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Antioxidant and Anticholinesterase Effects of Methanol Extract, And Consecutive Fractions of *Peganum harmala* L.

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Abstract

Peganum harmala L. is a medicinal plant growing in the region of Sidi Fredj of Souk Ahras city (Northeastern of Algeria), whose native populations commonly used this plant to treat various health conditions and illnesses. Up to now, no previous study on the beneficial effects of *P.harmala* in the Souk Ahras region has been conducted. Therefore, the present study aimed to investigate the phenolic and flavonoid composition, as well as the antioxidant activities of the methanol extract and its three fractions: chloroform, ethyl acetate and butanol from seeds of *P. harmala* using eight different methods. The inhibitory effect of the extracts against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was also determined. The results showed that the ethyl acetate fraction contained the highest amounts of total phenolic and total flavonoid (237.23±1.06 µg GAE/mg and 82.58±0.03 µg QE/mg, respectively). Important antioxidant activity was exerted by the extracts, especially the ethyl acetate fraction, which was the most effective. However, a weak antioxidant activity in Ferrous ions chelation was observed for all extracts with >800 µg/mL. The samples showed also a strong inhibitory effect against AChE and BChE, the chloroform fraction was the most potent with IC_{50} values of 14.69±0.10 µg/mL against AChE and 7.36±0.77 µg/mL against BChE comparable to that of galantamine. Conclusively, the obtained results proved the beneficial antioxidant and anticholinesterase activities of *P. harmala*-derived bioactive molecules.

Keywords: *Peganum harmala* L.; antioxidant & anticholinesterase effects; phenolic compounds; flavonoids.

1-Introduction

Oxidative stress associated with excessive production of reactive oxygen species (ROS) results mainly in the induction of various pathogenesis and diseases as evidenced by severe oxidative injuries to numerous biological functions of the body [1]. In addition, the progression of oxidative stress underlies neurodegenerative disorders, including Alzheimer's *disease* (*AD*) which is likely due to the aberrant deposition of accumulated amyloid β (Aβ) peptides and intracellular neurofibrillary tangle [2].

Aerobic organisms possess powerful enzymatic and non-enzymatic antioxidant defense systems able to act effectively against ROS-inducing the damaging effects. Further, the exogenous antioxidants, like vitamins and the major bioactive molecules (e,g, polyphenols, flavonoids, and carotenoids) derived from vegetables, cereals, and medicinal plants have been widely used by humans to enhance their immunity against pathogens [3]. Hence, many researchers have recently drawn great attention to the use of natural antioxidants as essential healthy alternatives instead of the commonly used synthetic antioxidants in food industries [4].

As reported, the bioactive substances (e,g, phenols, and flavonoids) derived mainly from plants exhibit a wide range of pharmacological effects and therapeutic benefits [5] against cancers, inflammation, and neurogenerative pathology [6]. Indeed, most of the beneficial effects of these compounds are ascribed to their antioxidant properties [7]. Moreover, the cholinesterase

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(Acetylcholinesterase and Butyrylcholinesterase) inhibitors, including only donepezil, rivastigmine and galantamine leading to increase brain levels of acetylcholine (a neurotransmitter involved in cholinergic activity and cognitive function) have been recently approved for the treatment of Alzheimer's disease [8,9], while their side effects limit their use [10]. Consequently, the use of new promising drugs originating from natural plant products becomes of the highest priority. In this context, the biological effects of plants usually used for traditional medicine have been well-documented as memory enhancers [11].

The natural plant extracts containing bioactive compounds have proved to effectively inhibit the enzymatic activity of AChE and BChE [12]. The natural plant compounds can, on one hand, interact with the active site and, as a result, lead to inhibit the activity of the specific enzyme [13]. *Peganum harmala* L. known as Harmal, Syrian rue, or wild rue is an herbaceous plant of the family *Zygophyllaceae* [14], and a widely distributed plant in North Africa, and Central Asia, the Middle East, Pakistan, and India [15].

Its seeds and the aérial parts have been used in traditional medicine as an emmenagogue, abortifacient, analgesic, and anti-inflammatory agent [16].A previous study investigating the phytochemical composition of its seeds reported the presence of alkaloids, flavonoids, polysaccharides, and anthraquinones [17]. The plant exhibit a wide spectrum of pharmacological effects, including antifungal, antibacterial, antioxidant, antiviral, antidiabetic, insecticidal, antineoplastic, hepatoprotective , and cytotoxic properties [18].

