechin (8.209 µg/mL) and catechin (2.429 µg/mL) are also detected in leaves, and epigallocatechin is the highest quantified compound in the leaf extract. These findings highlight the importance of organ-specific selection in phytochemical and pharmacological studies of *Acacia arabica*.

Rezumat

Studiul a avut ca obiectiv caracterizarea compoziției fitochimice, a activității antioxidante și a potențialului anticolinesterazic al extractelor metanolice din ramurile și frunzele speciei *Acacia arabica*, colectate din vestul Algeriei. Conținutul total de polifenoli și flavonoide a fost determinat prin metode colorimetrice. Activitatea antioxidantă a fost evaluată prin cinci teste: DPPH, ABTS, decolorarea β-carotenului, CUPRAC și chelatarea ionilor feroși. Activitatea inhibitorie asupra colinesterazelor a fost testată *in vitro*, iar compușii fenolici și distribuția lor au fost identificați prin LC-MS/MS. Frunzele au prezentat un conținut total mai ridicat de polifenoli și flavonoide, în timp ce extractul din ramuri a manifestat o activitate antioxidantă mai intensă. Ambele extracte au inhibat moderat acetilcolinesteraza (AChE), cu valori IC_{50} de 152,7 ± 0,85 µg/mL pentru frunze și 99,55 ± 0,79 µg/mL pentru ramuri. Inhibarea butirilcolinesterazei (BChE) a fost mai pronunțată în cazul extractului din ramuri (IC_{50} = 40,17 ± 0,93 µg/mL), valoare comparabilă statistic cu cea a galantaminei, utilizată ca standard (42,20 ± 0,35 µg/mL). Analiza LC-MS/MS a evidențiat catechina, epigalocatechina, acidul galic, acidul gentisic și vanilina drept compuși principali în ramuri, iar rutina și acidul salicilic în frunze.

Keywords: *Acacia arabica,* cholinesterase inhibitory activity, antioxidant potential, phytochemical composition, LC-MS/MS

Introduction

Plant-based natural compounds, due to their variety of biological applications and their use as possible drug candidates (1), have received significant scientific attention. The term "phytochemical" or "secondary metabolite" is commonly used to refer to compounds that are produced by plants as part of an adaptive defence mechanism in response to herbivorous insects and environmental stressors. Phytochemicals also serve as an additional resource of bioactive compounds, with potential applications in medicine and pharmaceutical research (1). Phenolics have received particular attention among the secondary metabolites, primarily due to their extensive free radical scavenging capabilities (2) and well-documented potential to reduce the risk of several degenerative diseases. Research has demonstrated that phenolic compounds can help mitigate oxidative stress; promote heart health; assist in

maintaining healthy blood sugar levels; and possibly aid in delaying onset or preventing neurodegenerative conditions such as Alzheimer's disease (2). Additionally, phenolic compounds exhibit anti-inflammatory activity and lower the risk of developing certain cancers (3). The various biological benefits attributed to these compounds have led to increased interest in natural antioxidants and stimulated the need for the development of reliable analytical techniques for evaluating the antioxidant capacity of plant extracts and individual phytochemicals (4, 5).

Several species of the genus *Acacia* have been widely used in traditional medicine (6) for the treatment of various health disorders, including infections, inflammatory conditions, diarrhoea, and malaria. *Acacia arabica* (taxonomic synonym *Vachellia nilotica***)** is a widely distributed species occurring across tropical and subtropical regions of the Old World, including North Africa, the Middle East, and South Asia. In Algeria, it grows under a broad range of ecological conditions, from coastal zones to semi-arid landscapes. The species has a long-standing reputation in traditional medicine, where different plant parts, such as the bark, leaves, pods, and flowers, have been used for generations to manage numerous health conditions. Traditional remedies prepared from *Acacia arabica* have been employed for respiratory complaints, diarrhoea, dysentery, fever, haemorrhoids, gallbladder disorders, eye diseases, tuberculosis, skin disorders, and certain gynaecological conditions (7, 8). Ethnobotanical surveys conducted in different regions have also documented the continued importance of *Acacia arabica* in traditional healthcare, particularly for the treatment of respiratory and gastrointestinal disorders (9).

Phytochemical studies have revealed that *Acacia arabica* is a rich source of phenolic compounds, particularly flavonoids and phenolic acids, which significantly contribute to its reported therapeutic potential (6). However, the accumulation and distribution of these bioactive metabolites can differ considerably between plant organs. For example, leaves and branches may display distinct phytochemical profiles, reflecting their specific physiological functions and defence mechanisms (10). Although increasing attention has been devoted to this species, investigations addressing the organ-specific distribution of phytochemicals and their related biological activities in *Acacia arabica* growing under Algerian environmental conditions are still scarce. Therefore, the present study aimed to characterize the chemical composition of leaves and branches of *Acacia arabica* collected from western Algeria and to assess their antioxidant and cholinesterase inhibitory activities through biochemical assays and LC–MS/MS analysis. This work is expected to provide further insights into the relationship between organ-dependent secondary metabolites and their associated biological activities.

Materials and Methods

Plant material

The branches and leaves of *Vachellia nilotica* (syn. *Acacia arabica*) were harvested in August 2021 from the campus of the University of Sciences and Technology of Oran Mohamed Boudiaf (USTO-MB), located in Bir El Djir (35°44′12″ N, 0°33′15″ W), Oran, Algeria.

Chemicals and reagents

Unless otherwise specified, all chemicals and solvents were of analytical grade. β-Carotene, Tween-40, linoleic acid, DPPH, ABTS, DTNB, galantamine, acetylthiocholine iodide, and butyrylthiocholine chloride were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Ethanol, methanol, n-hexane, and other analytical-grade solvents were obtained from Merck (Darmstadt, Germany).

Preparation of the extracts

Dried plant materials were ground using an electric grinder to obtain a fine, homogeneous powder. The powdered material was used directly for extraction without sieving. Before extraction, dried samples of branches and leaves (8 g) were immersed in 80 mL of 70% aqueous methanol and maintained at 60°C for 30 min under continuous reflux. The extracts were filtered, and the extraction procedure was repeated two additional times. The combined filtrates were concentrated to dryness using a rotary evaporator (100 mbar) at 45°C.

Phytochemical screening

Phytochemical screening was carried out on both methanolic extracts and powdered plant samples using standard analytical methods to identify the major constituents (11, 12).

