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Research Article

POLYPHENOLIC PROFILING BY LC–ESI–MS/MS AND BIOLOGICAL ACTIVITY ASSESSMENT OF *LATHYRUS OCHRUS* EXTRACTS

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ABSTRACT

Objective: The objective of this study was to analyze the polyphenolic composition and evaluate the antioxidant and antibacterial activities of *Lathyrus ochrus* extracts obtained using solvents of varying polarity.

Methods: Aerial parts of *L. ochrus* were extracted successively with cyclohexane, chloroform, and ethanol. Due to its poor solubility, the cyclohexane extract was excluded from biological assays. Total phenolics, flavonoids, and tannins were quantified spectrophotometrically. Antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching, and ferric reducing antioxidant power (FRAP) assays, with butylated hydroxytoluene, butylated hydroxyanisole, and ascorbic acid as reference antioxidants. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed for chemical profiling. Antibacterial activity of chloroform and ethanol extracts was evaluated against four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus cereus*) using the disk diffusion method.

Results: The ethanolic extract exhibited the highest total phenolic (41.9±0.2 µg GAE/mg), flavonoid (22.48±0.03 µg QE/mg), and tannin (15.33±0.03 µg CE/mg) contents. It showed strong antioxidant activity (DPPH IC_{50}=0.08 mg/mL; FRAP EC_{50}=0.14±0.02 mg/mL; and β-carotene bleaching inhibition=57.4%), comparable to reference standards. LC-MS/MS analysis revealed rutin, salicylic acid, chlorogenic acid, and p-coumaric acid as the major compounds. The chloroform extract exhibited stronger antibacterial activity than the ethanolic one, particularly against *S. aureus* (18.0 mm inhibition) and *B. cereus* (17.5 mm).

Conclusion: Solvent polarity significantly influenced the extraction of bioactive compounds. *L. ochrus* ethanolic extract is a promising source of natural antioxidants, while the chloroform extract may serve as a potential antibacterial agent. Further studies are warranted to isolate and characterize the active constituents.

Keywords: *Lathyrus ochrus*, Polyphenolic profiling, LC-MS/MS, Antioxidant activity, Bioactive compounds.

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INTRODUCTION

Throughout the documented ages, plants and plant-based products have played a crucial role in human life, serving both as essential foods and therapeutic agents as well. Since 2000, plant-based compounds have gained considerable popularity as valuable repositories of natural remedies, largely due to the high costs and numerous side effects associated with synthetic medicinal compounds [1]. In particular, plant secondary metabolites, including phenolics and flavonoids, exhibit significant biological activities such as antioxidant, antimicrobial, anticancer, and anti-mutagenic properties, all with the slightest detrimental effects on human health compared to their synthetic counterparts [2]. As a result, numerous researchers have redirected their attention to characterizing and investigating the biological effects of plants or their secondary metabolites [3,4]. Due to intense research activity, investigating untapped wild plants within various folk medicine systems shows high potential as a valuable reservoir of biologically active compounds for the development of drugs and functional foods.

Lathyrus species serve dual purposes, serving as both a food source and as traditional medicines. The tuber of the plant was documented in Renaissance herbals in Switzerland [5], while ethnomedicinally, it was used in Romania in love char, for wounds and stomach aches as a bath, also for sweetness, consumed primarily by children [6], for digestive problems in Bosnia and Herzegovina, and as a snack in Kosovo [7]. Furthermore, *Lathyrus* species play a pivotal role in traditional medicine, exhibiting analgesic properties through the seeds of *L. sativus* L., with anti-inflammatory attributes through aerial components of *L. cicero* L., and efficacy against rheumatism attributed to the leaves of L. rotundifolius Willd., particularly within the Turkish pharmacological context [8].

Earlier investigations of *Lathyrus* species have documented the existence of various biologically active compound, including phenolics, flavonoids, saponins, and polyunsaturated fatty acids [9,10].

Reactive oxygen species have a major role in the development of numerous neurodegenerative diseases such as autism, ischemia, Parkinson's syndrome, Alzheimer's disease, obesity, diabetes, cancer, cataracts, aging, and hepatic disorders [11]. In Algeria, Lathyrus genus is represented by 22 taxa. These herbaceous plants, either annual or perennial, often have climbing stems, compound leaves, and characteristic papilionaceous flowers [12].

Some species, such as *Lathyrus sativus* (grass pea), are cultivated for their protein-rich seeds. However, their excessive consumption can lead to lathyrism, a neurological disorder caused by the presence of the neurotoxin ODAP. Other species, like *Lathyrus odoratus* (sweet pea), are popular ornamental plants due to their fragrant flowers [13]. The species *Lathyrus* contain flavonoids [14] and fatty acids and protein [15].