Since there are no previous studies in this study area, the present study is, therefore, the first aimed to investigate the total bioactive components and the antioxidant and anticholinesterase activities of methanol extract/fractions from the seeds of *P. harmala* collected from the Sidi Fredj region of Souk Ahras city (Northeastern of Algeria).

2. Material and methods

2.1. Plant material

The seeds of *P. harmala* were collected in July 2019 during the flowering period from the region of Sidi Fredj located in the South-Eastern part of Souk Ahras city (North-East Algeria): 36° 0'58.94"N 8°13'54.03"E and 544 m (latitude N/longitude E/altitude) (Figure 1). The samples were air-dried in the shade and transformed to a fine powder by an electric blender.

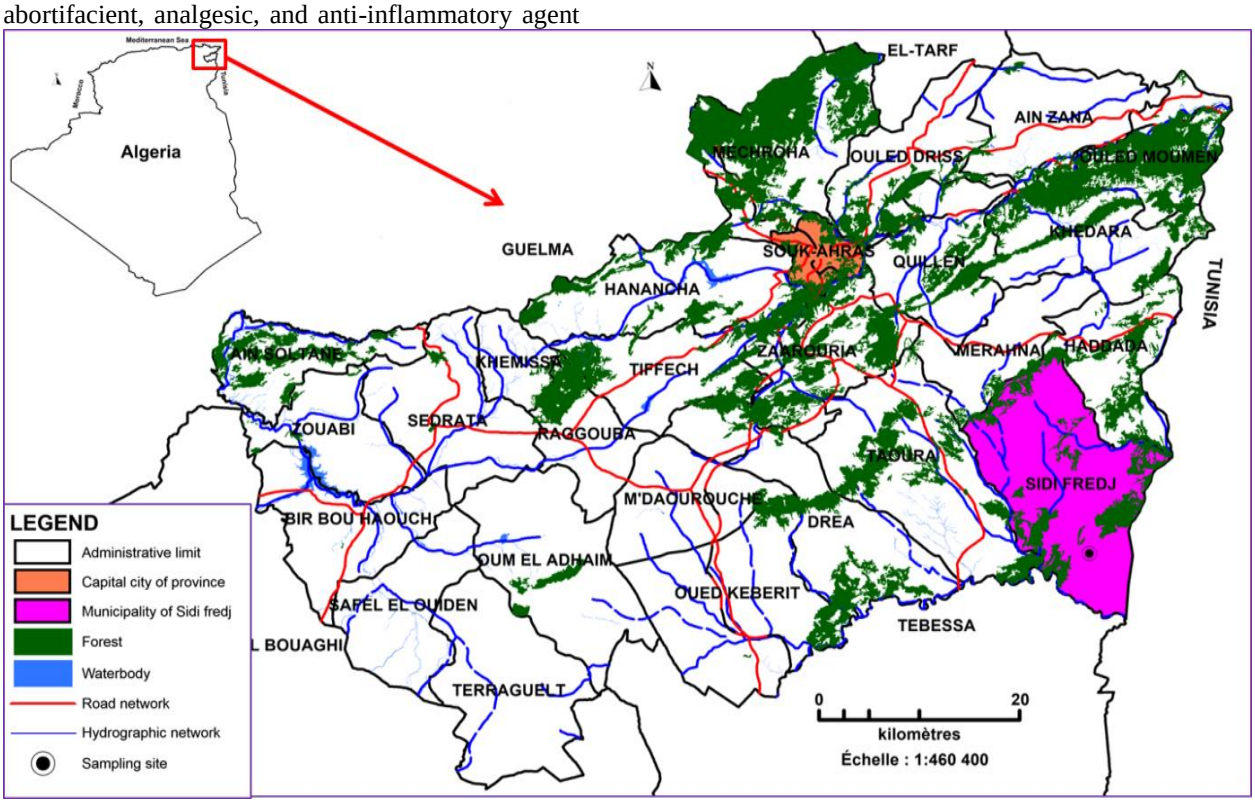

Figure 1. Geographical location of the study area (realized with Mapinfo Pro 12.0.2)

2.2. Preparation of extract/fractions

100 g of seeds powder were extracted in 500 ml of methanol /water (80:20, v/v) by maceration for 72h at room temperature under magnetic agitation. This experiment was performed three times with the renewal of the solvent. After filtration, the methanol was evaporated at 35 °C by a rotary evaporator to get the crude extract. The dried extract was afterward dissolved in distilled water and subjected to a liquid-liquid fractionation using increasing polarity solvents (chloroform, ethyl acetate and n-butanol). All fractions were concentrated under pressure in a rotary evaporator and kept at 4 °C until use. The yield of dried extracts was expressed as the ratio between the weights of obtained extract, and the plant powder and was determined using the following equation:

