International Journal of Secondary Metabolite
2025, Vol. 12, No. 2, 381–396
https://doi.org/10.21448/ijsm.1466689
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Exploring *Phlomis crinita* extracts: HPLC analysis, phenolic content, antioxidant and antimicrobial potentials

Abdelhakim Chelgham[1,2*], Abdelkader Saadi[2], Abdelaziz Merouane[3], Chawki Bensouici[4], Yavuz Selim Cakmak[5], Tânia C.S.P. Pires[6,7]

[1]Laboratory of Natural Bio-ressources, Hassiba Benbouali University of Chlef, Chlef, Algeria
[2]Department of Biotechnology, Faculty of Life and Natural Sciences, Hassiba Benbouali University of Chlef, Chlef, Algeria
[3]Department of Preparatory Class, Higher School of Saharan Agriculture, Adrar, Algeria
[4]Biotechnology Research Center (CRBt), Constantine, Algeria
[5]Department of Molecular Biology and Genetics, Faculty of Science and Letters, Aksaray University, Aksaray, Türkiye
[6]Mountain Research Centre, Polytechnic Institute of Bragança, Santa Apolónia Campus, Bragança, Portugal.
[7]Associate Laboratory for Sustainability and Technology in Mountains Region (SusTEC), Polytechnic Institute of Bragança, Santa Apolónia Campus, Bragança, Portugal

ARTICLE HISTORY

Received: Apr. 15, 2024

Accepted: Feb. 5, 2025

KEYWORDS

Phlomis crinita,
Herbal medicine,
HPLC-DAD,
Phenolic compounds,
Biological activity

Abstract: *Phlomis crinita* Cav. (*P. crinita*), known as " Khayat el-djerah " in Algerian folk medicine, is used for wound healing and abdominal pain relief. This study assessed the phytochemical profile, phenolic content, antimicrobial activity against five Gram-negative and three Gram-positive clinical bacterial strains, as well as *in vitro* antioxidant activity of hydroethanolic extracts from leaves (HLE), flowers (HFE), and rhizomes (HRE) of *P. crinita*. Fifteen phenolic compounds such as four flavonoids, trans-cinnamic acid, six cinnamic acid derivatives, and four benzoic acid derivatives were identified for the first time in *P. crinita* by HPLC-DAD, with quantitative differences among the analyzed parts. HRE exhibited high levels of total phenolics (262.97 ± 16.2 µg GAE/mg DW) and flavonoids (71.87 ± 3.25 µg QE/mg DW), while HLE had the highest flavonols content (18.89 ± 5.12 µg QE/mg DW). All extracts demonstrated strong antioxidant properties. HLE exhibited the highest potency, with IC_{50} values of 15.46 ± 0.45 µg/mL (DPPH) and 11.71 ± 0.50 µg/mL (ABTS). HLE exhibited good reducing power (FRAP $A_{0.5}$ = 40.07 ± 2.82 µg/mL), while HRE showed the best reducing power (Phenanthroline $A_{0.5}$ = 7.88 ± 1.63 µg/mL). All extracts revealed broad-spectrum antibacterial effects, and HRE exhibited the most potent activity against *Enterococcus faecalis*, with a minimum inhibitory concentration (MIC) value of 1.25 mg/mL. These results showed that *P. crinita* could be useful as source of bioactive compounds for pharmaceutical and food industry.

1. INTRODUCTION

Medicinal plants play a crucial role in traditional and modern healthcare, providing a wealth of bioactive compounds with therapeutic potential. In fact, natural products play a pivotal role as a source of drug compounds, and currently, a number of modern drugs derived from traditional herbal medicine are used in modern pharmacotherapy (Marrelli, 2021). Recently, there has been

*CONTACT: Abdelhakim CHELGHAM a.chelgham@univ-chlef.dz Hassiba Benbouali University, Faculty of Life and Natural Sciences, Department of Biotechnology, Chlef, Algeria

e-ISSN: 2148-6905

a growing interest in conducting phytochemical and pharmacological studies on traditional medicinal plants. The Lamiaceae family, a well-known source of bioactive molecules, has attracted significant attention. Among this family, the genus *Phlomis*, encompassing over 100 species of perennial herbs, is primarily found in regions around 40° north latitude including Algeria, Morocco, Spain, Greece, Turkey, and China (Li *et al*., 2010). Previous research has demonstrated that the genus *Phlomis* is rich in secondary metabolites, including flavonoids, phenolic acids, iridoids, phenylethylalcohol glycosides, phenylethanoid and benzyl glycosides, lignans, and terpenoids, and most of these classes belong to the polyphenol category and serve as powerful antioxidants due to their multiple hydroxyl groups (Amor *et al*., 2009a; Li *et al.*, 2010; Çalış & Başer, 2021). This has led to the exploration of the phytochemical and pharmacological properties of various *Phlomis* species, which have shown promise in the treatment of many illnesses. In fact, biological investigations of extracts of the genus *Phlomis* have demonstrated their diverse range of activities, including anti-inflammatory, immunomodulatory, free-radical scavenging, antimicrobial, anti-mutagenic, and anti-ulcerogenic effects (Limem *et al*., 2011).

Phlomis crinita Cav. (synonym: *P. biloba*), one of *Phlomis* species found in Algeria, is locally known as "Khayat el djerah" (wound healer) due to its traditionally used leaves for treating lesions and burns (Amor *et al*., 2009a; Boutennoun *et al*., 2023). Despite its ethnomedicinal significance, the phytochemical profile of *P. crinita* remains largely unexplored, with only a few studies examining its chemical composition. Given its traditional medicinal applications, there is a pressing need to investigate its phenolic profile and associated biological activities.

This study aimed to evaluate the phenolic composition, total phenolic content, flavonoid content, flavonol content, antioxidant capacity, and antibacterial efficacy of hydroethanolic extracts from the leaves (HLE), flowers (HFE), and rhizomes (HRE) of *P. crinita*. Specifically, the objectives were to: (1) identify and quantify phenolic compounds using high-performance liquid chromatography with diode array detection (HPLC-DAD); (2) determine total phenolic, flavonoid, and flavonol contents via spectrophotometric assays; (3) assess antioxidant activity using four methods: DPPH• free radical scavenging, ABTS•+ cationic radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and the Phenanthroline reduction method; and (4) evaluate antibacterial activity against clinical bacterial strains using the broth microdilution method.

2. MATERIAL and METHODS

2.1. Chemicals

High-quality, analytical-grade chemicals and solvents were used in this study, including: aluminum chloride, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid, α-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), chloroform, 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide, ethanol, ferric chloride, Folin-Ciocalteu reagent, gallic acid, hydrochloric acid (HCL), iodine, magnesium turnings, mercuric chloride, potassium bismuth iodide (Dragendorff's reagent), potassium acetate, potassium ferricyanide, potassium iodide, potassium persulfate, quercetin, sodium acetate, and trichloroacetic acid (Sigma-Aldrich, St. Louis, MO, USA). Sodium carbonate, ferric chloride, and phenanthroline were obtained from Biochem Chemopharma. Sodium hydroxide was obtained from PanReac AppliChem. Sulfuric acid was purchased from Honeywell International Inc. All chemicals and reagents were used without further purification.

2.2. Plant Material

After botanical identification of the species *P. crinita* by Professor Abdelkader Saadi (Faculty of Natural and Life Sciences, University of Chlef), fresh leaves, flowers, and rhizomes were collected during the flowering phase in June 2022 from a locality called Sidi Amer, located within Chlef province in the north-west of Algeria (latitude: 36°11'10.3"N, longitude:

1°18'18.2"E). The plant parts were removed from the stems, air-dried at room temperature for 15 days while avoiding exposure to direct sunlight, and then ground into a fine powder using a blender. The rhizomes were ground separately using a mortar and pestle. The resulting powders were kept in opaque containers at ambient temperature (25°C) until subsequent use.