First, the presence of tannins in the extracts was evaluated by mixing 5 mL of methanolic extract with 1 mL of 1% ferric chloride ($FeCl_3$) solution. The gallic tannins presence resulted in a blue color, while catecholic tannins produced a greenish-black color (13). Saponins were assessed using the foam index method. In this test, 0.5 g of plant material was mixed with 10 mL of distilled water and vigorously shaken. The persistence of foam for approximately 10 minutes confirmed the presence of saponins (14).

Flavonoids: For flavonoid detection, 1 mL of the extract was mixed with 1 mL of concentrated hydrochloric acid (HCl), followed by the addition of a few magnesium (Mg) chips. The appearance of a red, orange, or pink coloration indicated the presence of flavonoids (14).

Coumarins were detected by placing 1 mL of the extract in a water bath and sealing the container with filter paper impregnated with NaOH. After 20 minutes, the filter paper was examined under UV light (366 nm). The appearance of yellow fluorescent spots confirmed the presence of coumarins (5).

Alkaloids were identified in the methanolic extracts using Dragendorff's reagent. A few milliliters of the extract were placed in a test tube, and the reagent was added dropwise. The formation of an orange-red precipitate indicated the presence of alkaloids (16).

Steroids were detected using the Liebermann-Burchard reaction. A few drops of acetic anhydride were added to the methanolic extract, followed by two drops of concentrated sulfuric acid. The appearance of a reddish-blue color that gradually changed to green suggested the presence of steroids (11).

For quinone detection, 1 g of plant powder was mixed with 1 mL of hydrochloric acid and 5 mL of chloroform and left to stand for several hours. After filtration, diluted ammonia (1:2) was added to the filtrate. A coloration in the aqueous phase indicated the presence of quinones (17).

Finally, anthraquinones were tested by macerating 0.5 g of plant powder in water. After filtration, 1 mL of 10% ammonia was added. The development of a pink-red coloration confirmed the presence of anthraquinones. Quantitative determination of phytochemical constituents was subsequently performed according to established standard procedures (17).

Determination of total phenolic and flavonoid content

Phenolic contents in the crude extracts were expressed as micrograms of gallic acid equivalents (GAE) according to the following Singleton and Ross (1965) method (18). Briefly, 200 µl of the extract in methanol (1 mg of extract in 1 mL methanol) was mixed with 1 mL of Folin-Ciocâlteu's Reagent (10%). After 4 min, 800 µL of 7.5% Na_2CO_3 solution was added; the final solution was incubated at room temperature for 2 hours, and its absorbance was measured at 765 nm using a UVIKON UV/VIS spectrophotometer.

Total phenolic content (TPC) was calculated according to the following equation:

Absorbance = 0.002 Gallic acid (GAE) µg + 0.020 (R^2 = 0.9900) (Eq. 1)

Total flavonoid content of the extracts was measured according to the method described by Kim *et al*. (19), and results were expressed as µg catechin equivalents (CAE). Briefly, 500 µl of the solution (1 mg of extract in 1 mL methanol) was added to 1500 µL of distilled H_2O and 150 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of 10% $AlCl_3$ and 500 µL of 1 M NaOH were added to the same mixture. The absorbance was measured at 510 nm, and the total flavonoid content (TFC) was calculated as follows:

Absorbance = 0.035 Catechin (CAE) µg - 0.0394 (R^2 = 0.9922) (Eq. 2)

Calibration curves for both total phenolic content (TPC) and total flavonoid content (TFC) were constructed using 11 concentration points, including the blank (0 concentration).

Liquid Chromatography-Mass Spectroscopy

The LC-MS/MS analytical conditions and compound identification strategy were adapted from previously reported methods for plant extracts, with minor modifications according to Trad Khodja *et al.*, 2023 (20).

The analysis of the chemical profile of samples was conducted utilizing liquid chromatography–tandem mass spectrometry (LC-MS/MS) with a Triple Quadrupole Mass Spectrometer (Agilent 6460 LC-MS/MS) connected to an Agilent LCGC chromatographic system. Prior to LC–MS/MS analysis, the sample solutions were filtered through a 0.45 µm membrane filter to remove particulate matter that could interfere with chromatographic analysis. Chromatographic separation was achieved on a Poroshell 120 EC-C18 column (50 × 4.6 mm I.D., particle size 2.7 µm) at a column temperature of 30°C for operation. Mobile phase A consisted of 0.1% formic acid in deionized H_2O with ammonium formiate 5 mM and mobile phase B consisted of acetonitrile with 0.1% formic acid. Sample elution utilized a programmed gradient elution profile (Table I).

Table I

Gradient elution program used for LC–MS/MS analysis

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	85	15
5.0	75	25
15.0	25	75
16.0	0	100
20.0	0	100
22.0	85	15
40.0	85	15

The extract solutions were prepared at 2.0 mg/mL; the injection volume was 4.0 µL at a flow rate of 0.4 mL/min. The chemical investigation was conducted in Multiple Reaction Monitoring (MRM) using both positive and negative modes of electrospray ionization (ESI). The compounds were identified from the precursor ion (m/z) using unique fragmentation patterns and specific parameters from the instrument, as well as from 30 chemical standards with the same chemical structure, and by comparison of the obtained fragmentation data.

For LC–MS/MS sample preparation, 50 mg of dried extract was mixed with 1 mL of acetonitrile–methanol–H_2O mixture (1:1:1, v/v/v) and processed by vortex agitation until dissolved, followed by sonication if needed. Following dissolution, 0.8 mL of hexane was added to each Eppendorf tube, and the mixture was centrifuged at 7000 rpm for 5 min. The lower phase was then collected, diluted at a ratio of 1:4 (v/v) with water, filtered through a 0.45 µm membrane filter, and subjected to LC-MS/MS analysis.

LC-MS/MS method validation. The analytical performance of the LC–MS/MS method was evaluated in terms of linearity (slope and coefficient of determination, R^2), limit of detection (LOD), and limit of quantification (LOQ). The validation parameters for the analyzed phenolic and flavonoid standards are presented in Table II.