%Yield: (Weight of dry extract/ Weight of dry plant material) × 100

2.3. Quantification of total phenolic and total flavonoid contents

2.3.1. Total phenolic contents (TPC)

The content of total phenolic was determined in a 96-well microplate using Folin-Ciocalteu method as described by Müller et al. [19]. In brief, 100 µL of Folin–Ciocalteu reagent (1:10) and 75 µL of sodium carbonate (7.5%) were added to 20 µl of each extract (1 mg/ml). The microplate was incubated 2 h at room temperature in the obscurity. The absorbance was recorded at 765 nm. The results were expressed as µg of gallic acid equivalent per mg of extract (µg GAE/mg).

2.3.2. Total flavonoid contents (TFC)

The content of total flavonoid was estimated in a 96-well microplate using the aluminium nitrate method adopted from Topçu et al. [20]. In brief, 130 µL of methanol was added to 50 µl of each extract (1 mg/ml). After 40 min at room temperature, the absorbance was read at 415 nm. The results were expressed as µg quercetin equivalents per mg of extract (µg QE/mg).

2.4. Antioxidant activities

2.4.1. DPPH (Diphenyl‑1‑picrylhydrazyl) radical trapping activity

The free radical scavenging ability was established according to the method recorded by Aissani et al. [21]. In a 96-well microplate, 160 µl of the DPPH solution (0.1 mM) was added to 40 µl of each sample at various concentrations. After 30 min of incubation at room temperature in the obscurity, the absorbance was read at 517 nm using a multimode plate reader (EnSpire). Butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) and α-Tocopherol were considered as antioxidant standards. The inhibition percent was calcutated as follows:

Inhibition (%) = [(Abs control - Abs sample)/ Abs control] x 100 (1)

The results were represented as IC_{50} values (µg/mL) which corresponds to the concentration of 50% inhibition

2.4.2. ABTS (2,2'-azinobis (3-thylbenzothiazoline)-6-sulfonic) radical trapping activity

The anti-radical activity of extract/fractions against ABTS•+radical was realised by the method of Khalfallah et al. [22]. The ABTS•+ solution was obtained by combining 7 mM of ABTS in water with 2.45 mM of potassium persulfate, stored in the obscurity at room temperature for 16h. The ABTS•+ solution was diluted in water to an absorbance of 0.7 at 734 nm. 160 µl of this solution was added to 40 µL of each sample in methanol at various concentrations. After 10 min of incubation, the absorbance was recorded at 734 nm. The inhibition percent was calculated by applying above formula (1). BHA and BHT were used as antioxidant standards.

2.4.3. Galvinoxyl radical (GOR) trapping activity

GOR scavenging assay was done according to the method of Shi et al. [23]. In brief, 160 µL of galvinoxyl (0.1 mM in methanol) was mixed with 40 µL of each sample at various concentrations. After 120 min of incubation at room temperature in the obscurity, the absorbance was read at 517 nm. The inhibition percent was calculated by applying above formula (1). BHA and BHT were used as standards.

2.4.4. Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was established according to the method reported by Apak et al. [24]. In a 96-well microplate, 50 µL of $CuCl_2$ (10 mM), 50 µL of 7.5 mM neocuproine, and 60 µL of ammonium acetate (1 M) were mixed with 40 µL of extract/fractions. After 1 h of incubation, the absorbance was recorded at 450 nm. BHA and BHT were used as standards. The results were represented as $A_{0.5}$ values (µg/ml) referring to the concentration indicating 0.5 absorbances.

2.4.5. Reducing power activity

The reducing power of P. harmala extracts was assessed as indicated by Mamache et al. [25]. In brief, 40 µL of 0.2 M phosphate buffer (pH 6.6), and 50 µL of potassium ferricyanide (1%) were mixed with 10 µL of extract/fractions at various concentrations in the microplate. The mixture was incubated at 50°C for 20 min. Subsequently, 50 µl of trichloroacetic acid (10%), 40µl distilled water, and

10 µl of ferric chloride solution (0.1%) were added. The absorbance was read at 700 nm. Ascorbic acid and α-tocopherol were used as antioxidant standards.