2.3. Preparation of Extracts

The extraction was performed according to the method described by Merouane *et al*. (2020) with minor modifications. 10 g of fine powder from each sample (leaves, flowers, and rhizome) were macerated in 200 mL of ethanol (80%) at ambient temperature (25°C), while being shaken continuously for 24 hours using a WIS-10 shaking incubator (Daihan Scientific Co. Ltd., Korea). Subsequently, the mixtures were filtered through Whatman No. 1 filter paper, and the residues were rinsed twice with 10 mL aliquots of 80% ethanol. The resulting filtrates were then concentrated using a rotary evaporator (Büchi, Flawil, Switzerland) at temperatures below 48 °C under reduced pressure to remove ethanol. and then dried in an oven at 35 °C for 48 h to remove any remaining water. Each crude extract was then weighed, and the yield was calculated. Finally, the extracts were stored in a refrigerator at 4 °C until use for investigation.

The yield (%) of soluble constituents obtained through the hydroethanolic extraction was quantified using the equation below to assess extraction efficiency:

$$\% \text{ Yield} = (\text{Mass of the dried extract in mg} / \text{Initial mass of the sample in mg}) \times 100 \quad \text{(Eqn. 1)}$$

2.4. Qualitative Phytochemical Screening

Several standard tests were employed to highlight major phytoconstituents in the extracts, using methods described by Iqbal *et al*. (2015) and Saptarini *et al*. (2016).

2.4.1. *Tests for flavonoids*

A 1 g sample was extracted with 10 mL of 70% ethanol and filtered.

a. Shinoda test: a few turnings of magnesium and concentrated HCL (5 drops) were added to the ethanolic extract. The appearance of red to pink color after few minutes indicates the presence of flavonoids (Saptarini *et al*., 2016).

b. NaOH test: A few drops of 1N sodium hydroxide solution were added to the ethanolic extract. An intense yellow color which disappeared after adding dilute HCL indicates the presence of flavonoids (Saptarini *et al*., 2016).

2.4.2. *Test for terpenoids*

a. Salkowski test: approximately 100 mg of the crude extract was shaken with 2 mL of chloroform. Subsequently, 2 mL of concentrated sulfuric acid was added along the side of the test tube. The formation of a reddish-brown coloration at the interface indicates the presence of terpenoids (Iqbal *et al*., 2015).

2.4.3. *Test for tannins*

a. Ferric chloride test: A separate extraction was conducted for leaf, flower and rhizome samples, each weighing 0.5 g. These samples were individually mixed with 10 mL of distilled water and subjected to stirring, followed by filtration to obtain the aqueous extracts. Subsequently, a few drops of 5% ferric chloride solution were introduced to each filtrate. A black or blue-green coloration or precipitate was taken as a positive result for the presence of tannins (Iqbal *et al.*, 2015).

2.4.4. *Tests for alkaloids*

A few milligrams (about 15 mg) of each extract were separately stirred with 1% HCL (6 mL) in a water bath for 5 minutes and filtered. These filtrates were divided into three equal parts.

a. Dragendorff's test: A 1 mL aliquot of Dragendorff's reagent, a solution of potassium bismuth iodide, was introduced to a portion of the filtrate. The formation of an orange-red precipitate indicates the presence of alkaloids (Iqbal *et al*., 2015).

b. Mayer's test: To another aliquot of the filtrate, 1 mL of Mayer's reagent, composed of potassium mercuric iodide, was added. The subsequent formation of a cream-colored precipitate serves as an indicator for the presence of alkaloids (Iqbal *et al*., 2015).

c. Wagner's test: A solution was prepared by dissolving 2 g of potassium iodide and 1.27 g of iodine in 5 mL of distilled water and subsequently diluting this mixture to 100 mL with additional distilled water. Upon adding several drops of this reagent to the filtrate, the appearance of a brown precipitate confirms the presence of alkaloids (Iqbal *et al*., 2015).

2.5. Analyses of Phenolic Compounds (HPLC)

The method described by Fedoul *et al.* (2022) was used for HPLC analysis of phenolic compounds in *P. crinita* hydroethanolic extracts. An HP-Agilent 1290 Infinity HPLC system, equipped with a 250×4.6 mm C18 ultrasphere-ODS column and a diode array detector (DAD), was used with a mobile phase consisting of 3% acetic acid in water (A) and methanol (B). Samples (20 mg/mL) prepared in methanol were injected in 10 µL and detected at 278 nm. The elution gradient applied at a flow rate of 0.8 ml/min was: 93% A-7% B (0.1 min), 72% A-28% B (20 min), 75% A-25% B (8 min), 70% A-30% B (7 min) and the same gradient for 15 min was 67% A-33% B (10 min), 58% A-42% B (2 min), 50% A-50% B (8 min), 30% A-70% B (3 min), 20% A-80% B (2 min) and 100% B in 5 min until the end of the experimental cycle. Identification and quantification of phenolic compounds were achieved by comparison with standard phenolic compounds (Table 2). External calibration curves prepared for each standard were used to express individual phenolic component amounts in ppm (mg/kg extract).

2.6. Quantifying Phenolics, Flavonoids, and Flavonols

2.6.1. *Total phenolic content (TPC)*

TPCs of the extracts (HLE, HFE and HRE) were estimated with the method of Müller *et al*. (2010). Briefly, 20 µL of each extract (1 mg/mL) or gallic acid standard solution was mixed with 100 µL of Folin–Ciocalteu reagent. Then, 75 µL of sodium carbonate solution (7.5%) was added to the mixture. Following a 2-hour incubation in darkness, the reaction mixtures were assessed for absorbance at 765 nm with a microplate reader (Perkin Elmer, EnSpire, Singapore). TPC was then calculated against a gallic acid calibration curve (r^2 = 0.997) and expressed as µg gallic acid equivalents (GAE)/mg dry weight.

$$\text{TPC (µg GAE/mg dry weight)} = C_{GA} \times \text{Vol} / \text{Mass} \quad \text{(Eqn. 2)}$$

Where C_{GA}, Vol, and Mass are the concentration of gallic acid established from the calibration curve (µg/mL), the volume of the extract (mL) used in the assay, and the weight of the extract (mg) used in the assay, respectively.

2.6.2. *Total flavonoid content (TFC)*

TFCs of extracts (HLE, HFE and HRE) were quantified using a microplate assay adapted from the method described by Benouchenne *et al*. (2020). Briefly, 50 µL of each extract (1 mg/mL) or quercetin standard solution was combined with 130 µL of methanol, 10 µL potassium acetate solution (9.8%) and 10 µL of aluminium nitrate solution (10%), and mixed. After incubating for 40 minutes, the absorbance of the reaction mixtures was measured at 430 nm using a microplate reader (Perkin Elmer, EnSpire, Singapore). TFC was then calculated relative to a calibration curve constructed with quercetin as the standard (r^2 = 0.997) and expressed in µg quercetin equivalents (QE)/mg dry weight.

$$\text{TFC (µg QE/mg dry weight)} = Cq \times \text{Vol} / \text{Mass} \quad \text{(Eqn. 3)}$$

Where Cq, Vol, and Mass are the concentration of quercetin obtained from the calibration curve (µg/mL), the volume of the extract used in the assay (mL), and the weight of the extract (mg) used in the assay, respectively.