Table II

LC–MS/MS method validation parameters, including linearity (slope and R^2), limit of detection (LOD), and limit of quantification (LOQ) for the analyzed phenolic and flavonoid standards

Compound	Slope (S)	R^2	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	103.893	0.99357	0.0212	0.0643
Epigallocatechin	30.571	0.99891	0.0036	0.0109
Chlorogenic acid	79.725	0.99863	0.0045	0.0137
Catechin	66.880	0.99462	0.0178	0.0538
Gentisic acid	23.823	0.99791	0.0069	0.0209
Caffeic acid	244.718	0.99884	0.0038	0.0116
Syringic acid	57.460	0.99907	0.0031	0.0093
Vanillic acid	3.094	0.99857	0.0047	0.0143
Rutin	49.188	0.99772	0.0075	0.0228
Isoquercitrin	74.820	0.99876	0.0041	0.0124
p-Coumaric acid	88.298	0.99592	0.0135	0.0408
Sinapic acid	364.977	0.99969	0.0010	0.0031
Vanillin	150.584	0.99880	0.0040	0.0120
trans-Ferulic acid	137.603	0.99951	0.0016	0.0050
Taxifolin	68.774	0.99448	0.0182	0.0552
Salicylic acid	78.345	0.99820	0.0059	0.0180
o-Coumaric acid	33.638	0.99990	0.0003	0.0010
Baicalin	43.366	0.99914	0.0028	0.0086
Protocatechuic acid	278.320	0.99777	0.0074	0.0223
Quercetin	12.063	0.99309	0.0227	0.0691
Trans-cinnamic acid	24.888	0.99844	0.0052	0.0156
Naringenin	74.164	0.99948	0.0017	0.0052
Kaempferol	26.752	0.99695	0.0101	0.0305
Morin	335.860	0.99650	0.0116	0.0350
7-Hydroxyflavone	1084.261	0.99712	0.0095	0.0288
Chrysin	268.267	0.99843	0.0052	0.0157
Luteolin	37.599	0.99480	0.0172	0.0520
Biochanin A	687.527	0.99980	0.0007	0.0020
5-Hydroxyflavone	336.097	0.99834	0.0055	0.0166
Diosgenin	109.548	0.99821	0.0059	0.0179

LOD and LOQ were calculated according to the equations LOD = 3.3 × (σ/S) and LOQ = 10 × (σ/S), where **S** is the slope of the calibration curve and σ is the standard deviation of the response. All values are expressed in µg/mL.

Antioxidant activity

Bioactivity measurements were performed using a SpectraMax 340PC384 96-well microplate reader (Molecular Devices, Silicon Valley, CA, USA), and the data were analyzed using SoftMax Pro v5.2 software. Unless otherwise specified, all antioxidant and enzyme inhibitory assays were conducted using serial extract concentrations of 1.0, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL.

The overall antioxidant capacity was evaluated using the β-carotene–linoleic acid bleaching assay according to previously reported procedures (21, 22), with slight modifications. A solution containing 0.5 mg of β-carotene dissolved in 1 mL of chloroform was prepared and mixed with 200 mg of the emulsifier Tween 40 and 25 µL of linoleic acid. After complete evaporation of chloroform under vacuum, 50 mL of oxygenated distilled water was added while vigorously shaking the mixture to form an emulsion. Subsequently, 160 µL of this emulsion was distributed into the wells of a microplate containing 40 µL of either branch or leaf extracts of *A. arabica*. The same procedure was carried out for each extract at various concentrations. The initial absorbance was recorded at 470 nm. The microplate was then incubated at 50°C for 2 hours, after which the absorbance was measured again at the same wavelength. Ethanol served as the control. The IC_{50} value, defined as the concentration of extract required to achieve 50% antioxidant activity, was determined from the plot of antioxidant activity percentage versus extract concentration. α-Tocopherol and BHA were used as reference antioxidant standards for comparison. The bleaching rate (R) of β-carotene was calculated using the following equation:

$$R = \ln\frac{a}{b} \times 1/t \quad \text{(Eq. 3)}$$

Where: ln: natural log, a: initial absorbance, b: absorbance at 120 min, and t: total test time (120 min). The antioxidant activity was presented as percent inhibition using the following formula:

$$\text{Antioxidant activity \%} = [(\text{R control} - \text{R sample})/\text{R control})] \times 100 \quad \text{(Eq. 4)}$$

DPPH radical scavenging assay. The free radical scavenging activity of the extracts was evaluated using the DPPH radical assay according to previously described spectrophotometric methods (23, 24), with minor modifications. DPPH exhibits a strong absorption at 517 nm; however, this absorbance decreases when the radical is neutralized by an antioxidant compound.

In this assay, 120 µL of ethanol was mixed with 40 µL of the sample solution at different concentrations. The reaction was initiated by adding 40 µL of DPPH solution (0.4 mM) prepared in ethanol. The mixture was then incubated for 30 minutes at room temperature, after which the absorbance was measured at 517 nm. Ethanol was used as the blank control. The scavenging capability of the DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100 \quad \text{(Eq. 5)}$$

Where: Abs Control is the initial concentration of the DPPH, and Abs Sample s the absorbance of the solution containing the extract after reaction with DPPH. From the graph of DPPH radical scavenging effect percentage against extract concentration, the extract concentration providing 50% antioxidant activity (IC_{50}) was calculated. α-tocopherol and BHA were used as antioxidant standards for comparison of the activity.

ABTS cation radical decolorization assay. According to the method of Re *et al*., (1999) (25), the analysis of $ABTS^+$- scavenging activity was determined (with slight modifications). The $ABTS^+$-solution was prepared as follows: 7 mM $ABTS^+$-dissolved in distilled water was added to 2.45 mM potassium persulfate; the solution was kept in the dark at room temperature for 12-16 hours. The test consists of adding 160 µL of $ABTS^+$ solution to 40 µL of each sample solution (branches and leaves of *A. arabica*) at different concentrations. After 10 min, the percentage inhibition was measured at 734 nm.

The scavenging capability of $ABTS^+$ was calculated as follows:

$$ABTS^+ - \text{ scavenging effect (\%)} = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100 \quad \text{(Eq. 6).}$$

In this assay, A control refers to the absorbance of the $ABTS^+$-radical solution without extract, while A sample indicates the absorbance recorded in the presence of the tested sample. The IC_{50} value, defined as the extract concentration capable of scavenging 50% of $ABTS^+$-radicals, was obtained from the curve plotting the percentage of inhibition against extract concentration. Standard antioxidants, namely BHT and α-tocopherol, were used as positive controls for activity comparison.

Cupric reducing antioxidant capacity (CUPRAC) method. According to the CUPRAC method described by Apak *et al*. (2004) (26), the antioxidant activity of the extracts was determined using the cupric reducing antioxidant capacity (CUPRAC) assay. The reaction mixture consisted of 50 µL of 10 mM Cu(II) solution, 50 µL of 7.5 mM neocuproine, and 60 µL of ammonium acetate buffer (1 M, pH 7.0). Then, 40 µL of the extract at different concentrations was added to reach a final reaction volume of 200 µL. The mixture was allowed to stand for 1 h at room temperature, after which the absorbance was measured at 450 nm against a reagent blank. The absorbance values obtained at each extract concentration were plotted against concentration, and the $A_{0.50}$ value (the concentration required to achieve an absorbance of 0.50) was determined from the resulting calibration curve. The obtained values were compared with those of the standard antioxidants BHA and α-tocopherol.

