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CYTOTOXIC, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *Peganum harmala* L. EXTRACTS

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Peganum harmala L., known as 'Harmel', is a plant widely used in the traditional Algerian medicine.

Aim. The purpose of this work is to study the antioxidant, antiproliferative and antimicrobial potential of *Peganum harmala* extracts.

Methods. Colorimetric methods were used to quantify phenolic compounds, while the antioxidant activity was estimated *in vitro* using DPPH/ABTS radical scavenging assay, ferric reducing power, β-carotene bleaching assay, total antioxidant capacity, and ferrous iron chelating assay. The agar well diffusion and the broth microdilution method were used to evaluate the antibacterial activity and the MTT assay was used to test the cytotoxicity of the extracts.

Results. The ethanolic extracts of *Peganum harmala* L. showed the highest polyphenols content and the potent antioxidant, gave a good activity against Gram+ and Gram− bacteria and good antifungal effect and were more cytotoxic to the HeLa cell line.

Conclusions. It is concluded that selected plants could be a potential source of bioactive compounds with antioxidant, antimicrobial and antiproliferative potential. Hence, it is indicated to further investigate this plant *in vitro* as well as *in vivo* for new drug discovery.

Key words: *Peganum harmala*; polyphenols; antioxidant activity; antimicrobial effect; cytotoxic effect.

Natural products are attracting growing interest by phytotherapy researchers due to their safety and medicinal properties [1]. Medicinal plants have been used for many years as remedies for both human and animal ailments [2]. Aromatic and medicinal plants as the key source of complementary and alternative medicine have been recently bring many hopes in alleviating of symptomatology and curing associated with so many diseases [3]. The World Health Organization (WHO) reported that around 80% of the world's population uses traditional medicine for their primary health care [4]. The beneficial health effects of these plants are mainly related to the presence of phenolic compounds [5].

Polyphenols of medicinal plants are powerful antioxidants capable of fighting against free radicals (Reactive Oxygen Species — ROS), which are involved in the appearance of several pathologies such as cancer, diabetes, Alzheimer's disease, and cardiovascular disorders [6]. Polyphenols present a wide range of other bioactivities, including anti-inflammatory, anticarcinoma, antiviral and cardioprotective effects [7]. Phenolic compounds may also play a critical role as antimicrobial agents, a property that has received increased attention due to their potential effects against antibiotic-resistant bacteria [8].

Algeria, given its privileged biogeographical position and its extent

between the Mediterranean and sub-Saharan Africa, is considered among the countries known for their floristic diversity to which is added a secular tradition of traditional use of plants. Among the medicinal plants, the genus *Peganum* commonly called harmel, is a plant of the family *Zygophyllaceae* [9], is widely distributed in arid and semi-arid regions [10].

Peganum harmala L. is known for its wide use in folk medicine for the treatment of various diseases including diabetes and hypertension, lumbago, asthma, colic, hepatitis B, jaundice, and as an emmenagogue stimulant [11]. *P. harmala* has anti-carcinogenic [12], vasodilator, anti-protozoal, and anti-inflammatory effects [13, 14]. Because of their analgesic and antibacterial properties [15], their fruits are used in traditional medicine. In addition, alkaloid from *P. harmala* has antihemosporidian, antinociceptive and antineoplastic effects [16]. The seeds of *P. harmala* contains a large number of alkaloids and β-Carboline alkaloid and used in fever,abortion, red dye, diarrhea and many other human chronic diseases [17]. *Peganum harmala* seeds also comprises a large extent of total phenolic and total flavonoids component and act as strong antioxidant plant [18].

The main objectives of this study were to determine the antioxidant, cytotoxic and antimicrobial activities of extracts of the plant *P. harmala* L.

Materials and Methods

Plant material

Peganum harmala L. (Zygophyllaceae) seeds were collected during flowering stage El Hammadia, wilaya of Bordj Bou Arreridj. The identification of the plant was carried out by Pr. Boudjelal Amel and a voucher specimen (No. AB-65, 2018) was deposited at the University Mohamed BOUDIAF —M'Sila. The seeds of *P. harmala* L. were dried in dark, at room temperature for two weeks and powdered using electrical grinder and resulting powder was then stored until use.