Despite the importance of *Lathyrus* plants as a valuable source of phenolic compounds and our continued efforts to identify effective and safe antioxidant products [16,17], studies on their antioxidant potential remain limited. In continuation of our investigations on bioactive compounds [18-21], we report for the first time the antioxidant activity of Algerian Lathyrus ochrus. The antioxidant potential of chloroform and ethanol extracts was evaluated using three complementary assays and compared with standard antioxidants (Butylated hydroxytoluene [BHT], butylated hydroxyanisole [BHA], and ascorbic acid). In parallel, total polyphenol and flavonoid contents were precisely quantified. The antibacterial activity of the extracts was tested against four bacterial strains. Furthermore, liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was conducted on both extracts to characterize their phenolic composition. This comprehensive methodological framework provides valuable insights into the phytochemical profile and the antioxidant and antibacterial properties of *L. ochrus*.

METHODS

Plant collection and authentication

The aerial parts of the plant *L. ochrus* were collected in the Wilaya of Blida during the flowering season, from April to June 2023, at an altitude of 966 m (36°27′45″ N, 2°44′28″ E). After identification and authentication at the botanical laboratory of the University of Blida 1, the samples were dried in the open air. They were initially placed in the shade for 15 days to protect them from humidity, followed by an additional 2 weeks at ambient temperature. After drying, the aerial parts were finely ground using a knife grinder and stored in clean bags in a dark environment at room temperature (25°C±2°C) until further use.

Extraction method

80 g of plant material (leaves, stems, and flowers) were placed in a desiccator. Maceration was carried out for 24 h using three solvents of different polarities. The first solvent used was cyclohexane. After 24 h of maceration, the cyclohexane extract was recovered by filtration. The extract was then concentrated under reduced pressure in a rotary evaporator until a dry residue was obtained, weighing 1.19 g. After drying the plant material, a second maceration was performed using chloroform. The chloroform phase was recovered and concentrated to dryness, yielding a residue weighing 1.89 g. The same process was repeated with the third solvent, ethanol, and the obtained extract was concentrated to dryness, resulting in a residue weighing 4 g.

The cyclohexane extract was not included in the study due to its very low solubility, which prevented reliable evaluation in the biological assays.

Determination of the amount of antioxidant compounds

Determination of total phenolic content (TPC)

Total polyphenol content in plant extracts was estimated using the Folin-Ciocalteu method according to the method of Talbi *et al*. [22]. For this analysis, 200 µL of different solutions of extracts, diluted in ethanol (1 mg/mL for chloroformic and ethanolic extracts), are mixed with 1 mL of Folin-Ciocalteu reagent, previously diluted in distilled water (1:10). To the reaction, after 4 min, 800 µL of an aqueous sodium carbonate solution (75 mg/mL) are added to the mixture. The tubes are then kept in the dark at room temperature for 2 h. Absorbance is measured at 765 nm using a blank containing ethanol in place of the sample [23]. Gallic acid (0–75 µg/mL) is used as the standard for calibration, allowing the calculation of total polyphenol concentration in the extracts using the following formula [24]:

$$\text{Polyphenol content} = (C \times V)/M$$

Where:

- C: concentration of gallic acid (µg/mL)
- V: volume of extract solution (mL)
- M: mass of extract (mg).

Determination of total flavonoid content (TFC)

The TFC is determined using the aluminum trichloride ($AlCl_3$) method [25], with some modifications. A volume of 50 µL of a 10% hydro-ethanolic $AlCl_3$ solution is added to 1 mL of various extracts diluted in ethanol (0.5 mg/mL for ethanol and chloroform extracts). Subsequently, 50 µL of a 1 M ethanolic sodium acetate solution and 1.4 mL of distilled water are added. The mixture is incubated at room temperature for 1 h, after which the absorbance is measured at 420 nm against a blank containing ethanol instead of the sample.

Quercetin (0–30 µg/mL) is used as the standard, and the TFC in the extracts is calculated using the following formula:

$$\text{Flavonoid content} = (C \times V)/M$$

Where:

- C: concentration of quercetin (µg/mL)
- V: volume of extract solution (mL)
- M: mass of extract (mg).

Determination of total tannin content (TTC)

The content of condensed tannins was determined according to the method described by [26]. A volume of 400 µL of each extract was added to 3000 µL of a 4% vanillin solution in ethanol. The resulting mixture was vigorously shaken, and 1500 µL of concentrated hydrochloric acid was added. The mixture was then left to react at room temperature for 20 min. Absorbance was measured at a wavelength of 500 nm against a blank consisting of the 4% vanillin solution in methanol. A catechin stock solution was used as the reference standard for establishing the calibration curve and quantifying the condensed tannin content, expressed in micrograms of tannic acid equivalent per milligram of dry matter (µg CE/mg of dry matter).