2.6.3. *Total flavonol content (TFoC)*

The aluminium trichloride ($AlCl_3$) colorimetric approach was slightly modified for microplate determination in order to assess the TFoCs (Bendjedid, *et al*., 2020). A mixture of 50 µL of each extract (HLE, HFE and HRE) (1 mg/mL), 50 µL of aluminium trichloride solution (2%), 150 µL of sodium acetate solution (5%) was added. After the mixture was left in the dark for 150 min, the absorbance at 430 nm was measured using a microplate reader (PerkinElmer, EnSpire, Singapore). Using quercetin as the standard, a calibration curve (r^2 = 0.998) was used to quantify the flavonol content, which was then expressed in µg quercetin/mg dry weight by comparing it to the quercetin standard curve.

$$\text{TFoC (µg QE/mg dry weight)} = Cq \times Vol / Mass \quad \text{(Eqn. 4)}$$

Where Cq, Vol, and Mass are the concentration of quercetin established from the calibration curve (µg /mL), the volume of the extract used in the assay (mL), and the weight of the extract (mg) used in the assay, respectively.

2.7. Antioxidant Potential

The antioxidant activity of extracts (HLE, HFE and HRE) was evaluated using DPPH, ABTS, ferric reducing antioxidant power (FRAP), and phenanthroline assays compared to BHA, BHT, α-tocopherol, and ascorbic acid. Absorbance was read using a microplate reader (PerkinElmer, EnSpire, Singapore).

2.7.1. *DPPH scavenging assay*

The ability of radical scavenging was determined according to (Fatima Zohra *et al*., 2023) method, using the radical DPPH. A volume of 40 µL of varying concentrations of the samples (3.125 –200 µg/mL in a final volume of 200 µL) were incubated with 160 µL of 0.1 mM methanolic DPPH solution for 30 minutes at room temperature in the dark. Subsequently, the absorbance of the reaction mixtures was measured at 517 nm. The scavenging activity was expressed as IC_{50} values, reflecting the concentration of the sample needed to scavenge 50% of the DPPH free radicals. These values were calculated using the linear regression method from the curve of percent inhibition versus concentration.

Percentages of inhibition of the stable radical $DPPH^{\bullet}$ were determined by the following formula:

$$I = \left[\frac{A\ control - (A\ sample - A\ blank)}{A\ control}\right] \quad \text{(Eqn. 5)}$$

Where I represents the percentage inhibition; A_{sample}, A_{blank} and $A_{control}$ are the absorbance (after 30 min) of the sample (extract or standard antioxidant), blank, and control, respectively.

2.7.2. *ABTS scavenging assay*

An assessment of the radical scavenging capacity of the extracts towards ABTS cations was performed using the methodology described by Nickavar and Esbati (2012) with some modifications. Briefly, a pre-generated $ABTS^{\bullet+}$solution was employed for the assay. 40 µL of varying sample dillutions (3.125 –200 µg/mL in a final volume of 200 µL) were reacted with 160 µL of the $ABTS^{\bullet+}$solution within a 96-well microplate. The absorbance was read at 734 nm after 10 min of incubation. The scavenging activity of ABTS was expressed as the percentage of inhibition calculated using the preceding formula **(**Eqn. 5**).** Antioxidant activity was quantified as IC_{50} values.

2.7.3. *Ferric reducing antioxidant power assay (FRAP)*

The ability of the extracts to reduce ferric iron (Fe^{3+}) ions was determined using a microplate reader adaptation of the method described by Benouchenne *et al*. (2020). A volume of 10 µL of each sample at different concentrations (3.125 –200 µg/mL in a final volume of 200 µL) was mixed with 40 µL of phosphate buffer (0.2M, pH 6.6) and 50 µL of 1% potassium ferricyanide solution. The mixture was then incubated in an oven at 50°C for 20 min. After incubation, 50 µL of 10% tri-chloroacetic acid, 40 µL of distilled water, and 10 µL of 0.1% ferric chloride solution were added, and the absorbance was directly read at 700 nm. The results were calculated as $A_{0.5}$ values, defined as the concentration required for 50% reduction in absorbance, and determined from the absorbance curve plotted with different concentrations.

2.7.4. *Phenanthroline assay*

Additionally, the reducing ability of the extracts, as measured by Fe^{+2}-phenanthroline complex formation, was determined as described by Fatima Zohra *et al.* (2023). A volume of 10 µL of each sample at final concentration (3.125 –200 µg/mL in a final volume of 200 µL) were combined in a microplate well with 30 µL of 0.5% (in methanol) phenanthroline solution, 110 µL of methanol, and 50 µL of 0.2% $FeCl_3$ solution. These reaction mixtures were incubated at 30°C for 20 minutes and subsequently analysed for absorbance at 510 nm. We calculated $A_{0.5}$ values from the regression curves.

2.8. Antibacterial Activity – Broth Microdilution Method

Eight bacterial strains isolated from hospitalized patients at the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal) were investigated in this study. The strains included five Gram-negative bacteria: *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Morganella morganii*. Additionally, three Gram-positive strains were assessed: *Enterococcus faecalis*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (MRSA).

This study utilized the methodology described by Pires *et al*. (2018). The extratcs (HLE, HFE and HRE) were first of all dissolved in 5% (v/v) dimethyl sulfoxide (DMSO) and 95% of autoclaved distilled water to give a final concentration of 20 mg/mL for the stock solution. Then the samples were serially diluted to obtain the concentration ranges (10 to 0.03125 mg/mL). Microdilution method and the rapid piodonitrotetrazolium chloride (INT) colorimetric assay were used to determine minimum inhibitory concentration (MIC). For the determination of MBC (lowest concentration required to kill bacteria), 10 µL aliquots from wells exhibiting no colour change were plated on blood Agar (7% sheep blood) and incubated at 37°C for 24 hours. The lowest concentration with no growth on the agar plates determined the MBC. As positive controls, ampicillin, imipenem, and vancomycin were used.

2.9. Statistical Analysis

Statistical analyses were performed with the SPSS Statistics 27.0.1 programme. The data are presented as the mean values and standard deviations (SD) of the triplicate results. Data were analysed using one-way ANOVA with a significance level of $\alpha = 0.05$. Tukey's post-hoc test at a 95% confidence level was employed to identify specific groups responsible for significant differences.

3. RESULTS and DISCUSSION

3.1. Qualitative Phytochemical Screening

Initial phytochemical screening of the three hydroethanolic extracts revealed the presence of tannins, flavonoids, and terpenoids, while alkaloids were not detected (Table 1). These detected substances are well-known for their many biological actions, which include antibacterial, anti-inflammatory, and antioxidant characteristics. Similarly, no alkaloids were found in the phytochemical screening of *P. crinita* extracts by Amor et al. (2009b) even with diverse

solvents (methanol, ethyl acetate, lyophilized infusion) and a concentrated flavonoid fraction (total oligomer flavonoids).

Table 1. Qualitative screening of hydroethanolic extracts from organs of *Phlomis crinita*.

Phytoconstituents	Test	Plant's part		
		Leaves	Flowers	Rhizome
Flavonoids	Shinoda	+	+	+
	NaOH	+	+	+
Tannins	Ferric chloride	+	+	+
Terpenoids	Salkowski	+	+	+
Alkaloids	Mayer	-	-	-
	Wagner	-	-	-
	Dragendorff	-	-	-

Note: (+): Present; (-): Absent.

3.2. Analyses of Phenolic Compounds (HPLC)

Analysis of phenolic compounds in the hydroethanolic extracts of leaves, flowers, and rhizomes of the species *P. crinita* was conducted by HPLC-DAD and the identification of specific phenolic compounds relied on comparing their retention times and UV spectra to those of authentic standards (Table 2) analyzed under the same conditions.