Extracts preparation

To obtain the *aqueous extract (AqE)* of *P. harmala* L. following the method of Mbiantcha *et al*. [19] maceration has been made with 50 g of the seed powder in 500 mL of distilled water for 3 days. *The ethanolic extract (EtE)* was prepared from a mixture of 50 g of *P. harmala* L. powder in 200 mL of ethanol 80%; the mixture was shaken for 8 h and incubated overnight at room temperature. *The decoction extract (DcE)* was prepared according to the method of Robeson and Strobel [20] with some modification; 50 g of powdered plant material were mixed with boiling distilled water (200 mL) for 1 hour. All extracts were filtered through a Whatman No. 3 filter paper and evaporated using a rotary evaporator under vacuum at 40 °C until completely dried, to obtain AqE, EtE and DcE, which were stored at 4 °C until use.

Spectrophotometric estimation of phenolic compounds

The total phenolic content of the various extracts of *P. harmala* L. was determined using the Folin-Ciocalteu reagent. The results were expressed in µg of Gallic acid equivalent per mg of dry extract (µg of GAE/mg extract) [21].

Total flavonoid content was determined following the trichloride aluminum assay [22]. The results were expressed as µg quercetin equivalent per mg of dried extract (µg of QE/mg extract).

Total flavonols content in *P. harmala* L. extracts were determined according to the method of Kosalec et al. [23]. 0.5 mL of standard solution or extracts was separately mixed with 1.5 mL of ethanol and 0.1 mL of 10% methanolic aluminum chloride solution, then 0.1 mL of sodium acetate and 2.8 mL of water were added and incubated for 30 min at room temperature. The absorbance was measured at 415 nm. The results were expressed as µg of quercetin equivalent per mg of dried extract (µg of QE/mg of extract).

Antioxidant activity

The antioxidant activity was measured by using the phosphomolybdenum assay [24], which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green Phosphate / Mo (V) complex at acidic pH. The greenish color is measurable at 695 nm in the presence of reducing agent. The total antioxidant capacities were expressed as a µg equivalent of ascorbic acid per mg of dry extract (µg AAE/mg).

The 1.1-diphenyl- 2-picrylhydrazyl radical (DPPH) were also used for antioxidant activity. 375 µL of extracts or standard at the various concentrations was mixed with 125 µl of a methanol solution of DPPH (0.004%). After 30 min of incubation, the absorbance was recorded at 517 nm [25]. L′hydroxyanisolebutylé (BHA), L′hydroxytoluènebutylé (BHT) and Quercetin were used as standards; the scavenging activity was calculated according to equation (1):

$$\text{Inhibition}\ (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100. \quad (1)$$

To assess the scavenging activity of the extracts against the ABTS radical cation (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)),the reaction mixture included ABTS (7 mM) and potassium persulfate (2.45 mM). The extracts or standard (100 µL) were homogenized with 1.9 mL of ABTS solution and incubated for 7 min. The absorbance was measured at 734 nm [25]. The capacity of the extract to scavenge the ABTS radical was calculated according to the previous formula (1). Trolox was used as positive control, and the results were expressed in µmol g^{-1} of Trolox-equivalent antioxidant capacity (TEAC).

To assess the extract antioxidant properties in mechanisms involving iron, both *ferric reducing power (FRP)*and *ferrous iron chelation assays* were used. FRP was used to assess the extracts capability to reduce iron ions [26]. The absorbance was recorded at 700 nm and the EC_{50} value (effective concentration for that has an absorbance of 0.5) was calculated.

To evaluate extract-iron interaction, the ferrozine test was performed following the method of Decker and Welch [27], modified by Le et al. [21]. The red chromophore of the Fe^{+2}-ferrozine complex was measured spectrophotometrically at 562 nm against a blank. Ethylene diamine tetra acetic acid (EDTA) was used as reference chelator. The inhibition of ferrozine–Fe^{2+} complex formation was calculated according to equation (1). An IC_{50} value is defined as the inhibition concentration of tested material, which produces 50% of the maximal scavenging effect.

To assess the extracts antioxidant properties in lipidic like-systems, *the β–carotene/linoleic acid assay* was used. The antioxidant capacity of each extract was evaluated by measuring the inhibition of the volatile organic compounds and the conjugated diene hydro-peroxides arising from linoleic acid oxidation [28]. The antioxidant activity of samples and standards BHT (2 mg/mL) are calculated after 24h at 490 nm according to the following equation (2):

$$AA\% = (Abs_{test} / Abs_{BHT}) \times 100 \qquad (2)$$

where Abs_{test}: Absorbance of the extract; Abs_{BHT}: Absorbance at t = 0 in the presence of positive control BHT.

