

Evaluation of antioxidant and antimicrobial activities of chloroformic and methanolic extracts of 6 important medicinal plants collected from Northern of Iran

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Abstract

Background: The plant essential oils and extracts are possible sources of natural bioactive molecules and have been selected globally for new antimicrobial compounds, food preservatives, and alternatives to treat infectious disease.

Methods: In this research, antimicrobial activities of chloroformic and methanolic extracts of *Sophora flavescens*, *Rhaponticum repens*, *Alhagi maurorum*, *Melia azedarach*, *Peganum harmala*, and *Juncus conglomeratus* were evaluated against 8 bacteria (*S. aureus*, *B. subtilis*, *R. toxicus*, *P. aeruginosa*, *E. coli*, *P. syringae*, *X. campestris*, *P. viridiflava*) and 3 fungi (*Pyricularia oryzae*, *Fusarium oxysporum* and *Botrytis cinerea*) through disc diffusion method. Furthermore, the essential oil of plants with the highest antibacterial activity was analyzed utilizing GC/MS. Moreover, tested plants were exposed to screening for possible antioxidant effect utilizing DPPH test, guaiacol peroxidase, and catalase enzymes. Besides, the amount of total phenol and flavonoid of these plants was measured.

Results: Among tested plants, methanolic and chloroformic extracts of *P. harmala* fruits showed the highest antibacterial activity against tested bacteria. Also, the investigation of free radical scavenging effects of the tested plants indicated the highest DPPH, protein, guaiacol peroxidase, and catalase in *P. harmala*, *M. azedarach*, *J. conglomeratus* fruits, and *J. conglomeratus* fruits, respectively. In addition, the phytochemical analysis demonstrated the greatest amounts of total phenolic, and flavonoid compositions in *J. conglomeratus* and *P. harmala*.

Conclusion: The results indicated that these plants can act as a promising antimicrobial agent due to its short killing time.

Introduction

The plant essential oils and extracts are possible sources of natural bioactive molecules and have been selected globally for new antimicrobial compounds, food preservatives, and alternatives to treat infectious disease [1]. There are many researches about the antibacterial and antifungal activity of plant extracts and essential oils [2–6]. For example, Srinivasan et al. [7] measured the antimicrobial activity of fifty medicinal plants including *Eucalyptus globulus*. The results showed that *Eucalyptus globulus* has antimicrobial activity versus *Chromobacterium*, *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella paratyphi*, *S. typhi*, *Bacillus subtilis*, and *Staphylococcus aureus* bacteria and didn't show any antifungal activity on the tested fungus. Nagata et al. [8] investigated the antimicrobial activity of macrocarpals, which are phloroglucinol derivatives contained in *Eucalyptus* leaves versus a diversity of bacteria containing oral bacteria. Among tested bacteria, *P. gingivalis* presented the maximum sensitivity to macrocarpals. Furthermore, its trypsin-like proteinase activity and binding to saliva-coated hydroxyapatite beads were inhibited with macrocarpals. Hayet et al. [9] evaluated antibacterial activities of ethyl acetate, chloroform, butanol and methanol extracts of *peganum harmala* leaves against some pathogens containing 11 gram-positive and 6 gram-

negative bacteria and between tested extracts, methanol, and chloroform extracts exhibited a higher antibacterial activity versus gram-positive than gram-negative bacteria. Han and Guo [10] investigated the antibacterial activity of *Angelica sinensis* extract (AE), *Sophora flavescens* extract (SE), and herb pair *A. sinensis* and *S. flavescens* extract (HPE) and displayed HPE had strong antibacterial activity on *Escherichia coli*, *Staphylococcus aureus*, *Shigella castellani*, and *Chalmers*. Also, SE was moderately active to *E. coli*. Moreover, Sen and Batra [11] examined antimicrobial activity of ethanol, methanol, petroleum ether and water extracts of *Melia azedarach* L. leaves versus 8 human pathogens including *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxisporum*, and *Rhizopus stolonifera*. All the extracts indicated considerable activity versus all pathogens, but, the alcoholic extract exhibited maximum inhibitory concentration versus all the microorganisms. In addition, Ahmad et al. [12] described the antibacterial effect of *Alhagi maurorum* leaves extract and showed that the crude extract, chloroform, and ethyl acetate fractions showed prominent effects giving above 80% inhibition versus *Bacillus anthrax*. The crude extract displayed 80% inhibition versus *Shigella dysenteriae* and the ethyl acetate and crude extract was also well versus *Salmonella typhi* by 78.35% and 76.50% inhibition respectively.