In this study, a total of 15 distinct phenolic compounds were detected in *P. crinita* for the first time, including four flavonoids (quercetin, epicatechin, hesperidin, catechin hydrate), t-cinnamic acid, six cinnamic acid derivatives (chlorogenic acid, sinapic acid, rosmarinic acid, t-ferulic acid, caffeic acid, p-coumaric acid), and four benzoic acid derivatives (gallic acid, syringic acid, 4-hydroxy benzoic acid, 3-hydroxy benzoic acid). Notably, quercetin was found in moderate amounts in leaf and flower extracts, with concentrations of 284.98 and 208.63 mg/kg, respectively. Chlorogenic acid was found in moderate concentrations in leaf, flower, and rhizome extracts, with concentrations of 107.35, 77.665 and 42.609 mg/kg, respectively. Furthermore, certain phenolic compounds were detected in higher levels in the rhizome extract than in leaves and flowers, including sinapic acid (165.977 mg/kg), t-ferulic acid (124.13 mg/kg), and rosmarinic acid (88.259 mg/kg). All other compounds showed concentrations below 30 mg/kg. Additionally, sinapic acid, t-ferulic acid, and catechin hydrate were absent in leaves but present in flowers and rhizomes, highlighting the variability in phenolic compound distribution among different plant parts. At the tissue level, the localization of phenolic compounds is linked to their role in the plant and can be very characteristic. For instance, flavonoids are predominantly present in the epidermis of leaves (Hunt *et al*., 2021). Phenolic content and composition can vary significantly among organs and plant species. In most instances, their accumulation remains below 1% of the dry weight (Zagoskina *et al*., 2023).

Our results have revealed that the extracts contain several compounds, including derivatives of cinnamic acid and benzoic acid, which have been associated with antioxidant, anti-inflammatory, and antimicrobial effects (Garde-Cerdan *et al*., 2017). These compounds are widely used in industries such as the pharmaceutical and food industry. For example, sinapic acid and t-ferulic acid detected in the rhizome extract are considered important dietary phenolic acids and are among the most consumed by humans (Rahman *et al*., 2022). The presence of chlorogenic acid, previously reported to enhance cellular proliferation and epithelialization, key processes associated with wound healing (Sobha *et al*., 2023), aligns with the traditional medicinal use of *P. crinita* as a wound healer plant.

Additionally, other detected compounds, such as quercetin, have numerous beneficial effects on human health. This antioxidant flavonoid is believed to offer many health benefits, providing defence against ailments including lung cancer, osteoporosis, and cardiovascular problems

(Anand David *et al*., 2016). The health benefits of phenolic compounds are determined by their structure, solubility, conjugation with other phenolic compounds or other substances, and absorption, which in turn affects their metabolism (Zeb, 2020).

Table 2. HPLC-DAD profiling of phenolic compounds in hydroethanolic extracts from organs of *Phlomis crinita.*

N°	Phenolic compound	Correlation(r^2)	RT(min)	HLE	HFE	HRE
				Concentration (mg/kg DE)		
1	Quercetin #	0.99962	76.313	284.98	208.63	6.546
2	Chlorogenic Acid **	0.99970	16.239	107.35	77.665	42.609
3	Epicatechin #	0.99879	20.169	28.110	28.631	16.486
4	Rosmarinic Acid **	0.99907	70.655	20.658	7.533	88.259
5	Hesperidin #	0.99705	65.989	6.132	3.726	5.998
6	Sinapic Acid **	0.99925	37.264	N.D	13.272	165.97
7	t-Ferrulic Acid **	0.99993	37.202	N.D	9.773	124.13
8	Caffeic Acid **	0.99892	21.476	3.687	3.536	2.893
9	3-Hydroxy-Benzoic Acid *	0.99982	22.545	3.561	6.214	3.574
10	Syringic Acid *	0. 99839	22.628	1.762	2.716	1.427
11	Gallic Acid *	0. 99966	5.912	0.204	2.157	N.D
12	Catechin Hydrate #	0.99906	11.499	N.D	2.036	6.269
13	p-Coumaric Acid **	0.99982	33.597	1.577	N.D	N.D
14	4-Hydroxy-Benzoic Acid*	0.99994	17.647	1.456	1.52	0.579
15	t-Cinnamic Acid	0.99998	75.207	1.486	1.203	9.677
16	Benzoic Acid	0.99986	47.629	N.D	N.D	N.D

Note: HLE: Hydroethanolic leaf extract; HFE: Hydroethanolic flower extract; HRE: Hydroethanolic rhizome extract; #: Flavonoids; *Benzoic acid derivatives; **Cinnamic acid derivatives; Concentration: mg/kg= ppm; DE: of Dry extract; RT: Retention time of standard; N.D: Not determined.

Furthermore, previous research supports our findings. According to Merouane *et al*. (2020), phenolic acids (such as chlorogenic, rosmarinic, and benzoic acids) and flavonoids are primarily phenolic compounds in *Phlomis* species. In a recent study by Baali *et al*. (2024), a wide range of phenolic compounds were identified in *P. crinita*. These included rosmarinic, chlorogenic, and coumaric acids, along with their derivatives, as well as quercetin derivatives. Moreover, Zaabat *et al.* (2020) isolated chlorogenic acid from the aerial parts of *Phlomis bovei De Noé*. Our study is in accordance with the literature data.

3.3. Extraction Yield

As shown in Table 3, the hydroethanolic extracts obtained from the leaves, flowers, and rhizomes of *P. crinita* yielded 15.9%, 22.8%, and 5.5%, respectively. The extraction yield from medicinal plants can vary due to several internal parameters, including genotype, organ type, and age, as well as external factors such as climate, harvest timing, and storage duration. Additionally, extraction parameters such as temperature, duration, solvent choice, and solvent/feed ratio (S/F) play a crucial role to the final yield (Rostagno & Prado, 2013). For ethanol extracts, Roby *et al*. (2013) reported yields of 14% for thyme, 18% for sage, and 15% for marjoram.

Phenolic compounds have hydroxyl groups which make them more soluble in polar organic solvents (Aryal *et al*., 2019). Therefore, we selected ethanol as the solvent. However, while methanol with its higher polarity could potentially have offered greater yields, balancing the benefits, we opted for 80% ethanol as the greener alternative. According to previous literature, Merouane *et al*. (2020) found that the yield of the hydromethanolic extracts of *P. crinita* ranged from 21.66 to 26.56% (w/w) for the leaves and from 22.26 to 30.08% (w/w) for the floral parts.

Table 3. Yield of extraction and bioactive content of hydroethanolic extracts from organs of *Phlomis crinita.*

	Yield (% w/w)	Total Phenolics (µg GAE/mg DW)	Flavonoids (µg QE/mg DW)	Flavonols (µg QE/mg DW)
Leaves	15.9	168.80 ± 5.46^{a}	65.97 ± 6,38^{a}	18.89 ± 5.12^{a}
Flowers	22.8	81.64 ± 13.29^{b}	29.58 ± 1.98^{b}	6.92 ± 1.42^{b}
Rhizome	5.5	262.62 ± 16.2^{c}	71.87 ± 3.25^{a}	14. 76 ± 2.11^{ab}

Note: w/w: mg extract mass per mg sample mass; DW: dry weight of the extract; µg GAE/mg DW: expressed as µg gallic acid equivalents per mg of DW; µg QE/mg DW: expressed as µg quercetin equivalents (QE) per mg of DW; values are means ± standard deviation (n=3); a–c: indicates that values with different letters in the same column are significantly different (p<0.05).