The results were expressed as IC_{50} (the concentration required to inhibit 50% of the β-carotene bleaching). Synthetic antioxidant BHT was used as positive control and methanol and distilled water were used as a negative control.

Antiproliferative activity

HeLa cells(cervical cancer line, adherent) were used to investigate the cytotoxicity effect of *P. harmala* extracts, using MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) [29]. The cells were grown in RPMI 1640 medium (Gibco) supplemented with 10% (v/v) fœtal calf serum(FCS) and 2 mM L-glutamin with 400 µL antibiotic 50 U/mL of gentamycin and penicillin and 50 µg/mL of streptomycin (Sigma). The cultures were then maintained at 37 °C in an incubator at 5% CO_2 and saturated with water vapor 95% .

HeLa cells (4.44×10^5 cells/well) were seeded in 96-well cell culture plates for 48 hours in the presence or absence of extract (10 µL). After this period, 10 µL of MTT solution (1 mg/mL in phosphate-buffered saline; PBS) were added to each well and incubated for 4 h at 37 °C in a CO_2-incubator. 180 µL of the medium was removed from every well without disturbing the cell clusters. Formazan crystals were dissolved using 180 µL methanol/DMSO solution (50:50), and the preparations were thoroughly mixed on a plate shaker for 30 min in the room temperature with the cell containing formazan crystals.

Finally, after the dissolution of all crystals, the absorbance of each well was determined with a microplate reader (ELx 800) at 570 nm. The results were expressed as a percentage inhibition of cell proliferation and calculated according to the following formula (3):

$$\% \text{ Inhibition} = 100 - \% \text{ viability}, \qquad (3)$$

where % viability = $(Abs_{extract} \times 100)/Abs_{control}$

The IC_{50} of the extracts was determined as the drug concentrations that reduced cell number by 50% in treated compared to untreated cultures.

Antimicrobial activity

Microbial strain

The antibacterial activity was determined using clinical referenced strains bacteria, including Gram (+) and Gram (–) bacteria from the American Type Culture Collection (ATCC) standards. The strains were *Escherichia coli* (ATCC25922), *Salmonella thyiphimurium* (ATCC13311), *Proteus mirabilis* (ATCC35659), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC10876) and *Micrococcus luteus* (ATCC469). All the strains were obtained from the Laboratory of Bacteriology at Setif hospital, Algeria. Plant pathogenic fungus: *Fusarium culmorum*, *Aspergillus carbonarius*

(M333), *Aspergillus flavus* (NRRL 3251), *Penicillium glabrum* were obtained from the Laboratory of Applied Microbiology at the Ferhat Abbas University of Sétif, Algeria.

Antibacterial activity

The agar well diffusion method was employed for the determination of antibacterial activities of extract [30]. The extracts were dissolved in DMSO to obtain the doses of 9, 6 and 3 mg/well and sterilized by filtration trough 0.22 µm Nylon membrane filter. The bacterial strains were cultured in sterile distilled physiological water for 24 h. Petri dishes of sterile Mueller-Hinton agar were inoculated by the method of streak with the appropriate bacterial suspension (0.5 McFarland). Wells were cut into the agar and were loaded with 10 µL of extract.

Additionally, and for comparative purposes, standard gentamicin (10 mg/disc) and chloramphenicol (30 mg/disc) is used in the test as positive controls. All the plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition in millimeters.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The values of MIC, which represent the lowest extract concentration that completely inhibits the growth of microorganisms, were determined by a micro-well dilution method [31]. Briefly, 100 µL of Mueller Hinton broth (MHB) containing 0.02 g/L phenol red was dispensed into each well of a -96 well microplate. 100 µL of extract is then added to each well in the first column of the microplate except that of the second row. Serial twofold dilutions were carried out until the 12^{th} well of each row. Then, 100 µL of MHB without red phenol were added in the first well of the second row before diluting (positive controls). All the wells were inoculated, except those in the first row (negative controls), by introducing 100 µL of bacterial suspension (10^6 CFU)/mL). On the other hand, MHB without red phenol was put in the first row's wells. The microplate was wrapped loosely and then incubated at 37 °C for 24 hours. The appearance of a yellow color indicates a bacterial multiplication, while the persistence of the initial red color means the contrary.