LC-MS/MS analysis

The quantitative analysis of phenolic compounds in the *L. ochrus* chloroformic and ethanolic extracts was performed using LC-MS/MS (Agilent Technologies 1260 Infinity II system coupled with a 6460 Triple Quadrupole Mass Spectrometer). Separation was carried out on a Poroshell 120 SB-C18 column (3.0 × 100 mm, 2.7 µm), following the methodology described [27].

For sample preparation, 50 mg of the crude extract was placed into 2 mL Eppendorf tubes, and 2 mL of methanol was added. The mixture was stirred, followed by hexane extraction and centrifugation at 9000 rpm for 10 min. From the methanol phase of the resulting solution, a 100 µL aliquot was taken and diluted to 900 µL with a solution containing equal parts water and methanol (450 µL each). The diluted sample was then filtered and analyzed.

The LC-MS/MS analysis was conducted using a 5.12 µL injection volume, a flow rate of 0.400 mL/min, and a total runtime of 30 min. The mobile phase consisted of water (A) with 0.1% formic acid and 5.0 mM ammonium formate, and methanol (B) with 0.1% formic acid and 5.0 mM ammonium formate. The gradient program for the B phase was set as follows: 25% (1–3 min), 50% (4–12 min), 90% (13–21 min), and 3% (22–25 min). The column temperature was maintained at 40°C. The capillary voltage was set to 4000 V, with nitrogen as the nebulizing gas at a flow rate of 11 L/min, a pressure of 15 psi, and a gas temperature of 300°C.

Mass Hunter software was used to assess the data by comparing the retention time of the discovered substance with that of the standards. The calibration curves of the relevant standard were then used to carry out quantification.

Antioxidant activity assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The assessment of antioxidant activity focused on the ethanolic extract of *L. ochrus* and the reference antioxidant, ascorbic acid, using the

DPPH radical as a model. Absorbance was measured at 517 nm with a spectrophotometer monitoring the decrease in absorbance due to the reduction of the DPPH• radical, which results in a color change from violet (DPPH•) to yellow (DPPH-H). The intensity of this color change is inversely proportional to the ability of antioxidants to donate protons [28,29].

The experimental protocol followed Meriga *et al*. [30] with some modifications. A freshly prepared ethanolic solution of DPPH (0.03 mg/mL) was mixed with 400 µL of each extract chloroformic and ethanolic at different concentrations in ethanol, with a total volume of 1600 µL. After incubating the mixture in the dark at room temperature for 30 min, absorbance was measured at 517 nm against ethanol as a blank. The negative control contained all reagents except the extracts, which were replaced with an equal volume of ethanol. Solutions of different concentrations (0–0.2 mg/mL) of the reference antioxidant, ascorbic acid, were tested under the same experimental conditions as the positive control. Each crude extract was analyzed in triplicate. The percentage of DPPH radical scavenging activity was calculated using the formula provided by Kroyer and Hegedus [31]:

$$\% = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

β-carotene/linoleic acid bleaching assay

This spectrophotometric technique in the ultraviolet range, first developed by Marco [32], and later refined by Miller [33] measures the discoloration of β-carotene at 470 nm caused by oxidation from linoleic acid degradation products. The linoleic acid and β-carotene are dispersed in an aqueous phase using Tween. Oxidation is non-specifically triggered by heat (50°C), and the presence of antioxidants or plant extracts slows down the discoloration process. The antioxidant activity of our samples was determined using the method outlined by [34].

The antioxidant activity percentage is calculated using the following formula [35]:

$$AA\% = 1 - [\frac{Abs\,sample\,t=0 - Abs\,sample\,t=120}{Abs\,control\,t=0 - Abs\,scontrol\,t=120}] \times 100$$

Ferric reducing antioxidant power (FRAP)

The FRAP assay evaluates antioxidant capacity by measuring an extract's ability to reduce ferric ions (Fe^{3}+) to ferrous ions (Fe^{2}+) in the $K_3Fe(CN)_6$ complex. The reduction is quantified through the increase in blue color intensity at 700 nm [36].

The test was performed following the method of [37] with some modifications. A 200 µL volume of the extracts at various concentrations in ethanol was mixed with 500 µL of a 0.2 M phosphate buffer solution (pH=6.6) and 500 µL of a 1% aqueous $K_3Fe(CN)_6$ solution. The tubes were incubated at 50°C in the dark for 20 min, then cooled to room temperature. Afterward, 500 µL of a 10% aqueous trichloroacetic acid solution was added to stop the reaction, and the tubes were centrifuged at 3000 rpm for 10 min. 1.5 mL of the supernatant was collected and mixed with 300 µL of distilled water, followed by the addition of 60 µl of freshly prepared aqueous $FeCl_3$ solution. Absorbance readings were taken at 700 nm, using a blank containing ethanol instead of the sample. BHT (BHT) in a concentration range of 0–0.125 mg/mL was used as a positive control, subjected to the same experimental conditions.