2.4.6. Phenanthroline activity

The phenanthroline assay was evaluated by the method of Szydlowska-Czerniak et al. [26]. In brief, 30 µL O-phenanthroline (0.5% in methanol), 50 µL $FeCl_3$ (0.2%), and 110 µL of methanol were added to 10 µL of extract/fractions at various concentrations. After 20 min of incubation at 30°C in the obscurity, the absorbance was read at 510 nm. BHA and BHT were used as antioxidant standards.

2.4.7. β-carotene bleaching activity

The antioxidant capacity of the plant extracts to inhibit lipid peroxidation by the system β-carotene/linoleic acid was issued using the method of Mouffouk et al. [27]. To prepare the solution 0.5 mg of β-carotene was dissolved in 1 ml of chloroform.This mixture was combined with 25 µl of linoleic acid and 200 µl of Tween 40 and the solvent was extracted under vacuum using a rotary evaporator then, 50 ml of H_2O_2 was added and the absorbance of the solution was adjusted to 0.8-0.9 at 470 nm.160 µl of this solution was added to 40 µl of each sample at various concentrations in the microplate. The absorbance was read at 470 nm before and after incubation for 2 h at 50°C. BHA and BHT were used as antioxidant standards. Th inhibition percent was calculated using the following equation:

$$\text{Inhibition (\%)} = 1 - (A_{t=0} - A_{t=120} / A0_{t=0} - A0_{t=120}) \times 100$$

Where, $A_{t=0}$ and $A_{t=120}$ are the absorbances (470 nm) of the sample at 0 min and 120 min respectively. $A0_{t=0}$ and $A0_{t=120}$ represent the absorbances (470 nm) in the presence of positive control at 0 min and 120 min respectively.

2.4.8. Ferrous iron chelating activity

The metal chelating capacity of the extract/fractions was estimated as described by Saci et al. [28]. In a 96-well microplate, 40 µL of methanol, 40 µL of $FeCl_2$ (0.2 mM), and 40 µL of each sample at various concentrations were mixed. Then, 80 µL of Ferene (0.5 mM) was added. The mixture was incubated for 10 min at room temperature.

The absorbance was recorded at 593 nm. The inhibition percent was obtained using preceding equation (1). Ethylenediaminetetraacetic acid (EDTA) was used as standard.

2.5. Anticholinesterase activity

The inhibition of AChE and BChE by the crude extract/fractions was established by the method developed by Gali and Bedjou [29] in a 96-well microplate. In brief, 150 µL of sodium phosphate buffer (100 mM, pH 8.0) was mixed with 10µL of the extract/fractions in methanol at different concentrations or galantamine (positive standard) and 20 µL of AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) solution. After 15 min of incubation at 25°C, 10 µl of 0.5 Mm DTNB [5,5´-dithio-bis(2-nitrobenzoic) acid] and 10 µl of acetylthiocholine iodide (0.71 mM) or 10 µl of butyrylthiocholine chloride (0.2 mM) were added to the mixture. The absorbance was measured at 412 nm in every 5 min for 15 min. The inhibition percent of AChE or BChE was measured against a blank (phosphate buffer and methanol) using the following equation:

$$\text{Inhibition (\%)} = (E - S)/E \times 100$$

Where, E is the activity of the enzyme without extract, and S is the activity of the enzyme in the presence of the extract.

2.6. Statistical analysis

The data were displayed as mean±standard deviation, with three replicates. The results were statistically tested by one-way ANOVA with Tukey's test using GraphPad Prism software (version 9), where p values < 0.05 were considered significant.

3. Results

3.1. Extraction yield

As shown in Table 1, the methanol extract produced the highest yield, followed by the butanol fraction and the chloroform fraction, however the lowest yield was observed in the ethyl acetate fraction.

3.2. Total phenolic and total flavonoid contents

The contents of phenolic and flavonoid compounds were quantified in *P. harmala* crude extract and its fractions (Figure 2 & 3). The ethyl acetate fraction recorded the highest amount of total phenolic and total flavonoid (237.23±1.06 µg GAE/mg and 82.58±0.03 µg QE/mg, respectively), followed by the butanol fraction (197.23±0.29 µg GAE/mg and 57.98±0.70 µg QE/mg, respectively), and methanolic extract (144.97±0.34 µg GAE/mg and 43.62±0.42 µg QE/mg, respectively). The lowest amount (111.25±1.89 µg GAE/mg and 22.36±0.07 µg QE/mg, respectively) were found in chloroform fraction.