To determine MBC, the broth was taken from each well without visible microbial growths from the MIC are re-isolated on Muller-Hinton agar. The wells from the positive and negative control rows were also seeded on the same agar to ensure no bacteria growth in the wells. After 24 h of incubation in an oven at 37 °C, the number of colonies on the streaks was compared to those of the control dish seeded with streaks of dilutions of the bacterial inoculum. On reading, the lowest concentration of extract for which no microbial colony is observed (99.99% destruction) corresponds to MBC.

Antifungal susceptibility test

Antifungal activity was tested against four fungal strains using the agar diffusion method. The fungal suspension was obtained from spores after seven days at 28 °C and prepared in sterile distilled water (OD 0.12–0.15 at 525–530 nm). An aliquot of 0.1 mL of this fungal suspension was spread over the surface of the agar plate. Wells (7 mm diameter) were cut into the agar and impregnated with extracts (9, 6 and 3 mg/disk) or with positive controls like Rifampicin (5µg/well). The petri dishes were placed in an oven at a temperature of 28 ± 2 °C for a period of incubation of 10 days. For the determination of the effect of *P. harmala* L. extracts on radial growth against the tested fungus, the diameter of the disc growth was measured every 48 hours of incubation for 10 days.

Statistical analysis

The results are expressed as mean ± SD of triplicate measurements. The GraphPad Prism Software (version 8.00) was used for statistical analysis. Data were analyzed using Student's t-test and one-way analysis of variance (ANOVA), followed by Dunnett test for multiple comparison. The differences were considered significant at $P < 0.05$.

Results and Discussion

Quantitative analysis of phytochemicals

The extraction was performed using ethanol 80%, and water. The yield of extraction of the *P. harmala* L. extracts was significantly different ($P < 0.05$) (Table 1). The yield of extraction of the AqE and the EtE was similar to that found by Zainab et al. [32]. The extraction yield appears to be influenced by the several factors such as the polarity of the solvent [33].

Polyphenols, flavonoids and flavonols are very important compounds in plants because their therapeutic interest and their antioxidant activity have attracted most attention. The results obtained in this study (Table 1) showed a high level of phenolic, flavonoids and flavonols compounds in EtE. The DcE and AqE have average amounts of these compounds.

Table 1. **Yield and secondary metabolites content from *P. harmala* L. extracts**

Extract	Yield (%)	Total polyphenols (µg GAE/mg)	Total flavonoids (µg QE/mg)	Total flavonols (µg QE/mg)
Ethanolic	12.8 ± 0.40^c	102.68 ± 1.61^c	30.33 ± 1.06^c	108.05 ± 1.88^c
Aqueous	12.56 ± 0.67^c	65.92 ± 1.23^c	15.51 ± 0.50^c	46.39 ± 0.23^c
Decoction	10.62 ± 0.70^c	80.62 ± 0.59^c	20.45 ± 1.06^c	52.81 ± 0.87^c

Values were expressed as means ± SD ($n = 3$). Different letters in the same columns indicate significant difference (c: $P < 0.0001$). GA: Galic acid equivalents; QE: Quercetin equivalents.

Concerning the content of polyphenols in EtE and AqE it was almost similar that found by Zainab et al. [32]. It can be concluded that the phenolic or flavonoid compounds contained in the extracts were influenced by their solubility in the solvent used for extraction.

Antioxidant activity

The antioxidant properties of the extract of *P. harmala* was assayed *in vitro* using different antioxidant methods covering these mechanisms. The TAC of various extracts was estimated using phosphomolybdate assay. The present study reveals that the Ethanolic extract (EtE) showed higher antioxidant activity than aqueous extract (AqE and DcE) (Table 2).

Results showed that the extracts of *P. harmala* L. were able to decolorize the stable, purple-colored DPPH radical into yellow-colored DPPH-H. From the IC_{50} values obtained, the DcE and EtEwas found to exhibit the greatest scavenging activity compared to AqE. All extracts showed lower DPPH scavenging activity than BHT, BHA and quercetin used as standards antioxidants ($P< 0.01$). The comparison of our results with other works, shows that the EtE of the seeds of Algerian *P. harmala* showed a significant scavenging effect to what is obtained by the EtE from the Libyan *P. harmala* (IC_{50}: 179.62 ± 7.32 g/mL) [34]. The results are also compared with the IC_{50} of the ethanolic extract of *P. harmala* reported in Morocco by Khadhr et al. [35] (53.64 ± 0.5 g/mL).