Antibacterial activity

The antibacterial activity of the chloroform and ethanol extracts from the aerial parts of *L. ochrus* was tested *in vitro* using the disk diffusion method on agar medium. In this study, four bacterial strains were used: *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8039), and *Bacillus cereus* (ATCC 10876).

The extracts of chloroform and ethanol are prepared by dissolving 20 mg of each extract in 1 mL of DMSO to obtain stock solutions. Bacterial strains are cultured on Mueller-Hinton nutrient agar and incubated at 37°C for 24 h. Afterward, isolated and identical colonies are picked and suspended in 10 mL of sterile distilled water to create a homogeneous bacterial suspension, which is adjusted if necessary. For inoculation, a sterile swab is used to spread the bacterial suspension evenly across the agar surface in cross-striations. Discs impregnated with the extracts are placed on the agar, along with control discs containing gentamicin (positive control) and DMSO (negative control). The Petri dishes are incubated at 37°C for 18–24 h, and the results are assessed by measuring the inhibition zones around the discs after incubation. The diameters of these zones are measured with a ruler to evaluate the antibacterial activity of the extracts [38-40].

Statistical analysis

The results of the three measurements are expressed as mean ± standard deviation. Linear regression and one-way analysis of variance were performed to evaluate significant differences, with a significance level set at *p*<0.05. All analyses were carried out using XLSTAT software.

RESULTS AND DISCUSSION

Determination of the amount of antioxidant compounds

Determination of TPC

The determination of the total polyphenol content in *L. ochrus* extracts is estimated using the Folin-Ciocalteu. The results of the polyphenol content in the extracts were expressed in micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg of dry extract). The calibration curve of gallic acid is shown in Fig. 1.

As shown in Table 1, both extracts contained measurable amounts of polyphenols, the test revealed the presence of polyphenols in both extracts. We observe that the ethanol extract of *L. ochrus* exhibits the highest content (41.9±0.2 µg GAE/mg dry weight), followed by the chloroform extract (30.13±0.05 µg GAE/mg dry weight).

These results (Table 1) are consistent with a recurring observation that the largest proportion of phenolic compounds is found in polar extracts. This can be attributed to the higher solubility of polyphenols in polar solvents like ethanol, which facilitates the extraction of a greater quantity of these compounds compared to less polar solvents such as chloroform [41,42]. This trend aligns with previous studies highlighting the efficiency of polar solvents in extracting phenolic compounds from plant materials, including other *Lathyrus* species. Similar findings have been reported for *L. sativus* (grass pea), where polyphenolic composition and antioxidant activity varied significantly between different extraction methods and solvents [43,44]. Studies on *Lathyrus czeczottianus* and *L. nissolia* have also demonstrated the importance of solvent polarity in polyphenol extraction, with methanolic extracts showing higher TPC compared to less polar solvents [45]. Research on *Lathyrus maritimus* L. (beach pea) has further confirmed that extraction efficiency of condensed tannins and other phenolic compounds is significantly affected by solvent choice [46]. The differential extraction efficiency observed between ethanol and chloroform can be explained by the fact that flavonoid glycosides and more polar aglycones are

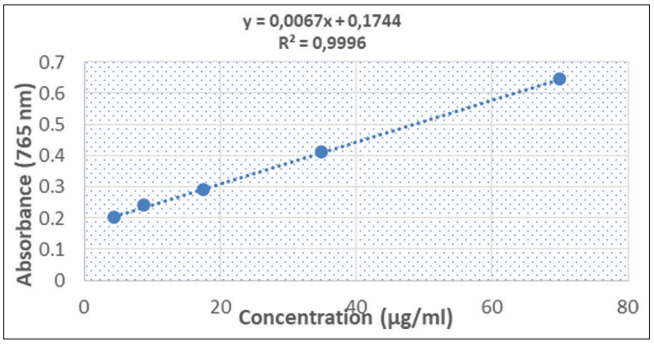

Fig. 1: Gallic acid calibration curve

Table 1: Total phenolic, flavonoid, and tannin contents of chloroform and ethanol extracts

Extracts	Total polyphenols content (µg GAE/mg dry extract)	Total flavonoid content (µg QE/mg dry extract)	Total tannin content (µg CE/mg dry extract)
Chloroform extract	30.13±0.05	19.56±0.1	10.33±0.05
Ethanol extract	41.9±0.2	**22.48±0.03**	15.33±0.03

Values expressed are means±standard deviation of three measurements n=3 ($p<0.05$)

preferentially extracted using alcohols or alcohol-water mixtures, while less polar flavonoids require organic solvents like chloroform [47].

Determination of TFC

The analysis revealed that the ethanolic and chloroformic extracts of the aerial parts of the *L. ochrus* plant contain a TFC of 22.48±0.03 µg QE/mg dry weight and 19.56±0.1 µg QE/mg dry weight, respectively. The results of the quercetin calibration curve are shown in Fig. 2.