3.3. Antioxidant capacity

The antioxidant potential of the crude extract and the different fractions of *P.harmala* seeds was reported using eight different methods and the results are depicted in Table 2 as IC_{50} and $A_{0.5}$ values.

Table 1. Different characteristics of the crude extract and the different fractions of *P. harmala*

Extract/Fraction	Yield (%)	Aspect	Color
Methanol	27.13	Paste	Brownish
Chloroform	0.38	Viscous	Blueish

Ethyl acetate	0.16	Solid	Yellowish
Butanol	7.77	Solid	Brownish

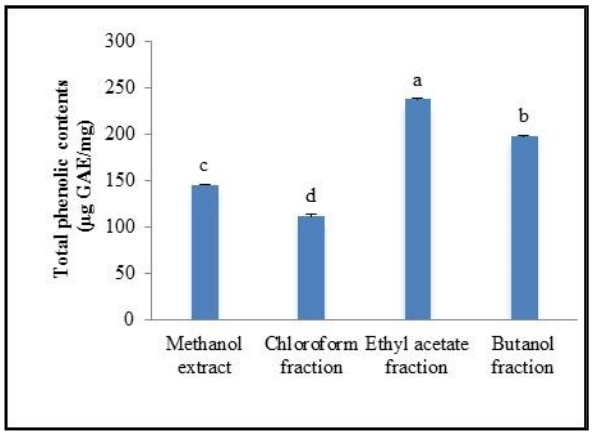

Figure 2. Total phenolic contents in the crude extract and the different fractions of *P. harmala*
[a-d]: Different superscript letters represent significant variation

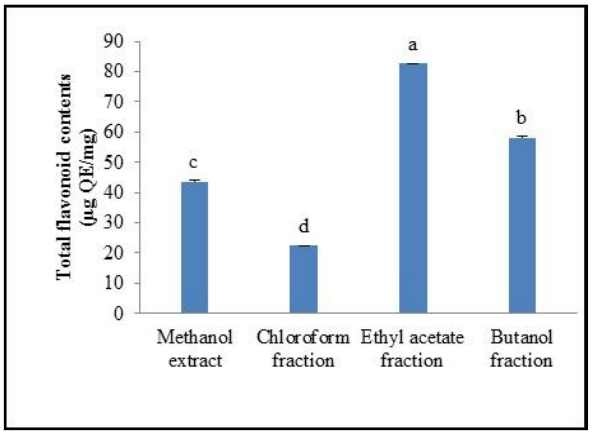

Figure 3. Total flavonoid contents in the crude extract and the different fractions of *P. harmala*
[a-d]: Different superscript letters represent significant variation

Table 2. Antioxidant activity of the crude extract and the different fractions of *P. harmala*

Extract/ Fraction	DPPH IC_{50} µg/mL	ABTS IC_{50} µg/mL	GOR IC_{50} µg/mL	Cuprac $A_{0.5}$ µg/mL	Reducing Power $A_{0.5}$ µg/mL	Phénanthr-oline $A_{0.5}$ µg/mL	β-carotene Bleaching IC_{50} µg/mL	Ferrous ion chelating IC_{50} µg/mL
Methanol	133.18±1.22^{e}	27.32±0.79^{e}	153.40±0.55^{d}	187.39±0.58^{e}	158.07±0.12^{e}	6.45±0.54^{c}	70.93±0.69^{e}	>800
Chloroform	187.57±0.87^{f}	35.02±0.57^{f}	305.59±0.41^{e}	110.49±0.51^{c}	89.61±0.90^{c}	5.73±0.95^{c}	34.45±1.52^{c}	>800
Ethyl acetate	92.02±1.30^{c}	10.22±0.26^{c}	45.93±0.75^{c}	90.57±0.84^{b}	87.83±0.56^{c}	3.78±0.64^{b}	27.21±0.99^{b}	>800
Butanol	108.36±0.83^{d}	19.91±0.96^{d}	47.03±0.93^{c}	123.03±0.35^{d}	129.58±0.62^{d}	5.95±0.82^{c}	40.32±0.56^{d}	>800
BHA	6.14±0.41^{a}	1.55±0.26^{a}	3.32±0.18^{a}	5.35±0.71^{a}	-	0.93±0.07^{a}	1.05±0.03^{a}	-
BHT	12.99±0.41^{b}	7.54±0.67^{b}	5.38±0.06^{b}	8.97.03±3.94^{a}	-	2.24±0.17^{ab}	0.91±0.01^{a}	-
a-Tocopherol	13.02±5.17^{b}	-	-	-	34.93±2.38^{b}	-	-	-
Ascorbic acid	-	-	-	-	6.77±1.15^{a}	-	-	-
EDTA	-	-	-	-	-	-	-	8.80±0.47