Similarly, to DPPH assay, ABTS assay revealed that EtE exerted a significantly higher antioxidant capacity ($P < 0.05$). In contrast, Wang et al. [36] found that some compounds, which have ABTS scavenging activity, did not show DPPH scavenging activity as in the case of the DcE.

The reducing power is associated with antioxidant activity; it may serve as a significant indicator of electron donating ability of phenolic compounds. *P. harmala* L. extracts showed considerable reducing power, indicating that they can act as electron donors and could react with free radicals to convert them into more stable products. The result showed that the EtE exhibited the highest reducing capacity followed by AqE and DcE. The reducing power of all extracts was lower than that of ascorbic acid, BHA and quercetin ($P < 0.01$) (Table 2). The chelating capacity of our extracts is important and probably due to the presence of antioxidant molecules capable of complexing with ferrous ions, it helps inhibit peroxidation. From the IC_{50} values obtained (Table 2), the potency of ferrous iron-chelating ability of DcE is statistically lower than those of the EtE and AqE extracts. IC_{50} values show that DcE exhibited potent chelating activities, but remained inferior to that of EDTA ($P < 0.01$).

Furthermore, the extracted phenolic compounds presented also the capacity to inhibit lipid peroxidation in the β-carotene/linoleic acid system. Results showed that all extracts gave a high inhibition and exhibited a high capacity to prevent the bleaching of β-carotene after 24 h of incubation as compared to the positive control (Fig. 1). In the present work, it was found that the EtE (73.98 ± 0.21%) were more powerful in inhibiting the β-carotene bleaching than AqE and DcE (38.61 ± 0.19% and 43.34 ± 0.89%, respectively). Ehanolic extracts of *P. harmala* L. containing higher amount of compounds which have a role in lipid peroxidation, compared with aqueous and decoction extracts. This results are in accordance with that found by Baghiani et al. [37]. The strong antioxidant effect of these compounds may be explained by the "polar paradox" phenomenon [38].

Antiproliferative activity

The effect of different concentrations of the extracts on HeLa cells survival was studied using the MTT reagent. The results obtained suggest that EtE potently suppressed

Table 2. **The *in vitro* antioxidant activity of *P. harmala* L. extracts, BHA, BHT, Gallic acid, ascorbic acid and EDTA**

Sample	DPPH · IC_{50} (µg/mL)	ABTS · + (µmol g^{-1} TEAC)	Iron chelating IC_{50} (mg/mL)	Total antioxidant activity (µg AAE/mg)	Reducing power EC_{50} (µg/mL)
Ethanolic	61.16 ± 0.82^{d}	2.107 ± 0.01^{c}	1.127 ± 0.06^{b}	123 ± 0.96^{b}	828 ± 0.13^{d}
Aqueous	178 ± 0.30^{d}	1.26 ± 0.01^{c}	1.478 ± 0.05^{b}	65.47 ± 2.19^{a}	848 ± 0.32^{d}
Decoction	108 ± 0.74^{d}	1.37 ± 0.018^{c}	0.74 ± 0.05^{b}	37.31 ± 1.05^{b}	969 ± 0.80^{d}
BHA	5.14 ± 0.17^{d}	/	/	/	34.01 ± 1.33^{d}
BHT	28.10 ± 0.17	/	/	/	/
Quercetin	1.13 ± 0.01^{d}	/	/	/	45.85 ± 0.41^{d}
EDTA	/	/	0.023 ± 0.05^{b}	/	/
Ascorbic acid	/	/		/	22.71 ± 0.2^{d}

Values were expressed as means ± SD ($n = 3$). Different letters in the same columns indicate significant difference (c: $P < 0.0001$). GA: Galic acid equivalents; QE: Quercetin equivalents.

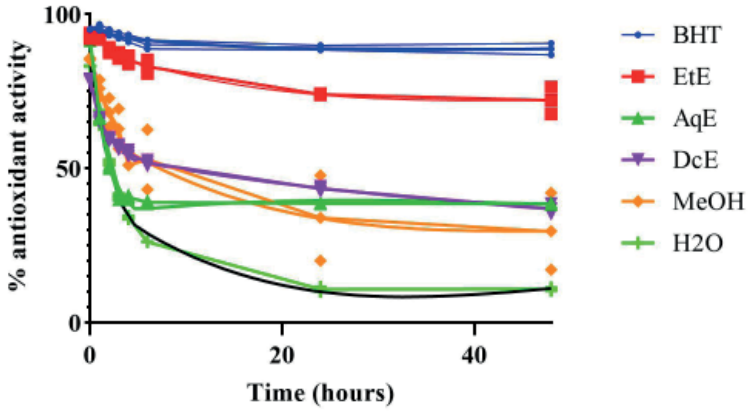

Fig 1. **Kinetics of β-carotene bleaching in the presence of AqE, EtE, DcE, water, methanol and BHT during 24 h**
AqE: aqueous extract, EtE: ethanolic extract, DcE: decoction extract, BHT: butylatedhydroxytoluene, MeOH: methanol