The analysis revealed that the ethanolic and chloroformic extracts of the aerial parts of the *L. ochrus* plant (Table 1) contain significant amounts of total flavonoids, with values of 22.48±0.03 µg QE/mg dry weight and 19.56±0.1 µg QE/mg dry weight, respectively. These findings indicate that the ethanolic extract possesses a slightly higher flavonoid content compared to the chloroformic extract. This difference can be attributed to the differential solubility of flavonoids in solvents of varying polarity; ethanol, being a polar solvent, is more effective in extracting a broader range of flavonoids – particularly polar compounds – than chloroform, which is less polar. This trend is consistent with previous studies reporting higher phenolic and flavonoid yields from plant materials using polar solvents [48,49]. The presence of flavonoids in both extracts underscores the potential antioxidant and bioactive properties of *L. ochrus*, aligning with findings from other species in the genus that demonstrate significant pharmacological and phytochemical activities [50,51]. These compounds may contribute to the plant's medicinal value and suggest possible applications in therapeutic or nutraceutical formulations. Furthermore, the use of a quercetin calibration curve (Fig. 2) for flavonoid quantification ensures reliability and reproducibility, providing a robust foundation for future phytochemical investigations of *Lathyrus* species.

Determination of TTC

According to the results presented in the Table 1, the ethanolic extract showed a tannin content of 15.33 ± 0.03 µg EC/mg dry weight, while the chloroform extract contained 10.33 ± 0.05 µg EC/mg dry weight. These results are consistent with observations from many researchers who have found that ethanol extracts generally exhibit higher tannin concentrations [52,53].

It can be concluded that tannin extraction is influenced by several factors, including their chemical nature, the type of solvent used, and operating conditions. However, tannin content may also vary depending on other parameters such as their sensitivity to degradation processes (oxidation, light exposure, etc.), the ripening stage of the fruits, cultivation and climatic conditions, as well as environmental factors such as soil pressure or predation [54,55].

The assay results also indicate that polyphenol content is higher than that of flavonoids and tannins. This disparity in the levels of polyphenols, flavonoids, and tannins among different extracts can be explained by the choice of extraction solvent and the assay conditions used [56].

It is possible that the extraction solvent used in each case has a different affinity for various types of polyphenolic compounds. Moreover, the assay conditions, such as the use of the Folin–Ciocalteu reagent for polyphenol determination, may lead to non-specific reactions with proteins, sugars, and other sulfur-containing compounds, potentially resulting in inaccurate measurements [57].

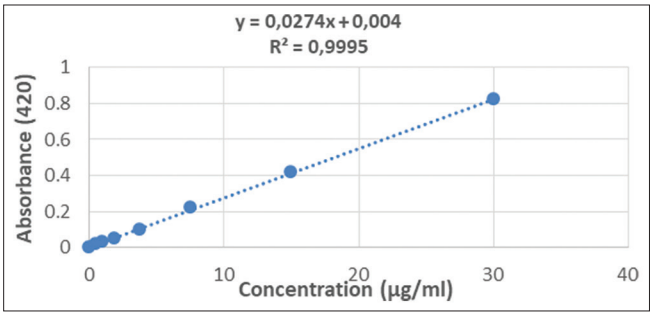

Fig. 2: The quercetin calibration curve

LC-MS/MS chemical profiling of *L. ochrus* extracts

The LC-MS analysis of the ethanol and chloroform extracts from *L. ochrus* (Table 2) revealed the presence of several phenolic acids and flavonoids, which are known to contribute to antioxidant and antibacterial properties. A total of 42 compounds were screened, of which 15 were detected across both extracts. The ethanol extract showed the highest rutin concentration (0.298 mg/g), approximately 3.3 times higher than that reported for *L. sativus* (0.089 mg/g [58]), indicating a relatively high level among *Lathyrus species*, followed by salicylic acid (0.081 mg/g), chlorogenic acid (0.077 mg/g, 71% higher than in *L. sativus*), and *p*-coumaric acid (0.077 mg/g, 175% higher than in *Lathyrus sylvestris* [59]. These results highlight a notable richness in phenolic acids, glycosylated flavonoids, and stilbenes, including the unique presence of resveratrol (0.021 mg/g), absent in *Lathyrus pratensis* and other species of the genus [60]. This feature serves as a distinctive chemotaxonomic marker, along with the complete absence of free quercetin, unlike *Lathyrus cicera*, which contains 0.034 mg/g [61].