Data are expressed as means ± standard deviation of three measurements; Analysis of variance (ANOVA) revealed significant effect ($p< 0.05$); Different superscript letters represent significant variations

The scavenging potential measured by DPPH assay showed that the ethyl acetate fraction exhibited the highest activity (IC_{50}: 92.02±1.30 µg/mL), compared with the other extracts but lesser activity than that in BHA, BHT and α-Tocopherol (IC_{50}: 6.14±0.41, 12.99±0.41 and 13.02±5.17 µg/mL, respectively), followed by butanol fraction and methanolic extract (IC_{50}: 108.36±0.83 and 133.18±1.22 µg/mL, respectively), however the chloroform fraction showed a weak activity (IC_{50}: 187.57±0.87 µg/mL).

Moreover, the results of Galvinoxyl radical (GOR) scavenging activity showed that the ethyl acetate and butanol fractions gives the highest activity with close IC_{50} values (45.93±0.75 and 47.03±0.93 µg/mL, respectively) compared with the other extracts but lesser activity than that in BHA and BHT (IC_{50}: 3.32±0.18 and 5.38±0.06 µg/mL, respectively), followed by methanolic extract (IC_{50}: 153.40±0.55 µg/mL), however the chloroform fraction exhibited a weak activity (IC_{50}: 305.59±0.41 µg/mL).

The scavenging capacity of ABTS also showed that the ethyl acetate fraction exhibited the greatest activity (IC_{50}: 10.22±0.26 µg/mL) among the extracts and closer to that in BHA and BHT (IC_{50}: 1.55±0.26 and 7.54±0.67 µg/mL, respectively), followed by butanol fraction (IC_{50}: 19.91±0.96 µg/mL), methanolic extract (IC_{50}: 27.32±0.79 µg/mL) and chloroform fraction (IC_{50}: 35.02±0.57 µg/mL).

The antioxidant activity using CUPRAC assay of the extract and different fractions was investigated as shown in Table 2, among the extracts the greatest activity was observed in the ethyl acetate fraction ($A_{0.5}$: 90.57±0.84 µg/mL) but lesser activity than that in BHA and BHT ($A_{0.5}$: 5.35±0.71 and 8.97±3.94 µg/mL, respectively), followed by chloroform and

butanol fractions ($A_{0.5}$: 110.49±0.51 and 123.03±0.35 µg/mL, respectively), however the methanolic extract exhibited a weak activity ($A_{0.5}$: 187.39±0.58 µg/mL).

The results for reducing power assay showed that the ethyl acetate and chloroform fractions gives the highest activity with close $A_{0.5}$ values (87.83±0.56 and 89.61±0.90 µg/mL, respectively) compared with the other extracts and further to the ascorbic acid and α-Tocopherol ($A_{0.5}$: 6.77±1.15 and 34.93±2.38 µg/mL, respectively), followed by butanol fraction ($A_{0.5}$: 129.58±0.62 µg/mL), while the methanolic extract exhibited the low activity ($A_{0.5}$: 158.07±0.12 µg/mL).

The antioxidant potential employing phenanthroline assay of the tested extracts and positive standards are as follows: BHA (0.93±0.07 µg/mL), BHT (2.24±0.17 µg/mL), ethyl acetate extract (3.78±0.64 µg/mL), chloroform (5.73±0.95 µg/mL), butanol extract (5.95±0.82 µg/mL) and methanolic extract (6.45±0.54 µg/mL).

The obtained results in Table 3 for β-carotene bleaching assay showed that among the extracts the ethyl acetate fraction was the most effective (IC_{50}: 27.21±0.99 µg/mL), followed by chloroform fraction (IC_{50}: 34.45±1.52 µg/mL), butanol fraction (IC_{50}: 40.32±0.56 µg/mL) and methanolic extract (IC_{50}: 70.93±0.69 µg/mL), however none of the extracts exhibited higher activity than those of BHA or BHT.

Concerning the Ferrous ions chelating assay all extracts exhibited a weak activity at 800 µg/mL, compared with EDTA (IC_{50}: 8.80±0.47 µg/mL).

3.4. Anticholinesterase activity

The inhibitory effect of the methanol extract/fractions of *P. harmala* on AChE and BChE was tested *in vitro* and the results were reported in Table 3 as percentages of inhibition at two final concentrations and IC_{50} values.