Furthermore, antioxidants can act as scavengers of free radicals and can neutralize potentially damaging reactive free radicals in body cells before they cause protein and lipid oxidation and might decrease potential mutation and so, help prevent cancer or heart diseases [13]. Plants include considerable extents of phytochemical antioxidants like flavonoids, phenolics, carotenoids, and tannins which can be utilized to scavenge the extra free radicals existing in the body [14]. Many researches have reported antioxidant effect of essential oils and plant extracts. For example, Hayet et al. [9] examined the antioxidant activity of ethyl acetate, chloroform, butanol and methanol extracts of *Peganum harmala* leaves and demonstrated methanol extract had the highest antioxidant activity. Nesrin and Tolan [15] proved the antioxidant effect of *Hyssopus officinalis*. However, it was lesser than butylated hydroxytoluene and ascorbic acid. Ahmad et al. [12] indicated that extracts/fractions from *Alhagi maurorum* leaves displayed powerful radical scavenging activity that it might be because of the existence of phenolic compounds in the plant.

This research studies the chemical composition, antioxidant effects, antimicrobial activities and phytochemical analysis of some important medicinal plants.

Materials And Methods

Plant materials

The studied plants in this research are displayed in Table 1. All plants were collected from the research field of Sari Agricultural and Natural Resources University (SANRU), located at 53° 04' E and 36° 39' N, and identified from flora resources. A botanist authenticated the samples (different parts of the mentioned plants) and the voucher specimen deposited in the laboratory.

Plant extracts preparation

Collection of plant materials comply with institutional guidelines, and whole plant materials were wild type including no necessary getting any licenses for application. The fresh selected parts of each plant were washed by distilled water, shade-dried and then powdered in a mechanical mill. Afterward, 10 g of powdered materials were soaked into 170 mL methanol and chloroform, separately. The plugged flasks of samples solution were placed for 48 h at room temperature by persistent shaking. The crude solutions filtered through glass funnel and then dried via a rotary vacuum evaporator at 40 °C temperature. Finally, the extracts were filter sterilized by a 0.22 µm Ministart (Sartorius) and stored at 4 °C before utilization [16].

Essential oils separation

Powdered samples (75 g) were exposed to hydro-distillation for 4 h, using a Clevenger-type apparatus. The essential oils were dehydrated by sodium sulfate anhydrous and stored at 4 °C before GC/MS analysis [17-19].

Gas Chromatography coupled to Mass Spectrometry (GC/MS) analysis

GC/MS analysis was performed on an Agilent Technologies 7890A (GC) coupled with Agilent Technologies 5975C, equipped with a fused silica capillary HP-5MS column (30 m × 0.25 mm iD, film thickness 0.25 µm). The oven temperature was increased from 50 to 220 °C at a speed of 15 °C min⁻¹, and then retained at 220 °C for 7 min; In the fallow, incremented to 260 °C at a speed of 15 °C min⁻¹. Transfer line temperature was 250 °C. Helium was used as the carrier gas, at a flow speed of 1 mL min⁻¹. The inlet temperature was 280 °C.

Antioxidant assays

Dry samples (0.5 g) were homogenized in extraction buffer (1 mL) containing; EDTA (1mM), PVP (1%) and sodium phosphate buffer (50 mM, pH = 7) by mortar and pestle. Afterward, the homogenates were centrifuged (Eppendorf centrifuge 5430R) at 10000 g for 15 min. Finally, the supernatant fractions were utilized for the measurement of protein content and enzyme activities [20].