Ferrous ions chelating activity. The chelating activity toward ferrous ions was evaluated using the ferrozine assay as described by (27) with slight modifications. In this assay, 80 µL of extract solution at various concentrations (dissolved in ethanol) was combined with 40 µL of $FeCl_2$ (0.2 mM) solution and 80 µL of 0.5 mM ferrozine. The mixture was vortexed thoroughly and incubated at room temperature for 10 min. Following the incubation period, the absorbance was measured at 593 nm. The percentage of metal chelation was calculated using the equation below.:

$$\text{the metal chelating activity (\%)} = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100 \quad \text{(Eq. 7)}$$

Where: A Control is the absorbance of the control devoid of sample, and A Sample is the absorbance of sample in the presence of the chelator. The extract concentration providing 50% metal chelating activity (IC_{50}) was calculated from the graph of Fe^{2+} chelating effects percentage against extract concentration. For comparison, EDTA was used as a reference chelating agent.

Anticholinesterase activity of the extracts

The inhibitory activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were evaluated according to the methods described by Ellman *et al*. (1961) and Tamfu *et al*. (2020) with slight modifications (4,28). AChE from electric eel (Type VI-S, EC 3.1.1.7, 425.84 U/mg protein, Sigma-Aldrich) and BChE from horse serum (EC 3.1.1.8, 11.4 U/mg protein, Sigma-Aldrich) were used as enzyme sources. Acetylthiocholine iodide (Sigma-Aldrich) and butyrylthiocholine chloride (Sigma-Aldrich)

were used as substrates, while 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich) was used for the detection of cholinesterase activity.

Briefly, 130 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of the extract solution (from branches and leaves of *A. arabica*) prepared in ethanol at different concentrations, and 20 µL of AChE or BChE solution were mixed and pre-incubated at 25°C for 15 min. Subsequently, 20 µL of DTNB solution (0.5 mM) was added to the mixture. The enzymatic reaction was then initiated by adding 20 µL of acetylthiocholine iodide (0.71 mM) or 20 µL of butyrylthiocholine chloride (0.2 mM).

Hydrolysis of the substrates produces thiocholine, which reacts with DTNB to generate the yellow-colored 5-thio-2-nitrobenzoate anion. The formation of this product was monitored by measuring the absorbance at 412 nm using a 96-well microplate reader. Galantamine was used as the positive control.

Statistical analysis

Unless otherwise specified, all antioxidant and anticholinesterase experiments were performed in technical triplicates, and the results were expressed as mean ± standard error of the mean (SEM). Extraction yield was determined from two independent extraction batches (n = 2) and is reported as mean ± standard deviation (SD). Statistical differences among the samples and reference standards were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test. Differences were considered statistically significant at $p < 0.05$. Values reported as ">200" or "NT" (not tested) were excluded from the statistical comparisons

Results and Discussion

Phytochemical screening

Phytochemical screening is an essential step that makes it possible to detect the presence in medicinal plants of products belonging to classes of physiologically active compounds. Phytochemical tests consist of detecting the different families of compounds that exist in the branches and the leaves of *A. arabica* by precipitation or coloration reactions using reagents specific to each family of compounds. The results obtained revealed the presence of flavonoids, coumarins, tannins, alkaloids, and anthraquinones in our samples. In addition, we detected the presence of saponins in the leaves. Furthermore, we noted the absence of quinones in our analyzed extracts.

Total phenolic and flavonoid

The total polyphenol (TPC) and total flavonoid (TFC) contents were determined using the linear regression equations obtained from the corresponding calibration curves. The results are expressed as mg gallic acid equivalents (GAE)/g extract for total polyphenols and mg catechin equivalents (CAE)/g extract for total flavonoids (Table III).

Table III

Total phenolic and flavonoid contents

Sample	**Total Phenolic Content** (mg GAE/g)	**Total Flavonoid Content** (mg CAE/g**)**
Branches	81.6 ± 0.28	43.4 ± 0.1
Leaves	104.7 ± 1.2	65.06 ± 0.90

Values are expressed as mean ± SEM (n = 3), based on technical triplicates.

According to the results (Table III), significant amounts of total phenol content (TPC) and total flavonoid content (TFC) were found in the branch extract: TPC = 81.6 ± 0.28 mg GAE/g and TFC = 43.4 ± 0.1 mg CAE/g. However, these amounts were lower than those observed in the leaf extract, which showed very high levels of phenolic and flavonoid contents: TPC = 104.7 ± 1.2 mg GAE/g and TFC = 65.06 ± 0.90 mg CAE/g.

Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS)

From the LC-MS/MS result, ten compounds were identified in the leaves and branches extracts of *Acacia arabica* among the 30 phytochemical standards employed (Table IV)**.** The branches extract exhibited generally higher polyphenol concentrations, particularly catechin and epigallocatechin, while rutin, salicylic acid, and trans-cinnamic acid were detected exclusively in the leaves extract, with some compounds common to both extracts.

Table IV

Quantitative analysis of natural compounds of *Acacia arabica* by LC-MS/MS (µg/mL)

Compound	**Retention Time (min)**	**Branches**	**Leaves**
Gallic acid	2.267	2.615899	ND
Epigallocatechin	2.592	82.97433	8.209259
Chlorogenic acid	2.813	ND	ND
Catechin	2.729	143.7995	2.429496
Gentisic acid	2.663	4.586931	ND
Caffeic acid	3.418	ND	ND
Syringic acid	3.640	0.91536	1.230511
Vanillic acid	3.531	ND	ND
Rutin	3.650	ND	0.688435
Isoquercitrin	4.462	ND	ND
p-coumaric acid	4.680	0.638237	ND

Compound	Retention Time (min)	Branches	Leaves
Sinapic acid	5.512	ND	ND
Vanillin	5.416	8.992595	ND
trans-ferulic acid	4.871	ND	ND
Taxifolin	5.963	ND	ND
Salicylic acid	6.912	ND	2.187129
o-coumaric acid	7.906	ND	ND
Baicalin	7.974	ND	ND
Protocatechuic acid	8.424	ND	ND
Quercetin	10.127	ND	ND
Trans-cinnamic acid	10.953	ND	1.173848
Naringenin	11.455	ND	ND
Kaempferol	11.544	ND	ND
Morin	11.933	ND	ND
7-Hydroxyflavone	12.088	ND	ND
Chrysin	13.693	ND	ND
Luteolin	13.887	ND	ND
Biochanin A	13.875	ND	ND
5-Hydroxyflavone	15.755	ND	ND
Diosgenin	21.001	ND	ND

ND: Non detectable.