3.4. Quantification of Total Phenolics, Flavonoids, and Flavonols

3.4.1. *Total phenolic content (TPC)*

Extracts from different plant parts exhibited significant differences (p<0.05) in TPC. The rhizome exhibited the highest levels (262.62 ± 16.2 µg GAE/mg DW), followed by the leaves with moderate levels (168.80 ± 5.46 µg GAE/mg DW). The flowers, however, showed the lowest levels (81.64 ± 13.29 µg GAE/mg DW) (Table 3).

Phenolic compounds, which include flavonoids, phenolic acids, stilbenes, coumarins, and tannins, are the most abundant and frequently found phytochemicals in the plant kingdom (Wuttisin *et al*., 2021). Several studies have examined the TPC of *P. crinita*. For instance, Merouane *et al*. (2020) reported higher TPC in hydromethanolic extracts of *P. crinita* leaves compared to flowers for three different populations which is consistent with our findings. They found a TPC of 117.96 ± 1.70 µg GAE/mg DW in hydromethanolic extract of leaves. However, there has been no previous investigation of the bioactive compounds in the rhizome. Our findings indicated that the rhizome of *P. crinita* had highest TPC levels (262.62 ± 16.2 µg GAE/mg DW), which makes it a promising source of natural compounds. In fact, Aryal *et al*. (2019) reported a comparable value of 292.65 ± 0.42 µg GAE/mg DW, putting our findings firmly within the expected range. The observed TPC differences with other reports could be because of varying concentrations of sugars, carotenoids, or ascorbic acid; it could also be because of extraction duration, geography, or extraction techniques, all of which can affect the amount of phenolics extracted (Aryal *et al*., 2019).

3.4.2. *Total flavonoid content (TFC)*

The TFCs were evaluated using the aluminium chloride spectrophotometric technique. This method relies on the unique interaction between aluminium and flavonoids (Wuttisin *et al*., 2021). Significant variations at p<0.05 in the levels of flavonoid content were observed among different parts of the plant (Table 3). The rhizome contained the highest level (71.87 ± 3.25 µg QE/mg DW) being the richest source of flavonoids, followed by the leaves (65.97 ± 6.38 µg QE/mg DW), and the flowers (29.58 ± 1.98 µg QE/mg DW).

Our findings indicated that TFCs in leaves and flowers of *P. crinita* were higher than those reported by Merouane *et al*. (2020). They measured a TFC of 42.72 ± 0.53 mg QE/g DW and 15.85 ± 0.40 mg QE/g DW in methanol extracts of leaves and flowers, respectively, from a *P. crinita* population in Medjadja. In contrast, Baali *et al*. (2024) reported a higher TFC of 82.28 ± 0.44 mg QE/g DW in the methanolic extract of flowering tops of *P. crinita*. Given their wide-ranging applications in nutraceuticals, pharmaceuticals, medicine, and cosmetics, flavonoids have deservedly attracted considerable attention. By virtue of its anti-inflammatory, anti-mutagenic, anti-carcinogenic, and free radical scavenging activities, as well as its capacity to regulate the activity of important cellular enzymes (Panche *et al*., 2016).

Flavonoid profiles, as determined by HPLC-DAD analysis, which identifies and quantifies specific flavonoid compounds, indicated a higher concentration in the leaf extract. However, TFC measurements suggested greater abundance in the rhizome extract. This discrepancy may

be due to the diverse nature of flavonoids, which include subgroups like flavonols, flavones, and isoflavones (Eljabboury *et al*., 2023). Rhizomes might contain a higher proportion of flavonoid subgroups not detected by HPLC-DAD.

3.4.3. *Total flavonol content (TFoC)*

The leaves showed significantly higher levels of TFoC (18.89 ± 5.12 µg QE/mg DW) compared to the flowers (6.92 ± 1.42 µg QE/mg DW) at a significance level of $p<0.05$. The rhizome showed high levels (14. 76 ± 2.11 µg QE/mg DW) (Table 3). Interestingly, this result aligns with the fact that flavonols are found in lower concentrations than other phenolics (El Gharras, 2009).

The concentration in HFE aligns with a prior study by Merouane *et al*. (2019), who reported a similar flavonol content of 6.22 ± 0.05 µg RE/mg in a hydromethanolic extract of *P. crinita* flowers. Interestingly, the concentration of flavonols in HLE is almost six-fold higher than that reported for hydromethanolic leaf extracts (3.39 ± 0.06 µg RE/mg DW) in the same study. It's worth noting that the consumption of flavonols is linked to several health advantages, such as antioxidant properties and decreased chances of developing vascular disease (Panche *et al*., 2016). Quercetin, kaempferol, myricetin, and fisetin are among the most studied flavonols (Panche *et al*., 2016).

3.5. Antioxidant Potential

Four distinct *in vitro* assays were employed to evaluate the antioxidant effects of leaf, flower, and rhizome hydroethanolic extracts of *P. crinita*. This included assessing their ability to scavenge free radicals like $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, potentially via electron or hydrogen donation. Additionally, Phenanthroline and FRAP assays were conducted to explore the samples' ability to reduce iron ions through electron transfer mechanisms (Szydłowska-Czerniak *et al*., 2008). The antioxidant potential of extracts was compared to that of BHA, BHT, α-tocopherol and ascorbic acid. The sample which has a lower IC_{50} or $A_{0.5}$ presented a high antioxidant activity. A summary of the results can be found in Figure 1.

DPPH assay measures the ability of antioxidants to donate an electron to a stable free radical, reflecting their free radical scavenging activity. Based on the findings reported in Figure 1.A, HLE had significant antioxidant potential, more promising than BHT with IC_{50} values of 15.46 ± 0.45 µg/mL and 22.32 ± 1.19 µg/mL, respectively. Other plant parts (HRE and HFE) showed interesting antioxidant capabilities. The observed antioxidant activity supports the established understanding of phenolic compounds as prominent antioxidant agents. Regardless of whether HLE had lower levels of TPC as compared to HRE (Table 3), it exerted superior antioxidant activity, which may be attributed the diverse range of compounds present (not limited to phenolics) that can function according to a particular mode of action (Bakhouche *et al*., 2021). In comparison to a study conducted by Merouane *et al*. (2020), which reported variation in antioxidant activity between flowers and leaves, as well as among populations of *P. crinita*, HLE demonstrates approximately 4.78-fold greater DPPH scavenging activity than the leaf hydromethanolic extract from the Ouled Benabdelkader population of *P. crinita*. Furthermore, HFE exhibits three times higher DPPH scavenging activity compared to the flower hydromethanolic extract from the Medjadja population of *P. crinita.*

ABTS assay measures antioxidant capacity by monitoring the ability of antioxidants to quench the free radical $ABTS^{+}$ via a proposed mixed mechanism of hydrogen atom transfer (HAT) and single electron transfer (SET) reactions, leading to a characteristic color change from dark green to pale green (Ilyasov *et al*., 2020). Figure 1.B. reveals the strong antioxidant potential of HLE, evidenced by its IC_{50} value (11.71 ± 0.50 µg/mL) remarkably similar to that of BHA (7.54 ± 0.69 µg/mL). Furthermore, HFE and HRE exhibited moderate antioxidant activity. This test confirms the first test that HLE has good antioxidant activity.