the proliferation of HeLa cells: 36.48% at 0.015 mg/mL and to 97.99% at 2 mg/mL (Fig. 2), this shows that they are potent inhibitors of cancer cell growth. While the growth of the same cells exposed to aqueous and decoction extracts was not significantly reduced. The IC_{50} values of EtE, DcE and AqE were evaluated to 0.028 ± 0.004 mg/mL, 0.242 ± 0.040 mg/mL and 0.230 ± 0.014 mg/mL, respectively.

The EtE induced a significant decrease in cell viability and presented lower IC_{50} (< 30 µg/mL) for HeLa cell compared to AqE and DcE, so we can consider them as active and potential source of cytotoxic molecules toward HeLa cancer cells. While, the aqueous and decoction extracts can be considered as poorly cytotoxic for HeLa cells since the IC_{50} values are in the range (100–1 000 µg/mL). Cytotoxic effects of the crude seed extract of this plant, such as aqueous and hydro-alcoholic extracts have been previously reported, indicating considerable cytotoxic potential [39]. These cytotoxic natural products may be able to play a vital role in treating selected cancers by working in synergy with conventional chemotherapeutic drugs [40].

Antibacterial activity

Faced with the problems of bacterial resistance to synthetic antibiotics, much work has been done on the antimicrobial

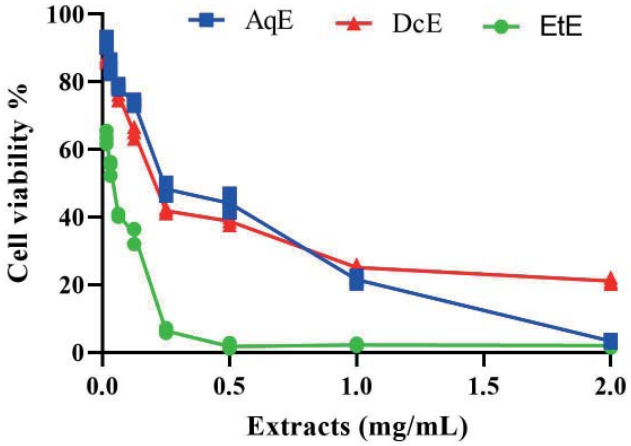

Fig. 2. ***P. harmala*** **L. extracts inhibited the viability of HeLa cell line in a dose-dependent manner**
Cell viability was determined using MTT assay and expressed as means ± SD.
AqE: aqueous extract, EtE: ethanolic extract, DcE: decoction extract

power of natural plant extracts. The *in vitro* antibacterial activity of AqE, EtE and DcE was evaluated using the agar diffusion method. Based on the results obtained, it is observed that the zones of inhibitions increase with the increase in the dose of the extracts. In addition, all our extracts were effective against all bacterial strains examined compared to the commercial antibiotics (gentamicin and ciprofloxacin) (Fig. 3). These results are in agreement with the bibliographic data of Guergour [41]. According to Biyiti et al. [42], an extract is considered active if its inhibition zone is greater than or equal to 10 mm. From these results, it can be seen that the Gram– bacteria possess strong resistance compared to Gram+ bacteria, which can be attributed to the difference in the morphology of the cell wall [43]. The hydroalcoolic extract was found to be the most effective of the tested solvents, with maximum activity (zone of inhibition) against all strains tested compared to the aqueous extracts. Similar results were observed by Djarmouni et al. [44], they also used different solvent extracts against a wide-range of microorganisms.The same results was also reported by Arif et al. [45], and the antimicrobial activity was tested on four different bacteria using a disc diffusion assay.

The results of MIC and MBC of *P. harmala* L. extracts are shown in Table 3. As can be noted in this finding, EtE showed a higher antibacterial activity against *S. thyiphimurium* and *M. luteus* with a MIC value of 1.5625 mg/mL. The results indicate that the CMB / MIC activity ratio is less than or equal to 4 for each given extract, when the CMB/MIC ratio of a substance is less than or equal to 4, this substance is considered to be bactericidal, while it is said to be bacteriostatic if this ratio is greater than four [46]. These results states that all the extracts tested are bactericidal against the bacterial strains tested. With the exception of AqE appear to have a bacteriostatic effect on *Bacillus aureus* and bactericidal on *S. thyiphimurium* and *M. luteus*.