Table 3. Anticholinesterase activity of the crude extract and the different fractions of *P. harmala*

Samples	AChE (% Inhibition)			BChE (% Inhibition)		
	100 µg/ml	200 µg/ml	IC_{50} (µg/ml)	100 µg/ml	200 µg/ml	IC_{50} (µg/ml)
Methanol	62.20±1.93	71.14±2.03	65.38±1.47^{e}	76.14±0.38	78.82±1.64	24.76±0.86^{d}
Chloroform	88.25±0.46	91.90±0.57	14.69±0.10^{b}	94.95±0.11	95.75±0.78	7.36±0.77^{a}
Ethyl acetate	81.31±051	86.15±0.64	24.57±0.78^{c}	88.94±1.18	93.84±0.40	12.14±1.10^{b}
Butanol	83.47±0.11	86.02±0.66	36.01±0.18^{d}	85.15±1.75	89.92±1.46	17.60±0.31^{c}
Galantamine	91.80±0.20	94.77±0.34	6.27±1.15^{a}	73.57±0.77	78.95±0.58	34.75±1.99^{e}

Data are expressed as means ± standard deviation of three measurements; Different superscript letters represent significant variations (Tukey test, $p< 0.05$)

The crude extract and all fractions showed a high potential for inhibiting AChE. The chloroform fraction was the most active against AChE with an IC_{50} of 14.69±0.10 µg/mL, and a similar value to the galantamine (IC_{50}: 6.27±1.15 µg/mL), followed by ethyl acetate fraction (IC_{50}: 24.57±0.78 µg/mL), butanol fractions (IC_{50}: 36.01±0.18 µg/mL) and methanolic extract (IC_{50}: 65.38±1.47 µg/mL). We can observe that the inhibition of BChE by methanol extract and its fractions was generally more effective than that for AChE. Further, the chloroform fraction exhibited the most significant activity against BChE with an IC_{50} of 7.36±0.77, followed by ethyl acetate fraction (IC_{50}: 12.14±1.10 µg/mL), butanol fraction (IC_{50}: 17.60±0.31 µg/mL) and methanolic extract

(IC_{50}: 24.76±0.86 µg/mL) showing better effect than the galanthamine (IC_{50}: 34.75±1.99 µg/mL).

4. Discussion

In this study, the extraction yields varied significantly depending on the parameters of liquid-liquid fractionation of polyphenols, the extraction solvent as well as the extraction method. These parameters directly influence the biological activities of the final extracts [30]. A yield of 27.13% of the methanolic extract was close to that obtained by Atrooz et al. [15] and Rezzagui et al. [31] reporting an extraction yield equal to 19.9% and 20.18% respectively. Several previous studies have reported that the combination of water and organic solvent (methanol) provides relatively higher percentages of extractable compounds as compared to other solvents [32].

Furthermore, the bioactive phenolic compounds quantified in the methanolic extract and its subsequent fractions were better extracted by polar solvents, ethyl acetate, and butanol than solvents with low polarity, like chloroform. Our findings are in line with those of Bouchoukh et al. [33] who found the highest amounts of total phenolic and total flavonoid in the ethyl acetate fraction followed by the butanol fraction since the lowest value was obtained with the chloroform fraction. Additionally, the content of phenolic compounds in *P. harmala* has been reported in several preceding studies, including that of Abbas et al. [34] reporting a content of 142.03±0.1 mg GAE /g for total phenolic and 0.81±0.02 mg of QE /g for total flavonoid. Another study has also reported high amounts of TPC and TFC in methanol extract with a value of 650.38±30.3 mg GAE /g and 70.49±0.56 mg

QE /g respectively [35], and accordingly, a value of total phenolics content of 0.89 GAE mg /g was previously found in the methanol extract [15]. Similarly, Radjah et al. [36] have found the content of TPC and TFC as 72.454±0.214 mg GAE/g DW and 1.706 mg QE/g DW respectively in the methanolic extract, suggesting thus that such variability can be related to several factors, like geographical location and various environmental conditions.

In this study, the antioxidant activity of the plant extract/fractions was evaluated by eight complementary methods instead of a single technique to provide more conclusive results on the antioxidant properties, and this coincides with the previously performed studies [37]. Consequently, the methanolic extract and its subsequent fractions displayed a potent antioxidant activity by trapping free radicals, preventing β-carotène bleaching, and acted as reductants.