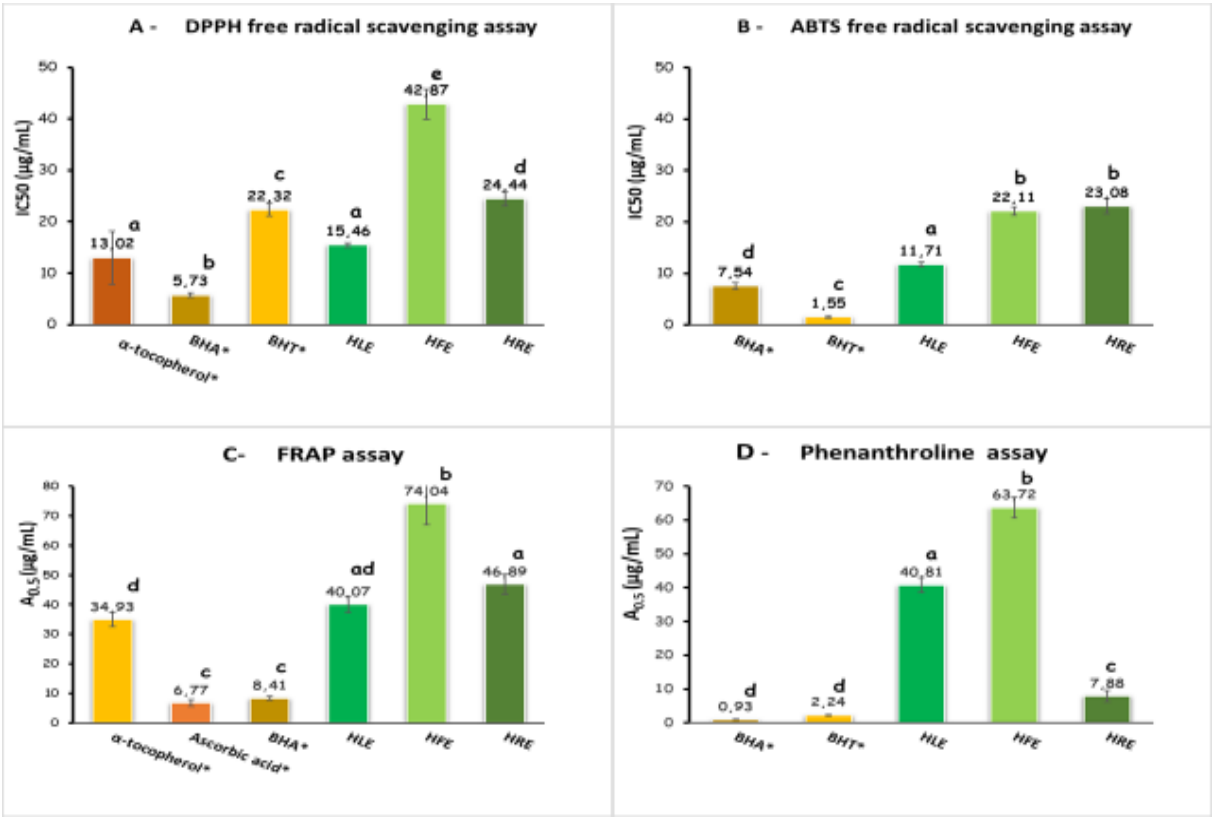

Figure 1. Antioxidant potential of hydroethanolic extracts from organs of *Phlomis crinita* using *in vitro* tests: **A:** DPPH free radical scavenging, **B**: ABTS free radical scavenging, **C**: FRAP assay, **D**: Phenanthroline assay. HLE: Hydroethanolic leaves extract; HFE: Hydroethanolic flowers extract; HRE: Hydroethanolic rhizome extract; *: Reference compound; BHT: butylatedhydroxytoluene; BHA: butylated hydroxyanisole; Values (means of three parallel measurements ± SD) followed by different lower-case letters (a-c) in the same bar graph for each test are significantly different at $p<0.05$.

The results of the FRAP assay, presented in Figure 1.C, confirm the findings of the previous tests. HLE (40.07 ± 2.82 µg/mL) and HRE (46.89 ± 3.48 µg/mL) exhibits higher reducing power $A_{0.5}$ than that of BHT (>50 µg/mL) and near to α-tocopherol (34.93 ± 2.38 µg/mL). These results may be due to the presence of electron-donating substances, since the reducing-power method follows the mode of action of electron transfer.

Phenanthroline assay measures antioxidant capacity via ferric iron (Fe^{+3}) reduction to ferrous iron (Fe^{+2}), and formation of the orange-red Fe^{2+}-phenanthroline complex (Fatima Zohra *et al*., 2023). In this assay, all extracts possess the ability to reduce iron ions, but HRE (6.95 ± 1.90 µg/mL) exhibited the highest activity, which was near to BHA (2.24 ± 0.17 µg/mL). On the other hand, HLE and HFE showed lower activity (Figure 1.D).

Overall, all extracts exhibited significant scavenging activity against DPPH and ABTS free radicals, and remarkable reducing power in the FRAP assay. Interestingly, HRE displayed the highest iron-reducing capacity in the Fe^{+2}-phenanthroline assay. These findings highlight the importance of utilizing multiple complementary assays when evaluating complex mixtures like crude extracts. This is because individual compounds within the mixture can function according to specific modes of action, and there can be potential synergistic interactions (Bakhouche *et al*., 2021). Flavonoids and phenolic acids identified in our HPLC-DAD analysis are among the compounds responsible for the antioxidant potential.

The abundance of antioxidant effects in any bioactive compound is a sign of its potential utility as a medicine or food supplement. Therefore, based on our findings, it's evident that different extracts of *P. crinita* hold great promise as sources of natural antioxidants, offering a safer alternative to potentially harmful and carcinogenic synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) (Akbaba, 2021).

3.3. Antibacterial Activity

In this study, we assessed the antibacterial activity of HLE, HFE, and HRE against clinical isolates using MIC and MBC. The values are shown in Table 4.

MIC values revealed that, all eight bacterial strains were inhibited by at least one extract type, except *Pseudomonas aeruginosa* which remained resistant at the highest tested concentration (10 mg/mL). These MIC studies revealed promising activity, particularly against Gram-positive bacteria (*E. faecalis*, *L. monocytogenes*, MRSA). Interestingly, HRE with its higher TPC compared to HLE (Table 3), exhibited strongest activity against two Gram-positive bacteria. Also, HRE exhibited the lowest MIC value (1.25 mg/mL) against *E. faecalis*, indicating the greatest susceptibility of *E. faecalis* to this extract. Even Gram-negative bacteria responded well to HRE, particularly against *M.* morganii (2.5 mg/mL) and *P. mirabilis* (5 mg/mL) compared to other extracts (10 mg/mL). This finding aligns with higher TPC of HRE suggesting a potential link between total phenolics and antibacterial activity. However, this correlation wasn't observed for *E. coli* and *L. monocytogenes* implying that other compounds besides phenolics also contribute.

Table 4. Antimicrobial potential of hydroethanolic extracts from organs of *Phlomis crinita* against clinical bacterial strains

							Positive Control					
	HLE (mg/mL)		HFE (mg/mL)		HRE (mg/mL)		Ampicillin (10mg/mL)		Imipenem (1mg/mL)		Vancomycin (1mg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria												
Escherichia coli	5	>10	>10	>10	>10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Klebsiella pneumoniae	5	>10	2.5	>10	5	>10	10	>10	<0.0078	<0.0078	n.t.	n.t.
Morganella morganii	10	>10	10	>10	2.5	>10	>10	>10	<0.0078	<0.0078	n.t.	n.t.
Proteus mirabilis	10	>10	10	>10	5	>10	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	0.5	1	n.t.	n.t.
Gram-positive bacteria												
Enterococcus faecalis	2.5	>10	5	>10	1.25	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Listeria monocytogenes	5	>10	10	>10	>10	>10	<0.15	<0.15	n.t.	n.t.	0.25	0.5
MRSA	2.5	>10	5	>10	2.5	>10	<0.15	<0.15	n.t.	n.t.	0.25	0.5

Note: MIC: minimal inhibitory concentration (mg/mL), MBC: minimal bactericidal concentration (mg/mL), n.t: not tested, MRSA: Methicillin resistant *Staphylococcus aureus*.