Antifungal susceptibility test

Fungi and yeasts may cause serious pathologies that affect humans, among which we can mention mycotoxicosis. The results obtained, show that after 48 h of incubation at 28 °C, all extracts inhibited fungal growth except that of *A. flavus*. The incubation time of fungal cultures treated with *P. harmala* L. extracts is calculated during a period of 10 days. During this period, it appears that the zones of inhibition produced by extracts against filamentous fungi tested remained clear during the 10 days of incubation. EtE manifested a strong activity by diameters of inhibition varying between 20 to 9 mm. However, the DcE and AqE only inhibited *A. carbonarius* during the entire incubation period by diameters of inhibition varying between 10 to 20 mm and 9 to 13 mm, respectively. On the other hand, absence of inhibition on the 6^{th} day and for the entire remaining incubation period against *F. culmorum* and *P. glabrum* fungi who developed resistance against DcE and AqE with the disappearance of the inhibition zones due to sporulation of the fungus. It should be noted that the positive control (Rifampicin) is not active against all fungi tested with a dose of 5 µg/disc. Our results are in agreement with

Table 3. **The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values (mg/mL) of the *P. harmala* L. extracts against three human pathogen bacteria strains tested in microdilution assay**

Extracts	CMI and CMB	*B. cereus*	*S. thyiphimurium*	*M. luteus*
Ethanolic	CMI CMB CMB/CMI	3.125 6.25 2	1.5625 6.25 4	1.5625 1.5625 1
Aqueous	CMI CMB CMB/CMI	3.125 200 64	12.5 3.125 0.25	3.125 12.5 4
Decoction	CMI CMB CMB/CMI	3.125 12.5 4	12.5 12.5 1	12.5 12.5 1