Antioxidant activity

Table V presents the results of experiments measuring the antioxidant activity of samples and reference substances in various tests: the β-carotene-linoleic acid assay, the DPPH assay, the $ABTS^+$, the CUPRAC assay, and the metal chelating assay. The results are expressed as IC_{50} (inhibitory concentration at 50%) and as percentage inhibition at 200 µg/mL. The samples include branches and leaves of *A. arabica*, as well as reference substances such as α-tocopherol, BHA, and EDTA. In the β-carotene linoleic acid method, both the methanol extract of acacia branches (IC_{50}: 7.03 ± 0.24 µg/mL) and the methanol extract of leaves (IC_{50}: 8.79 ± 0.17 µg/mL) showed potential antioxidant activity, but it was lower compared to BHA (IC_{50}: 1.50 ± 0.03 µg/mL) and α-tocopherol (IC_{50}: 2.10 ± 0.05 µg/mL). The obtained results, as shown in Table V, indicate that the methanolic extract of *A. arabica* branches exhibited the highest radical-scavenging activity, with IC_{50} values of 11.51 ± 0.40 µg/mL in the DPPH• assay and 9.86 ± 0.63 µg/mL in the $ABTS^+$ assay. Under the experimental conditions used, these values were lower than those obtained for the reference antioxidants α-tocopherol (IC_{50}: 38.20 ± 0.50 and 34.75 ± 0.55 µg/mL, respectively) and BHA (IC_{50}: 19.50 ± 0.30 and 12.70 ± 0.10 µg/mL, respectively), indicating a higher radical-scavenging capacity of the branch extract in these two assays. This activity may be associated with the combined contribution of different antioxidant constituents present in the extract, particularly phenolic compounds, although the specific contribution or possible synergistic interactions among these constituents cannot be established from the present results alone. In the CUPRAC assay, the methanolic extracts of *A. arabica* branches and leaves showed relatively high cupric ion (Cu^{2+}) reducing capacity, with $A_{0.50}$ values of 13.52 ± 0.22 and 15.93 ± 0.14 µg/mL, respectively. These values were lower than those obtained for α-tocopherol (60.45 ± 0.30 µg/mL) and BHA (25.40 ± 0.42 µg/mL), indicating a higher reducing capacity of both extracts under the experimental conditions used. The branch extract showed slightly greater reducing capacity than the leaf extract, as reflected by its lower $A_{0.50}$ value.

In the metal chelating assay, branch and leaf extracts also demonstrated strong antioxidant activity (with IC_{50} values of 13.85 ± 0.69 µg/mL and 17.01 ± 0.52 µg/mL), respectively, but lower than the standard compound EDTA (IC_{50}: 5.50 ± 0.25 µg/mL) (Table V).

Table V

Antioxidant activities of branches and leaves of *A. arabica*

Samples/ Standards	β-Carotene IC_{50}	β-Carotene Inh.	DPPH IC_{50}	DPPH Inh.	ABTS IC_{50}	ABTS Inh.	CUPRAC $A_{0.50}$	CUPRAC Abs.	Metal IC_{50}	Metal Inh.
Branches of *Acacia*	7.03 ± 0.24^{b}	92.72 ± 0.83^{bc}	11.51 ± 0.40^{d}	89.05 ± 0.23^{a}	9.86 ± 0.63^{c}	90.73 ± 0.85^{a}	13.52 ± 0.22^{d}	2.58 ± 0.08^{c}	13.85 ± 0.69^{b}	89.00 ± 1.17^{b}
Leaves of *Acacia*	8.79 ± 0.17^{a}	92.09 ± 0.91^{c}	14.02 ± 0.54^{c}	88.62 ± 0.46^{a}	11.62 ± 0.35^{bc}	89.45 ± 0.79^{a}	15.93 ± 0.14^{c}	2.63 ± 0.06^{c}	17.01 ± 0.52^{a}	87.24 ± 0.82^{b}

Samples/ Standards	β-Carotene IC_{50}	β-Carotene Inh.	DPPH IC_{50}	DPPH Inh.	ABTS IC_{50}	ABTS Inh.	CUPRAC $A_{0.50}$	CUPRAC Abs.	Metal IC_{50}	Metal Inh.
α-Tocopherol	2.10 ± 0.05^{c}	95.73 ± 0.44^{ab}	38.20 ± 0.50^{a}	84.25 ± 0.36^{b}	34.75 ± 0.55^{a}	83.63 ± 0.34^{b}	60.45 ± 0.30^{a}	1.21 ± 0.10^{b}	NT	NT
BHA	1.50 ± 0.03^{c}	96.20 ± 0.28^{a}	19.50 ± 0.30^{b}	87.90 ± 0.44^{a}	12.70 ± 0.10^{b}	88.93 ± 0.21^{a}	25.40 ± 0.42^{b}	2.35 ± 0.22^{a}	NT	NT
EDTA	NT	NT	NT	NT	NT	NT	NT	NT	5.50 ± 0.25^{c}	94.40 ± 0.35^{a}

Values are expressed as mean ± SEM (n = 3 technical replicates). Statistical comparisons were performed independently within each column using one-way ANOVA followed by Tukey's HSD post hoc test. Values within the same column that do not share a common lowercase superscript letter are significantly different ($p < 0.05$). The technical replicates represent repeated measurements of the same sample and therefore reflect analytical variability rather than biological variability.
NT: not tested.

Anticholinesterase activity

Acetylcholinesterase is an important therapeutic target in the treatment of Alzheimer's disease. It has been found that *A. nilotica* has an effect on central nervous system activities due to its potent acetylcholinesterase inhibitory activities. More investigations are required on the treatment of Alzheimer's (27). This study evaluated the anticholinesterase activity of *Acacia arabica* using the Ellman method (4); galantamine was used as a positive control for Alzheimer's disease. Extracts from various parts of *Acacia arabica* showed moderate inhibition of the acetylcholinesterase enzyme (AChE). Specifically, the leaf extract exhibited an inhibitory activity of IC_{50}: 152.7 ± 0.85 µg/mL, while the branch extract showed an IC_{50} value of 99.55 ± 0.79 µg/mL. These values were higher compared to those of galantamine, which had an inhibitory activity of IC_{50}: 5.50 ± 0.20 µg/mL. Extracts from various parts of *Acacia arabica* showed moderate inhibition of the acetylcholinesterase enzyme (AChE). Specifically, the leaf extract exhibited an IC_{50} value of 152.7 ± 0.85 µg/mL, while the branch extract showed an IC_{50} value of 99.55 ± 0.79 µg/mL. These values were higher than those of galantamine, which showed an IC_{50} value of 5.50 ± 0.20 µg/mL. The branch extract exhibited strong butyrylcholinesterase (BChE) inhibitory activity, with an IC_{50} value of 40.17 ± 0.93 µg/mL, and its activity was statistically comparable to that of galantamine (42.20 ± 0.35 µg/mL), used as the reference standard. In contrast, the leaf extract showed lower BChE inhibitory activity, with an IC_{50} value of 100.1 ± 1.12 µg/mL (Table VI).