All MBC values were higher than the highest tested concentration (10 mg/mL). While our MIC results suggest the extracts can inhibit bacterial growth at the tested concentrations, their ability to kill all bacteria remains inconclusive.

The increasing resistance of bacteria is a growing problem. This includes bacteria like *Enterococcus*, which is a leading cause of bloodstream and urinary tract infections (Chakraborty *et al*., 2015). Standard antibiotics can be very effective, but the emergence of resistant strains necessitates exploring alternative treatment options. Despite showing a higher MIC value (1.25 mg/mL) compared to standard antibiotics, our study found HRE to be effective against *Enterococcus faecalis.*

4. CONCLUSION

This study investigated the phenolic profile, bioactive content, and biological activities of *P. crinita*, a plant widely used in Algerian traditional folk remedies. The analyses revealed a

mixture of beneficial flavonoids and phenolic acids in the leaves, flowers, and rhizomes of this species, with notable quantitative variability and lower qualitative variability. The bioactive screening showed an abundance of phenolics, flavonoids, and flavonols in various parts of this medicinal herb. On the other hand, these parts exhibited strong antioxidant properties, exceeding in some cases those of synthetic antioxidants, highlighting this plant as a promising and superior source of natural antioxidants. The antibacterial properties of this species were appreciable against a panel of clinically relevant bacteria. This combination of antioxidant and antibacterial properties suggests a promising future for *P. crinita* extracts as therapeutic agents, with potential applications in the food, pharmaceutical, and cosmetic industries. This investigation significantly contributes to the existing knowledge on the secondary metabolites of *P. crinita* and the *Phlomis* genus.

Acknowledgments

We are grateful to the Directorate General for Scientific Research and Technological Development (DGRSDT) of Algeria for its financial support.

Abdelhakim Chelgham and Abdelkader Saadi are deeply indebted to Dr. Abdallah Noui for his instrumental role in cultivating fruitful cooperation with international research groups. They also thank Dr. Mohamed Kouidri for his intellectual support.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Abdelhakim Chelgham: Design, Data Collection and Processing, Data interpretation, Literature review, Writing the original draft. **Abdelkader Saadi**: Supervision, Conception, Design, Critical Review. **Abdelaziz Merouane**: Conception, Design. **Chawki Bensouici:** Design, Data Processing. **Yavuz Selim Cakmak:** Design, Data Processing. **Tânia Pires:** Design and Data Collection. All authors read and approved the manuscript.

Orcid

Abdelhakim Chelgham https://orcid.org/0000-0003-3835-304X
Abdelkader Saadi https://orcid.org/0000-0001-5458-9197
Abdelaziz Merouane https://orcid.org/0000-0003-0900-4625
Chawki Bensouici https://orcid.org/0000-0003-4612-4642
Yavus Selim Cakmak https://orcid.org/0000-0001-8954-5485
Tânia C.S.P. Pires https://orcid.org/0000-0002-3954-3833

REFERENCES

Akbaba, E. (2021). Characterization of bioactive and antioxidant composition of mountain tea (*Sideritis montana* ssp. *montana*): Microwave-assisted technology. *International Journal of Secondary Metabolite, 8*(2), 159-171. https://dx.doi.org/10.21448/ijsm.926926

Amor, I.L.-B., Boubaker, J., Sgaier, M.B., Skandrani, I., Bhouri, W., Neffati, A., … Chekir-Ghedira, L. (2009a). Phytochemistry and biological activities of *Phlomis* species. *Journal of Ethnopharmacology*, *125*(2), 183-202. https://doi.org/10.1016/j.jep.2009.06.022

Amor, I.L.-B., Skandrani, I., Boubaker, J., Sghaïer, M.B., Neffati, A., Bhouri, W., … Chekir-Ghedira, L. (2009b). Investigation of biological activity of polar extracts isolated from *Phlomis crinita* Cav ssp. Mauritanica Munby. *Drug and Chemical Toxicology*, *32*(1), 38-46. https://doi:10.1080/01480540802416265

Anand David, A.V., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy Reviews*, *10*(20), 84–89. https://doi.org/10.4103/0973-7847.194044

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, *8*(4), 96. https://doi.org/10.3390/plants8040096

Baali, F., Boudjelal, A., Smeriglio, A., Righi, N., Djemouai, N., Deghima, A., Bouafia, Z., & Trombetta, D. (2024). *Phlomis crinita* Cav. From Algeria: A source of bioactive compounds possessing antioxidant and wound healing activities. *Journal of Ethnopharmacology*, *331*, 118295. https://doi.org/10.1016/j.jep.2024.118295

Bakhouche, I., Aliat, T., Boubellouta, T., Gali, L., Şen, A., & Bellik, Y. (2021). Phenolic contents and in vitro antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte Limonium delicatulum. South African Journal of Botany, *139*, 42–49. https://doi.org/10.1016/j.sajb.2021.01.030

Bendjedid, S., Djelloul, R., Tadjine, A., Bensouici, C., & Boukhari, A. (2020). In vitro assessment of total bioactive contents, antioxidant, anti-alzheimer and antidiabetic activities of leaves extracts and fractions of *Aloe vera*. *Chiang Mai University Journal of Natural Sciences*, *19*, 469-485. https://doi.org/10.12982/CMUJNS.2020.0031

Benouchenne, D., Bellil, I., Akkal, S., Bensouici, C., & Khelifi, D. (2020). LC–MS/MS analysis, antioxidant and antibacterial activities of Algerian fir (Abies numidica de LANNOY ex CARRIÈRE) ethylacetate fraction extracted from needles. *Journal of King Saud University - Science*, *32*(8), 3321–3327. https://doi.org/10.1016/j.jksus.2020.09.017

Boutennoun, H., Boussouf, L., Balli, N., Makhlouf, L., Madani, K., Desdous, N., … Al-qaoud, K. (2023). In vitro Antioxidant and Anti-Inflammatory Effects of the Hydro-Methanolic Extract of *Phlomis crinita* from North Algeria. *Jordan Journal of Chemistry*, *18*(2). https://jjc.yu.edu.jo/index.php/jjc/article/view/645

Çalış, İ., & Başer, K.H.C. (2021). Review of studies on *Phlomis* and *Eremostachys* species (Lamiaceae) with emphasis on iridoids, phenylethanoid glycosides, and essential oils. *Planta Medica*, *87*(14), 1128–1151. https://doi.org/10.1055/a-1527-4238

Chakraborty, A., Pal, N.K., Sarkar, S., & Gupta, M.S. (2015). Antibiotic resistance pattern of *Enterococci* isolates from nosocomial infections in a tertiary care hospital in Eastern India. *Journal of Natural Science, Biology, and Medicine*, *6*(2), 394-397. https://doi.org/10.4103/0976-9668.160018

El Gharras, H. (2009). Polyphenols: Food sources, properties and applications – a review. *Int. International Journal of Food Science & Technology*, *44*(12), 2512-2518. https://doi.org/10.1111/j.1365-2621.2009.02077.x

Eljabboury, Z., Bentaib, R., Dajic Stevanovic, Z., Ousaaid, D., Benjelloun, M., & Ghadraoui, L. (2023). *Ammi visnaga* (L.) Lam.: An overview of phytochemistry and biological functionalities. *Trends in Phytochemical Research*, *7*(3),141-155. https://doi.org/10.30495/tpr.2023.1987739.1347