Here, the methanolic extract revealed remarkable antioxidant activity against the DPPH trapping with an IC_{50} of 133.18±1.22 µg/mL. This result was lower than that obtained with the methanolic extract of *P. harmala* by Khlifi et al. [38] and Abbas et al. [34], but close to that of El Abed et al. [39] and Benarous et al. [40] and higher than that of Atrooz et al. [15] and Radjah et al. [36]. In contrast, the work performed by Baghiani et al. [41] claimed that the methanolic extract is more effective than the ethyl acetate fraction of the same plant.

The obtained results of the antioxidant activity of the plant extract/fractions evaluated by the ABTS assay are similar to those found by Benarous et al. [40]. Accordingly, the methanolic extract of *P. harmala* showed a potent antioxidant activity (IC_{50} = 44.63±1.08 µg/mL), and unlike, the study of Khlifi et al. [38] recorded a weak antioxidant activity of the methanolic extract with an IC_{50} of 90.7±1.83 µg/mL.

Moreover, the reducing power activity of the plant compounds, a valuable antioxidant marker, [42] determined in the crude extract and all fractions showed remarkable reducing properties via iron, and this coincides with study results reported by Guergour [43] and Mazandarani et al. [44]. However, the ferrous ions chelating assay revealed a weak antioxidant activity in the crude extract and all fractions, and this agrees with the findings of Baghiani et al. [41].

On the other hand, the results obtained by the β-carotene assay indicated that the ethyl acetate, chloroform, and butanol fractions are more active than the methanolic extract. Also, the ethyl acetate fraction exhibited a strong antioxidant activity with IC_{50} of 27.21±0.99 µg/mL, and this is alike to that reported by Baghiani et al. [41], and unlike that of Elansary et al. [45] reporting a highly potent antioxidant activity in the methanolic extract of *P.harmala* leaves as determined by the β-carotene assay.

On top of that, the CUPRAC, GOR, and Phenanthroline assays showed an interesting antioxidant activity in the crude extract and all its fractions, in particular, the ethyl acetate fraction displaying the best activity ($A_{0.5}$: 90.57±0.84 µg/mL). This result concords with that of Taştekin [46], reporting the highest antioxidant activity determined by CUPRAC assay in the ethyl acetate fraction of *P. harmala* growing in Turkey.

The tested extracts of *P. harmala* revealed a very higher inhibitory activity against AChE and BChE, owing to the presence of bioactive inhibitory phytomolecules. The chloroform fraction displayed the most potent effect against AChE and BChE, despite its low content in phenolic and flavonoid compounds. These findings suggest the involvement of other bioactive compounds such as alkaloids and coumarins in the inhibition of these enzymes. A similar result was found in the ethyl acetate and butanol fractions, suggesting the role of the flavonoid content against the cholinesterase activity. In addition, the extracts inhibited a more effective effect on BChE than AChE, and hence their activity explains the specificity to BChE rather than AChE. Results showed also a good inhibitory effect of the methanolic extract against AChE (IC_{50}: 65.38±1.47 µg/mL) which is similar to that reported in the study of Ali et al. [47] conducted on the methanolic extract of Egyptian *P.harmala*, showing an important AChE inhibitory activity (IC_{50}: 68 µg/mL). The BChE effect of the methanolic extract of the aerial parts of Tunisian *P.harmala* has recently been reported by Edziri et al. [48]. As reported, the β-carboline alkaloids are the main bioactive compound of *P. harmala* having a potent effect against AChE [49]. Among β-carboline molecules, harmaline which is a common dihydro b-carboline type has interesting pharmacological effects, and is able to interact with numerous enzymes and neurotransmitters, including topoisomerase I, and monoamine oxidase-A [50].

5. Conclusion

In the light of the results obtained, it can be concluded that the crude extract and all fractions of *P. harmala* seeds have an important antioxidant activity mainly the ethyl acetate fraction that can be assigned to their phenolic contents since high amounts of total phenolic and flavonoids have been recorded in the extracts. The results also revealed a powerful anticholinesterase especially the chloroform fraction exhibiting an effect comparable to or even more potent than the standard used. From these outcomes, it can be suggested that *P. harmala* seeds can constitute a prominent source of bioactive molecules for human health purposes, both in pharmaceutics and nutritional industrial products.

The study needs to be completed by future research investigating the phytochemical analysis of the bioactive molecules using HPLC and LC-MS methods.

6. Conflicts of interest

There are no conflicts to declare

7. References

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