Table VI

Cholinesterase inhibitory activity of the samples[a]

Samples/Standards	AChE IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	BChE IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
Branches of *Acacia*	99.55 ± 0.79^{b}	63.30 ± 0.61^{b}	40.17 ± 0.93^{b}	80.07 ± 0.77^{a}
Leaves of *Acacia*	152.7 ± 0.85^{a}	55.43 ± 0.97^{c}	100.1 ± 1.12^{a}	63.17 ± 0.57^{b}
Galantamine	5.50 ± 0.20^{c}	89.25 ± 0.48^{a}	42.20 ± 0.35^{b}	79.43 ± 0.60^{a}

Values are expressed as mean ± SEM (n = 3). Statistical comparisons were performed independently within each column using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. Values within the same column that do not share a common lowercase superscript letter are significantly different ($p < 0.05$).

The LC-MS/MS analysis of *Acacia arabica* methanolic extracts revealed a pronounced organ-specific distribution of phenolic and flavonoid compounds between leaves and branches, reflecting the metabolic specialization of different plant tissues. Among the ten identified compounds, some were common to both organs, while others were detected exclusively in either leaves or branches. Catechin and epigallocatechin were also detected in both branches and leaves. Syringic acid, catechin, and epigallocatechin were detected in both plant parts, although at different concentrations. Branches exhibited a richer and more diverse phenolic profile compared to leaves. Catechin (143.79 µg/mL) and epigallocatechin (82.97 µg/mL) were the dominant compounds in branches, indicating that these tissues are particularly abundant in flavan-3-ols.

These compounds are well-known for their antioxidant, anti-inflammatory, and cardioprotective properties (29, 30) and have also been reported to participate in plant defence against microbial and environmental stress. Other branch-predominant compounds included gallic acid, gentisic acid, p-coumaric acid, and vanillin, all recognized for antimicrobial, anti-inflammatory, and neuroprotective activities (31, 32, 33). Comparable organ-specific distribution patterns have been observed in other *Acacia* species, including *A. nilotica* and *A. senegal*, where

catechin and epigallocatechin are predominant in woody tissues and contribute substantially to their pharmacological properties (34, 35). In contrast, leaves exhibited a more simplified yet characteristic phytochemical profile, with rutin, salicylic acid, and trans-cinnamic acid being exclusively identified, whereas epigallocatechin was detected at a lower concentration (8.20 µg/mL). These leaf-associated metabolites have been widely recognized for their antioxidant, anti-inflammatory, and vascular-protective activities (36), and they may also participate in plant defense signalling and adaptation mechanisms under environmental stress conditions in photosynthetically active tissues (37).

The LC-MS/MS findings provided complementary information to the results obtained from total phenolic content (TPC) and total flavonoid content (TFC) assays. Despite the higher TPC (104.7 ± 1.2 mg GAE/g) and TFC (65.06 ± 0.90 mg CAE/g) values observed in leaves, branches showed higher levels of certain individual bioactive metabolites and a more diverse chemical composition. It should be noted, however, that the TPC and TFC values are expressed in mg/g extract, whereas the LC-MS/MS concentrations are reported in µg/mL of the analytical solution. Therefore, these datasets cannot be directly compared quantitatively. Instead, the LC-MS/MS results provide complementary information on the distribution of selected individual phytochemicals between the two plant organs. These results highlight that biological activity may be influenced not only by the overall abundance of phenolic compounds but also by the qualitative composition of metabolites and possible synergistic interactions among them (34, 38).

Antioxidant assays reinforced the LC–MS/MS observations. Branch extracts showed the strongest DPPH and $ABTS^{\bullet+}$ scavenging activities (IC_{50}: 11.51 ± 0.40 µg/mL and 9.86 ± 0.63 µg/mL, respectively), surpassing standard antioxidants α-tocopherol and BHA. However, these findings should be interpreted with caution, since crude plant extracts are complex mixtures of phytochemicals, and matrix effects or interactions among their constituents may influence the responses obtained in colorimetric antioxidant assays.

Both branches and leaves displayed notable reducing power in the CUPRAC assay ($A_{0.50}$: 13.52 ± 0.22 µg/mL and 15.93 ± 0.14 µg/mL, respectively) and significant metal-chelating activity. Since the antioxidant assays are based on different analytical principles, the results were expressed using different endpoints (IC_{50} for DPPH and ABTS assays, and $A_{0.50}$ for the CUPRAC assay). Therefore, the antioxidant performance of the extracts was interpreted qualitatively across assays rather than through direct numerical comparison of IC_{50} and $A_{0.50}$ values. For this reason, differences in antioxidant potency among DPPH, ABTS, and CUPRAC assays should be interpreted as qualitative trends rather than direct quantitative differences, since each assay is based on a different reaction mechanism.

Although the leaf extract exhibited higher total phenolic content (TPC) and total flavonoid content (TFC), the Folin–Ciocâlteu and $AlCl_3$ colorimetric assays are widely recognized as non-specific methods that may be influenced by matrix constituents and other reducing or complexing compounds present in plant extracts. Consequently, these assays may partially overestimate the apparent total phenolic and flavonoid contents and do not necessarily reflect the qualitative composition of individual metabolites. In contrast, LC-MS/MS provides selective identification and quantification of specific phytochemicals. Therefore, the apparent discrepancy between the colorimetric assays and the LC-MS/MS results may be partly explained by the limited specificity of the colorimetric methods, in addition to differences in phytochemical composition and the different units and analytical bases used to report the two datasets. In the present study, LC-MS/MS analysis revealed that the branch extract contained markedly higher levels of catechin and epigallocatechin, together with detectable amounts of gallic acid and gentisic acid, whereas these compounds were absent or present at much lower levels in the leaf extract. Since catechin and epigallocatechin are among the most potent naturally occurring antioxidants, their higher abundance in the branch extract most likely contributed to its superior antioxidant activity despite its lower TPC and TFC values (39).