In contrast, the chloroform extract, although less diverse in phenolics, was found to contain trans-ferulic acid (0.0292 mg/g), salicylic acid (0.0272 mg/g), rutin (0.2701 mg/g), kaempferol-3-glucoside (0.013 mg/g), naringenin (0.0106 mg/g), and fisetin (0.0019 mg/g). Some of these, particularly naringenin and kaempferol derivatives, are associated with antimicrobial activity, especially against Gram-positive [62], which supports the higher antibacterial performance of the chloroform extract against *S. aureus* and *B. cereus (Table* 3*).*

This exceptional phenolic composition, characterized by an infinite rutin/quercetin ratio and a remarkable diversity of hydroxycinnamic acids – with protocatechuic and salicylic acid concentrations 191% and 326% higher, respectively, than in *L. sylvestris* [63], suggests a metabolic adaptation specific to Mediterranean conditions. It endows *L. ochrus* with an estimated antioxidant potential 25–50% greater than other species in the genus [64], positioning this legume as a privileged source of bioactive compounds for nutraceutical and pharmaceutical applications, particularly in cardiovascular protection and combating oxidative stress.

Antioxidant activity

DPPH free radical scavenging activity

The antioxidant capacity of the plant extracts was evaluated using the DPPH free radical scavenging assay (Table 4). The ethanolic extract demonstrated significant antioxidant activity, with an IC_{50} value of 0.08±0.008 mg/mL, (Table 4) which is comparable to that of the standard antioxidant ascorbic acid (IC_{50}=0.04±0.01 mg/mL). In

Table 2: LC-MS/MS analysis of ethanol and chloroform extracts of *Lathyrus ochrus* (mg/g extract)

No.	Compound	RT (min)	Ethanol extract (mg\g)	Chloroform extract (mg\g)
1	Shikimic acid	1.4391	ND	ND
2	Gallic acid	3.7900	ND	ND
3	Protocatechuic acid	6.1577	0.0348	ND
4	Catechin	6.9360	ND	ND
5	Chlorogenic acid	7.7969	0.0767	0.0078
6	Hydroxybenzaldeyde	7.8004	0.0183	0.00180
7	Vanillic acid	7.8153	ND	ND
8	Caffeic acid	8.3298	0.0224	ND
9	Syringic acid	8.5063	ND	ND
10	Caffeine	8.3026	ND	ND
11	Vanillin	8.7214	ND	ND
12	p-coumaric acid	9.7073	0.0765	0.00658
13	Salicylic acid	9.8207	0.0808	0.02717
14	Taxifolin	9.6382	ND	ND
15	Polydatin	9.8229	0.0016	ND
16	Resveratrol	9.8156	0.0208	ND
17	trans-ferulic acid	10.2212	ND	0.0292
18	Sinapic acid	10.3656	ND	ND
19	Scutellarin	11.1993	ND	ND
20	o-coumaric acid	11.5025	ND	ND
21	Coumarin	11.6036	ND	ND
22	Protocatehuic ethyl ester	11.5255	ND	ND
23	Rutin	11.5043	0.2978	0.2700
24	Isoquercitrin	11.5532	0.0019	0.0018
25	Hesperidin	12.2771	ND	ND
26	Quercetin-3-D-xyloside	12.0633	ND	ND
27	Kaempferol-3-glucoside	13.1565	ND	0.0130
28	Fisetin	13.2768	ND	0.0018
29	Baicalin	13.5955	ND	ND
30	trans-cinnamic acid	14.4519	ND	ND
31	Quercetin	14.1836	ND	ND
32	Naringenin	14.3619	ND	0.0105
33	Hesperetin	16.1488	ND	ND
34	Morin	15.6891	ND	ND
35	Kaempferol	16.4958	ND	ND
36	Baicalein	17.0828	ND	ND
37	Luteolin	17.8160	ND	ND
38	Biochanin A	17.9260	ND	ND
39	Chrysin	17.8171	ND	ND
40	Capcaicin	17.7555	ND	ND
41	Dihydrocapcaicin	18.6325	ND	ND
42	Diosgenin	23.3816	ND	ND

ND: Not detected, RT: Retention time

Table 3: The diameter of inhibition growth zone, of *Lathyrus ochrus*

Bacterial strain	Chloroform extract	Ethanol extract	Gentamicin* (control)
Escherichia coli	–	6.5	44.0
Pseudomonas aeruginosa	15.5	12.0	33.5
Staphylococcusaureus	18.0	11.0	26.5
Bacillus cereus	17.5	16.5	24.0

*Compared with Gentamicin as positive control

Table 4: Antioxidant activity expressed by the IC_{50} (μg/mL)

Samples	DPPH IC_{50} (mg/mL)
Chloroformic extract	0.66±0.05
Ethanolic extract	0.08±0.008
Ascorbic acid	0.04±0.01

Values expressed are means±standard deviation of three measurements n=3 ($p<0.05$)

contrast, the chloroform extract showed considerably lower activity (IC_{50}=0.66±0.05 mg/mL) and did not reach 50% inhibition at the tested concentrations, indicating poor radical scavenging potential. As a result, it was excluded from further analyses.