Fatima Zohra, H., Bendif, H., Chawki, B., Alsalamah, S., Zaidi, B., Bouhenna, M., … Boufahja, F. (2023). Phytochemicals, antioxidant and antimicrobial potentials and LC-MS analysis of *Centaurea parviflora* Desf. extracts. *Molecules*, *28*(5), 2263. https://doi.org/10.3390/molecules28052263

Fedoul, F., Meddah, B., Larouci, M., Tir Touil, A., Merazi, Y., Bekhti, N., … Selim, Y. (2022). Medicinal applications, chemical compositions, and biological effects of Algerian *Ocimum basilicum* L.var genovese with the conversion of experimental doses to humans. *Journal of Applied Biotechnology Reports*, *9*(2), 671-683. https://doi.org/10.30491/jabr.2021.290237.1401

Garde-Cerdan, T., Gonzalo-Diago, A., & Pérez-Álvarez, E. (2017*). Phenolic compounds: Types, effects and research*. Nova Science Publishers.

Hunt, L., Klem, K., Lhotáková, Z., Vosolsobě, S., Oravec, M., Urban, O., Špunda, V., & Albrechtová, J. (2021). Light and CO2 Modulate the Accumulation and Localization of Phenolic Compounds in Barley Leaves. *Antioxidants*, *10*(3). https://doi.org/10.3390/antiox10030385

Ilyasov, I.R., Beloborodov, V.L., Selivanova, I.A., & Terekhov, R.P. (2020). ABTS/PP decolorization assay of antioxidant capacity reaction pathways. *International Journal of Molecular Sciences*, *21*(3), 1131. https://doi.org/10.3390/ijms21031131

Iqbal, E., Kamariah, A., & Lim, L. (2015). Phytochemical screening, Total phenolics and Antioxidant Activities of Bark and Leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University - Science*, *27*(3), 224-232. https://doi.org/10.1016/j.jksus.2015.02.003

Li, M.-X., Shang, X.-F., Jia, Z.-P., & Zhang, R.-X. (2010). Phytochemical and biological studies of plants from the Genus *Phlomis*. *Chemistry & Biodiversity*, *7*(2), 283–301. https://doi.org/10.1002/cbdv.200800136

Limem, I., Harizi, H., Ghedira, K., & Chekir-Ghedira, L. (2011). Leaf extracts from *Phlomis crinita* Cav. Subs. mauritanica Munby affect immune cell functions in vitro. *Immunopharmacology and Immunotoxicology*, *33*(2), 309-314. https://doi.org/10.3109/08923973.2010.504926

Marrelli, M. (2021). Medicinal Plants. *Plants*, *10*(7), 1355. https://doi.org/10.3390/plants10071355

Merouane, A., Fellag, S., & Noui, A. (2020). Variation of phenolic content and antioxidant activity in organs and populations of *Phlomis crinita* L. *Revista Cubana de Plantas Medicinales*, *25*(4), e1123. https://www.medigraphic.com/cgibin/new/resumenI.cgi?IDARTICULO=102686

Merouane, A., Saadi, A., Noui, A., & Bader, A. (2019). Evaluation of phenolic contents and antioxidant properties of the leaves and flowers of *Phlomis biloba* Desf. *International Food Research Journal*, 26, 167-173. https://www.proquest.com/docview/2224303131?pq-origsite=gscholar&fromopenview=true&sourcetype=Scholarly%20Journals

Müller, L., Gnoyke, S., Popken, A.M., & Böhm, V. (2010). Antioxidant capacity and related parameters of different fruit formulations. *LWT - Food Science and Technology*, *43*(6), 992–999. https://doi.org/10.1016/j.lwt.2010.02.004

Nickavar, B., & Esbati, N. (2012). Evaluation of the antioxidant capacity and phenolic content of three *Thymus* Species. *Journal of Acupuncture and Meridian Studies*, *5*(3), 119–125. https://doi.org/10.1016/j.jams.2012.03.003

Panche, A.N., Diwan, A.D., & Chandra, S.R. (2016). *Flavonoids: An overview*. *Journal of Nutritional Science*, *5*, e47. https://doi.org/10.1017/jns.2016.41

Pires, T.C.S.P., Dias, M.I., Barros, L., Alves, M.J., Oliveira, M.B.P.P., Santos-Buelga, C., & Ferreira, I.C.F.R. (2018). Antioxidant and antimicrobial properties of dried Portuguese apple variety (*Malus domestica Borkh.* Cv Bravo de Esmolfe). *Food Chemistry*, *240*, 701–706. https://doi.org/10.1016/j.foodchem.2017.08.010

Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., … Uddin, M.S. (2022). Role of phenolic compounds in human disease: Current knowledge and future prospects. *Molecules*, *27*(1), 233. https://doi.org/10.3390/molecules27010233

Roby, M.H.H., Sarhan, M.A., Selim, K.A.-H., & Khalel, K.I. (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industrial Crops and Products*, *43*, 827–831. https://doi.org/10.1016/j.indcrop.2012.08.029

Rostagno, M.A., & Prado, J.M. (Eds.). (2013). *Natural Product Extraction: Principles and Applications*. Royal Society of Chemistry. https://doi.org/10.1039/9781849737579-FP001

Saptarini, N.M., Herawati, I.E., & Permatasari, U.Y. (2016). Total flavonoids content in acidified extract of flowers and leaves of Gardenia. *Asian Journal of Pharmaceutical and Clinical Research*, *9*(1), 213-215. https://journals.innovareacademics.in/index.php/ajpcr/article/view/12979/6407

Sobha, K., Dmp, P., Sajja, R., & Anantha, R. (2023). Phytoconstituents of *Chromolaena odorata* (L.) leaf extract for the synthesis of copper oxide/copper nanoparticles and

evaluation of their biological potential in wound healing. *Trends in Phytochemical Research*, *7*(3), 186-206. https://doi.org/10.30495/tpr.2023.1990359.1363

Szydłowska-Czerniak, A., Dianoczki, C., Recseg, K., Karlovits, G., & Szłyk, E. (2008). Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta*, *76*(4), 899–905. https://doi.org/10.1016/j.talanta.2008.04.055

Wuttisin, N., Nararatwanchai, T., & Sarikaphuti, A. (2021). Total phenolic, flavonoid, flavonol contents and antioxidant activity of Inca peanut (Plukenetia volubilis L.) leaves extracts. *Food Research*, *5*(1), 216-224. https://doi.org/10.26656/fr.2017.5(1).346

Zaabat, N., Hay, A.-E., Michalet, S., Skandrani, I., Chekir-Ghedira, L., Dijoux-Franca, M.-G., & Akkal, S. (2020). Chemical composition, antioxidant, genotoxique and antigenotoxic potentials of *Phlomis Bovei* De Noé Aerial Parts. *Iranian Journal of Pharmaceutical Research*, *19*(1), 282-291. https://doi.org/10.22037/ijpr.2019.15197.12938

Zagoskina, N.V., Zubova, M.Y., Nechaeva, T.L., Kazantseva, V.V., Goncharuk, E.A., Katanskaya, V.M., Baranova, E.N., & Aksenova, M.A. (2023). Polyphenols in plants: Structure, biosynthesis, abiotic stress regulation, and practical applications (Review). *International Journal of Molecular Sciences*, *24*(18). https://www.mdpi.com/1422-0067/24/18/13874

Zeb, A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, *44*(9), e13394. https://doi.org/10.1111/jfbc.13394