These findings suggest that the higher levels of the detected flavan-3-ols in branches may have contributed, at least in part, to the stronger radical-scavenging and redox activities observed in the branch extract. However, this interpretation should be considered as an association rather than a direct quantitative relationship, since the LC-MS/MS concentrations are reported in µg/mL of the analytical solution and were not normalized to the amount of extract (29, 36). Anticholinesterase assays also aligned with the phytochemical profiles. Branch extracts exhibited higher inhibition against both acetylcholinesterase (AChE; IC_{50}: 99.55 ± 0.79 µg/mL) and butyrylcholinesterase (BChE; IC_{50}: 40.17 ± 0.93 µg/mL) than leaf extracts; this enhanced activity was associated with the higher abundance of catechin, epigallocatechin, and vanillin detected in the branch extract. Although these compounds have previously been reported to exhibit cholinesterase-inhibitory and neuroprotective properties, the present study demonstrates only a correlation between their occurrence and the observed enzyme inhibition, rather than a direct causal relationship (32, 40). Leaf extracts displayed moderate inhibition, which may be associated with the presence of rutin and salicylic acid, highlighting complementary mechanisms across plant

organs. Recent LC/MS-MS studies on *Acacia* and related *Fabaceae* species support the organ-specific distribution of bioactive phenolics. Catechin, epigallocatechin, rutin, and other flavonoids are consistently reported as dominant in branches and bark, while glycosylated flavonoids and soluble phenolic acids predominate in leaves (35, 38, 41). Solvent polarity and extraction methods also strongly influence phenolic recovery, reinforcing the appropriateness of methanol for obtaining a comprehensive profile in the present study (37).

It is noteworthy that chlorogenic acid, caffeic acid, and quercetin, which have been reported in some *Acacia* species, were not detected in the present samples. This apparent discrepancy may be attributed to differences in species, geographical origin, plant organ, environmental conditions, harvest period, as well as extraction and analytical procedures. Recent metabolomic investigations have demonstrated considerable qualitative and quantitative variation in the phenolic composition among *Acacia* species, indicating that the occurrence of individual phenolic compounds is highly dependent on these factors rather than being a universal characteristic of the genus (38, 40). Therefore, the absence of these compounds in the present study most likely reflects natural phytochemical variability rather than inconsistency with previous reports.

Only ten compounds were detected under the analytical conditions used in this study. The absence of the remaining targeted compounds does not necessarily indicate that they were completely absent from the extracts. Their concentrations may have been too low for detection. The preliminary hexane defatting step used during LC-MS/MS sample preparation may have affected the recovery of relatively lipophilic compounds, although the extent of any potential loss cannot be determined from the present data. Differences in plant material and analytical conditions can influence which metabolites are detected. Therefore, the LC-MS/MS profile presented here should be considered representative of the compounds detected under the experimental conditions applied in this study.

Overall, these findings indicate that *Acacia arabica* is a rich source of bioactive phenolic compounds with organ-specific accumulation patterns. Branches are particularly enriched in flavan-3-ols and other protective metabolites, while leaves provide unique flavonoids and phenolic acids contributing to antioxidant and enzyme-inhibitory activity. The results emphasize that both qualitative and quantitative phytochemical composition should be considered when assessing the therapeutic potential of plant extracts, and further studies should focus on isolation, structural characterization, and biological evaluation of these metabolites (38, 41). Future studies should include bioassay-guided fractionation and evaluation of isolated compounds to identify the constituents associated with the observed cholinesterase inhibitory activity and to clarify possible synergistic interactions among the detected phytochemicals.

This study has some limitations. The biological activities were evaluated using crude methanolic extracts and *in vitro* assays only; therefore, the contribution of individual phytochemicals could not be determined directly. In addition, all experiments were performed using technical triplicates on the same extract rather than independent biological replicates. Consequently, the reported variability reflects analytical precision under the experimental conditions and should not be interpreted as biological variation among different plant samples. Furthermore, the therapeutic potential of the extracts should be confirmed through *in vivo* studies before any clinical relevance can be inferred.

Conclusions

The methanolic extracts obtained from *Acacia arabica* proved to be a valuable source of flavonoids and phenolic compounds and showed considerable antioxidant and anticholinesterase activities. LC–MS/MS characterization highlighted clear differences in the phytochemical composition of leaves and branches. The branch extract was particularly rich in catechin, epigallocatechin, gallic acid, and gentisic acid, whereas rutin and salicylic acid were detected only in the leaves, suggesting that these compounds may also contribute to the biological properties of this organ. These observations indicate that the biological activities of *Acacia arabica* are influenced not only by the overall phenolic and flavonoid contents but also by the nature and relative abundance of individual constituents. The stronger antioxidant activity recorded for the branch extract may be explained, at least in part, by its higher levels of catechin and epigallocatechin. Nevertheless, because the biological assays were carried out on crude extracts rather than purified compounds, the specific contribution of each constituent cannot be confirmed.

Taken together, the present results provide additional scientific support for the traditional medicinal use of *Acacia arabica*. The antioxidant and cholinesterase inhibitory activities observed in this study are in agreement with the ethnomedicinal uses previously reported for this species and suggest that its phenolic constituents are likely to play an important role in its traditional therapeutic properties. Overall, these findings identify *Acacia arabica* as a promising source of bioactive compounds and provide a useful basis for future pharmacological investigations.

Notably, this study adds to the limited literature on the anticholinesterase activity of *Acacia arabica* extracts

collected in Algeria. These findings highlight the importance of evaluating different plant organs to identify those with the greatest therapeutic potential. Future studies should focus on bioassay-guided fractionation, isolation of active constituents, mechanistic investigations, *in vivo* validation, and assessment of environmental and seasonal variations in phytochemical composition to further clarify their therapeutic potential and optimize the medicinal use of this species.

Acknowledgement

This work is supported by High Teaching and Scientific Research Ministry www.mesrs.dz.
The authors are greatful to Mugla University, Faculty of Science, Department of chemistry for opening its laboratory.

Funding

Not applicable.