The high antioxidant activity of the ethanolic extract is likely attributable to its content of phenolic compounds, which are known to act as efficient free radical scavengers due to the presence of multiple hydroxyl groups capable of donating hydrogen atoms or electrons. Structural features of flavonoids, such as the presence of ortho-dihydroxy substitutions on the B-ring (3′,4′-OH) and/or a hydroxyl group at position 3 on the C-ring, are associated with enhanced antioxidant activity [65,66].

When compared to other species of the *Lathyrus* genus, the ethanolic extract of the studied species exhibits comparable or superior antioxidant activity. For example, *L. sativus* and *L. odoratus* have been reported to possess moderate antioxidant activity, with IC_{50} values ranging from 0.20 to 0.35 mg/mL in similar DPPH assays [67,68]. This suggests that the studied species may represent a more potent source of natural antioxidants within the genus. Such variability in antioxidant activity among *Lathyrus* species may be attributed to differences in phenolic content and flavonoid profiles, influenced by environmental, genetic, and extraction factors.

β-carotene/linoleic acid bleaching assay

The antioxidant activity of the extracts was further assessed using the β-carotene-linoleic acid bleaching assay. This method monitors the oxidative degradation of β-carotene in the presence of the extract, ethanol (negative control), and BHA as a positive control. Absorbance was measured at 470 nm at 30-min intervals over a period of 2 h. The progressive decrease in absorbance indicates continuous oxidation of β-carotene, with significant variation observed between control and test groups.

After 120 min of incubation, the ethanolic extract inhibited β-carotene oxidation by 57.4%, while the chloroform extract achieved 52.3% inhibition. In comparison, BHA produced a slightly higher inhibition of 60.28% (Table 5). These findings indicate that the extracts contain bioactive compounds capable of delaying the oxidative degradation of β-carotene, although their effect remains less pronounced than that of the synthetic antioxidant.

The observed activity may be influenced by the differential solubility and polarity of the bioactive constituents in the extract. As the β-carotene-linoleic acid assay involves an emulsion system consisting of lipids dispersed in an aqueous phase, the partitioning behavior of phenolic and flavonoid compounds plays a critical role in their antioxidant effectiveness [69,70]. Hydrophilic compounds may remain in the aqueous phase and exert less activity in the lipid environment where oxidation occurs.

When compared to other species of the *Lathyrus* genus, the antioxidant performance of the ethanolic extract in this assay appears consistent with previously reported data. For instance, *L. sativus* and *L. cicera* extracts have shown β-carotene bleaching inhibition values ranging between 45% and 55%, depending on extraction solvent and assay conditions [71,72]. These findings support the antioxidant potential of the studied *Lathyrus* species and highlight the relevance of solvent selection in optimizing antioxidant extraction.

Table 5: Antioxidant activity of extracts evaluated by the β-carotene bleaching assay

Samples	β-carotene inhibition (%)
Chloroformic extract	52.3
Ethanolic extract	57.4
BHA	60.3

FRAP

The reducing power of the plant extracts was assessed by measuring their effective concentration (EC_{50}), defined as the extract concentration required to reach an absorbance of 0.5 at 700 nm, corresponding to 50% of maximal reducing capacity (Table 6). A lower EC_{50} value indicates greater iron-reducing efficiency and, consequently, stronger antioxidant potential.

Among the tested samples, the ethanolic extract exhibited the highest reducing activity, with an EC_{50} of 0.14±0.02 mg/mL. In contrast, the chloroform extract displayed the weakest reducing capacity, with an EC_{50} of 0.33±0.07 mg/mL. For comparison, BHT`, used as the positive control, demonstrated the strongest reducing power, with an EC_{50} of 0.06±0.01 mg/mL.

A concentration-dependent increase in reducing power was observed for all extracts, suggesting a direct correlation between extract concentration and electron-donating ability. This activity is primarily attributed to phenolic compounds, which possess hydroxyl groups capable of donating electrons to reduce Fe^{3+} to Fe^{2+}. As such, antioxidants function as reducing agents that stabilize and neutralize reactive species [73,74].

Comparable results have been reported for other species of the *Lathyrus* genus. For instance, methanolic extracts of *L. sativus* and *L. czeczottianus* have shown moderate to strong reducing power, with EC_{50} values ranging between 0.12 and 0.25 mg/mL depending on the extraction protocol and plant part used [75,76]. The ethanolic extract examined in this study aligns well with these findings, further supporting the presence of redox-active phytochemicals, particularly polyphenols, in *Lathyrus* species.

The results obtained from the DPPH radical scavenging assay, the β-carotene bleaching method, and the FRAP assay collectively demonstrate that the ethanolic extract possesses the highest antioxidant potential among the tested samples. This trend is consistent with the total phenolic and flavonoid content of the extracts, suggesting a strong correlation between antioxidant activity and the presence of polyphenolic compounds [77].

Previous studies have emphasized that phenolic compounds, particularly phenolic acids and flavonoids, are among the most significant secondary metabolites contributing to antioxidant capacity in plants [78,79]. These compounds exert their antioxidant effects primarily through mechanisms such as free radical scavenging, metal ion chelation, and inhibition of lipid peroxidation, attributed to the presence of hydroxyl groups that can donate hydrogen atoms or electrons [80,81].