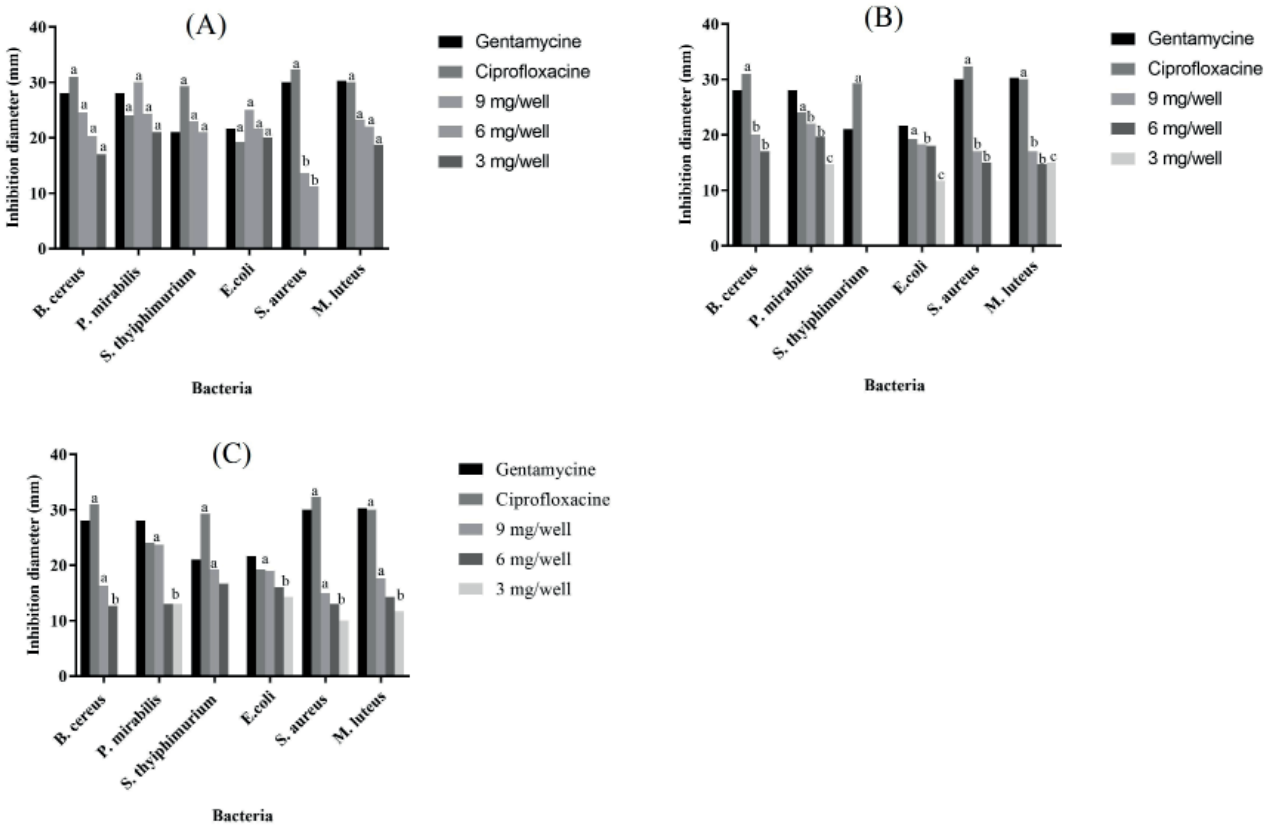

Fig 3. **Zone of inhibition of the *P. harmala* L. seeds extracts and control (ciprofloxacin, gentamycin) against six bacterial strains**

Values were expressed as means ± SD ($n = 3$). Analysis of variance (ANOVA) revealed significant effect ($P < 0.05$). Different letters (b, c and d) significant difference while 'a' indicate a non-significant difference ($P > 0.05$)

those of Diba et al. [47] and Guergour [41] who showed a greater effect of the alcoholic extract of *P. harmala* L. on the sporulation of the yeast Candida and most species of the genus *Aspergillus*. Decreases in inhibition diameter, by prolonging the incubation time, may indicate that the fungi have adapted to the constituents of the *P. harmala* L. extracts and began to develop resistance to the extracts.

Conclusion

In conclusion, *Peganum harmala* L. seeds were extracted with various solvents including water and ethanol. From the present work, it could be concluded that the solvent play an important role in the extraction of the plant constituents. *P. harmala* L. extracts were found to be rich in phenolic compounds and

flavonoids. In terms of antioxidant activity, it is concluded that *P. harmala* L. extracts possessed potent antioxidant activity and could be utilized as new natural antioxidant in food and therapeutics. The cytotoxic and antiproliferative effect show that the extracts have significant *in vitro* cytotoxic activity on HeLa cell line. The results of the present investigation suggest that *P. harmala* L. extracts have a significant antitumor activity, and could be an important basis for the design and synthesis of new antitumor drugs. This study showed that *P. harmala* L. may be a potential source of antimicrobial drug against the strains tested. This is particularly important in the fight against the recent multi-resistant organisms with drugs.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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ЦИТОТОКСИЧНА, АНТИОКСИДАНТНА ТА АНТИМІКРОБНА ДІЯ ЕКСТРАКТІВ *Peganum harmala* L.

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Peganum harmala L., відома як «Harmel», є рослиною, що її широко використовують у традиційній алжирській медицині.

Мета. Вивчити антиоксидантний, антипроліферативний та антимікробний потенціал екстрактів пегану гармали.

Методи. Для кількісного визначення фенольних сполук використовували колориметричні методи, тоді як антиоксидантну активність оцінювали *in vitro* за допомогою аналізу поглинання радикалів DPPH/ABTS, відновлювальної сили заліза, аналізу відбілювання β-каротину, загальної антиоксидантної здатності та аналізу хелатування двовалентного заліза. Для оцінювання антибактеріальної активності використовували дифузію з лунки з агаром і метод мікророзведення бульйону, а для перевірки цитотоксичності екстрактів застосовувадт MTT-аналіз.

Результати. Етанольні екстракти *Peganum harmala* L. показали найвищий вміст поліфенолів і потужний антиоксидант, високу активність проти Грам-позитивних і Грам-негативних бактерій і хороший протигрибковий ефект і були більш цитотоксичними для лінії клітин HeLa.

Висновки. Обрані рослини можуть бути потенційним джерелом біоактивних сполук з антиоксидантним, антимікробним та антипроліферативним потенціалом. Таким чином, показано подальше дослідження цієї рослини *in vitro*, а також *in vivo* для відкриття нових ліків.

Ключові слова: *Peganum harmala*, поліфеноли, антиоксидантна активність, антимікробна дія, цитотоксичний ефект.