Availability of data and materials

The data generated in the present study are included in the tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Elshafie HS, Camele I, Mohamed AA. A comprehensive review on the biological, agricultural and pharmaceutical properties of plant-based secondary metabolites based-plant origin. Int J Mol Sci. 2023;24(4):3266.
2. Kalaivani T, Mathew L. Free radical scavenging activity from leaves of Acacia nilotica (L.) Willd. ex Delile, an Indian medicinal tree. Food Chem Toxicol. 2010;48(1):298-305.
3. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am J Clin Nutr. 2005;81(1 Suppl):215S-217S.
4. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95.
5. Moulay Y, Ghiaba Z, Saïdi M. Phenolic content and antioxidant activity of extracts from Acacia arabica barks. PhytoChem BioSub J. 2014;8(1):20-25.
6. Ali A, Akhtar N, Khan BA, Khan MS, Rasul A, Shahiq-uz-Zaman, et al. Acacia nilotica: a plant of multipurpose medicinal uses. J Med Plants Res. 2012;6(9):1492-1496.
7. Duke JA. Handbook of legumes of world economic importance. New York: Plenum Press; 1981.
8. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed. Vol 1. Dehra Dun: Bishen Singh Mahendra Pal Singh; 1975. p. 521-528.
9. Ullah R, Alqahtani AS, Noman OMA, Alqahtani AM, Ibenmoussa S, Bourhia M. A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. Saudi J Biol Sci. 2020;27(10):2706-2718.
10. Maina S, Ryu DH, Bakari G, Misinzo G, Nho CW, Kim HY. Variation in phenolic compounds and antioxidant activity of various organs of African cabbage (Cleome gynandra L.) accessions at different growth stages. Antioxidants (Basel). 2021;10(12):1952.
11. Harborne JB. Phytochemical methods. London: Chapman and Hall; 1973.
12. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res. 2008;7(3):1019-1024.
13. Karumi Y, Onyeyili PA, Ogugbuaja VO. Identification of active principles of Momordica balsamina (balsam apple) leaf extract. J Med Sci. 2004;4(3):179-182.
14. N'Guessan K, Kadja B, Zirihi GN, Traoré D, Aké-Assi L. Phytochemical screening of selected Ivorian medicinal plants used in the Krobou region (Agboville, Côte d'Ivoire). Sci Nat. 2009;6(1):1-15 [Available in French].
15. Bruneton J. Pharmacognosie, phytochimie des plantes médicinales. 3rd ed. Paris: Tec & Doc; 1999.
16. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. Iran J Pharm Res. 2003;2(2):77-82.
17. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. London: Chapman & Hall; 1998.
18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-158.
19. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem. 2003;51(22):6509-6515.
20. Anissa Trad Khodja E, El Hamid Khabtane A, Arhab R, Benouchenne D, Sabri Bensaad M, Bensouici C, et al. In vitro assessment of antioxidant, neuroprotective, anti-urease and anti-tyrosinase capacities of Tamarix africana leaves extracts. J Tradit Chin Med. 2023;43(2):252-264.
21. Marco GJ. A rapid method for evaluation of antioxidants. J Am Oil Chem Soc. 1968;45(9):594-598.
22. Djebili S, Taş M, Bouguerra A, Kucukaydin S, Ceylan O, Duru ME, et al. Volatile compound profile and essential oil composition of three wild Algerian aromatic plants with their antioxidant and antibiofilm activities. J Food Meas Charact. 2022;16(2):987-999.
23. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200.
24. Tel-Çayan G, Duru ME. Chemical characterization and antioxidant activity of Eryngium pseudothoriifolium and E. thorifolium essential oils. J Res Pharm. 2019;23(6):1106-1114.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-1237.
26. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E using their cupric ion reducing capability in the

presence of neocuproine: the CUPRAC method. J Agric Food Chem. 2004;52(26):7970-7981.
27. Decker EA, Welch B. Role of ferritin as a lipid oxidation catalyst in muscle food. J Agric Food Chem. 1990;38(3):674-677.
28. Tamfu AN, Ceylan O, Kucukaydin S, Duru ME. HPLC-DAD phenolic profiles, antibiofilm, antiquorum sensing and enzyme inhibitory potentials of Camellia sinensis (L.) O. Kuntze and Curcuma longa L. LWT. 2020;133:110150.
29. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727-747.
30. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition and antioxidant potential of grain legume seeds: a review. Food Res Int. 2017;101:1-16.
31. Abedi F, Razavi BM, Hosseinzadeh H. A review on gentisic acid as a plant-derived phenolic acid and metabolite of aspirin: comprehensive pharmacology, toxicology, and some pharmaceutical aspects. Phytother Res. 2020;34(4):729-741.
32. Batiha GES, Akhtar N, Alsayegh AA, Abusudah WF, Almohmadi NH, Shaheen HM, et al. Phenolic acids and their pharmacological potential: a review. J Pharm Pharmacol. 2022;74(12):1809-1835.
33. Olatunde A, Mohammed A, Ibrahim MA, Tajuddeen N, Shuaibu MN. Vanillin: a food additive with multiple biological activities. Eur J Med Chem Rep. 2022;5:100055.
34. Ali A, Cottrell JJ, Dunshea FR. LC-MS/MS characterization of phenolic metabolites and their antioxidant activities from Australian native plants. Metabolites. 2022;12(11):1016.
35. Okoro S, Kawo A, Arzai A. Phytochemical screening, antibacterial and toxicological activities of Acacia senegal extracts. Bayero J Pure Appl Sci. 2012;5(1):163-170.
36. Ganeshpurkar A, Saluja A. The pharmacological potential of rutin. Saudi Pharm J. 2017;25(2):149-164.
37. Elshamy S, Handoussa H, El-Shazly M, Mohammed ED, Kuhnert N. Metabolomic profiling and quantification of polyphenols from leaves of seven Acacia species by UHPLC-QTOF-ESI-MS. Fitoterapia. 2024;172:105741.
38. Aryal B, Adhikari B, Aryal N, Bhattarai BR, Khadayat K, Parajuli N. LC-HRMS profiling and antidiabetic, antioxidant, and antibacterial activities of Acacia catechu (L.f.) Willd. Biomed Res Int. 2021;2021:7588711.
39. Zheleva-Dimitrova D, Sinan KI, Etienne OK, Ak G, Sharmeen JB, Dervisoglu G, et al. Comprehensive chemical characterization and biological evaluation of two Acacia species: A. nilotica and A. ataxacantha. Food Chem Toxicol. 2021;156:112446.
40. Al-Rajhi A, Qanash H, Bazaid AS, Binsaleh NK, Abdelghany TM. Pharmacological evaluation of Acacia nilotica flower extract against Helicobacter pylori and human hepatocellular carcinoma in vitro and in silico. J Funct Biomater. 2023;14(4):237.
41. Elshamy S, Kuhnert N, El-Shazly M, Ziemah J, Handoussa H. Comparative metabolomic study of twelve Acacia species by UHPLC-q-tof-ESI-MS coupled with chemometrics in correlation with antibacterial activity. Fitoterapia. 2025;181:106378.

.