The superior performance of the ethanolic extract may thus be attributed to its higher efficiency in extracting these hydrophilic antioxidant compounds compared to less polar solvents such as chloroform. The data also support findings from other *Lathyrus* species, where methanolic or aqueous extracts generally exhibit stronger antioxidant activities due to richer polyphenolic profiles [75,76].

Antibacterial activity

The antibacterial activities of *L. ochrus* extracts obtained using chloroform and ethanol were evaluated against four bacterial strains—*E. coli, P. aeruginosa, S. aureus*, and *B. cereus* – using the agar well diffusion method (Table 3). The positive control (a broad-spectrum antibiotic) produced inhibition zones ranging from 24.0 mm to 44.0 mm, depending on the bacterial strain.

Table 6: Reducing power of *L. ochrus* extracts expressed as EC_{50} values (mg/mL)

Samples	Ferric reducing antioxidant power EC_{50} (mg/mL)
Chloroformic extract	0.33±0.07
Ethanolic extract	0.14±0.02
BHT	0.06±0.01

Values expressed are means±standard deviation of three measurements n=3 ($p<0.05$)

- *E. coli*: No inhibition was observed with the chloroform extract, whereas the ethanol extract exhibited a minimal average inhibition zone of 6.5 mm
- *P. aeruginosa*: Chloroform extract showed a greater inhibitory effect (15.5 mm) than the ethanolic extract (12.0 mm)
- *S. aureus*: Chloroform extract produced the highest inhibition among the tested extracts (18.0 mm), compared to 11.0 mm for ethanol extract
- *B. cereus:* Both extracts demonstrated moderate activity, with chloroform extract showing slightly higher efficacy (17.5 mm vs. 16.5 mm).

Overall, the chloroform extract exhibited superior antibacterial activity in three of the four tested strains, indicating the likely presence of lipophilic bioactive compound with strong membrane-disruptive properties [82,83]. This extract was particularly effective against Gram-positive bacteria (*S. aureus* and *B. cereus*), achieving 70% and 73% of the activity of the reference antibiotic, respectively. The relatively high susceptibility of these Gram-positive strains could be attributed to their less complex cell wall structure compared to Gram-negative bacteria, which possess an outer membrane that acts as a barrier to hydrophobic molecules [84,85].

In contrast, *E. coli* exhibited no susceptibility to the chloroform extract, suggesting limited permeability or absence of suitable intracellular targets. The ethanolic extract, while generally less potent, showed modest and relatively uniform inhibitory effects across all tested strains, indicating the presence of more polar antibacterial agents with broader but milder efficacy.

These findings support previous studies on *Lathyrus* species, which have reported variable antibacterial activity depending on the solvent used and the strain tested [75,76]. The differential extraction of bioactive constituents depending on solvent polarity is a well-documented phenomenon, with non-polar solvents like chloroform preferentially extracting terpenoids, alkaloids, and other hydrophobic secondary metabolites [86].

Given the relatively high activity of the chloroform extract against Gram-positive pathogens, this fraction could serve as a promising lead for the isolation of potent antimicrobial agents. Future investigations should focus on bio-guided fractionation, cytotoxicity profiling, and determination of minimum inhibitory concentrations to fully characterize the therapeutic potential of these extracts.

CONCLUSION

This study provides the first detailed polyphenolic profiling and biological activity assessment of *L. ochrus* extracts from Algeria. Among the two solvents used, the ethanolic extract demonstrated the highest concentrations of total phenolics, flavonoids, and tannins, which directly correlated with its strong antioxidant activity observed in DPPH, β-carotene bleaching, and FRAP assays. LC-MS/MS analysis revealed a rich composition of phenolic acids and flavonoids, with particularly high levels of rutin and salicylic acid, supporting the extract's potent antioxidant potential. While the chloroform extract exhibited weaker antioxidant properties, it showed greater antimicrobial efficacy, particularly against Gram-positive strains such as *S. aureus* and *B. cereus*. These results underscore the influence of solvent polarity on the extraction efficiency of bioactive compounds and position *L. ochrus* as a promising candidate for the development of natural antioxidant and antibacterial agents. Future studies should focus on the isolation and characterization of specific active constituents and evaluate their potential in pharmacological applications through *in vivo* testing and mechanistic studies.

AUTHORS' CONTRIBUTIONS

Meriem Aissaoui and Abderrahmane Mezrag were responsible for the laboratory work, sample collection, data collection, data analysis, and drafting of the manuscript. Ilyas Yildiz and Ramazan Erenler provided the LC–MS/MS data with the respective standards. Chahrazed Esseid contributed to the translation and assisted in manuscript preparation. All authors contributed equally to this work.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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