Measurement of catalase (CAT)

Catalase was examined using evaluating the primary rate of disappearance of H₂O₂, according to the Chance and Meahly [21] method. The reaction mixture included phosphate buffer (2.5 mL, 50 mM, pH = 7), H₂O₂ (0.1 mL, 1%) and enzyme extracts (50 µL), diluted until retain the evaluations in the linear range of the analysis. The absorbance of the reaction mixtures was investigated at 240 nm via spectrophotometer (Biochrom WPA Biowave II UV/Visible), and the reduction of H₂O₂ was because of the reduction in absorbance at 240 nm. The activity was stated as µmole activity mg⁻¹ protein.

Measurement of guaiacol peroxidase

Guaiacol peroxidase (GPX) activity was examined according to the Upadhyaya et al. [22] method. The reaction combination included phosphate buffer (2.5 mL, 50 mM, pH = 7), H₂O₂ (1 mL, 1%), guaiacol (1 mL, 1%), and enzyme extracts (20 µL). The absorbance of the reaction mixtures was investigated at 470 nm via spectrophotometer (Biochrom WPA Biowave II UV/Visible), and the increment in absorbance at 470 nm was followed for 1 min. The activity was stated as mmole activity mg⁻¹ protein.

Measurement of protein

Protein concentrations were specified based on the Bradford [23] method, by Bovine Serum Albumin (BSA) as standard protein.

2, 2- Di-Phenyl-1-Picryl Hydrazyl (DPPH) scavenging evaluate

The antiradical activity of the methanol extract of samples was evaluated through a spectrophotometer, using Liyana-Pathirana and Shahidi [24] method. A solution of 0.135 mM DPPH in methanol was made, and 1.0 mL of this solution was blended with 1.0 mL of the methanol extract of samples in methanol including 40-270 µg of the methanol extract. The reaction mixtures were vortexed completely and placed for 30 min in the gloomy at room temperature. The mixes absorbance was evaluated spectrophotometrically at 517 nm. Ascorbic acid was utilized as a reference. The capability to scavenge DPPH radical was computed using the following equation:

$$\text{DPPH}^\circ \text{ scavenging assay (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100$$

Where Abs_{control} is the absorbance of DPPH radical + methanol; and Abs_{sample} is the absorbance of DPPH radical + samples methanol extract. The radical scavenger activity was stated as the extent of antioxidants essential to reduce the primary DPPH absorbance by 50% (IC₅₀). The IC₅₀ amount for any sample was investigated graphically through plotting the percent disappearance of DPPH as a function of the sample concentration.

Phytochemical analysis

Total Phenolic Content (TPC) of the test samples was assayed using Yu et al. [25] Folin – Ciocalteu method, utilizing gallic acid as standard. Briefly, double distilled water (900 µL) was added to the methanolic solution of test samples (100 µL, 100 µg mL⁻¹). Then, Folin – Ciocalteu reagent (500 µL) was added, followed using the adding sodium carbonate (1.5 mL, 20%). The volume of the mix was reached to 10 mL by distilled water. The mixture was afterward incubated at room temperature for 2 h. Thereafter, the absorbance was assayed via spectrophotometer (Biochrom WPA Biowave II UV/Visible) at 725 nm. The same method was repeated for the standard solutions of gallic acid. Based on the evaluated absorbance, the concentration of phenolic content was determined from the calibration line. Finally, the total phenolic content of methanol extracts was stated as mg Gallic Acid Equivalent (GAE) g⁻¹ dry matter.

For the evaluation of flavonoid, the colorimetric aluminum chloride method was utilized [26]. Each sample in methanol (0.5 mL, 1:10 g mL⁻¹) was blended with methanol (1.5 mL), potassium acetate (0.1 mL, 1 M), aluminum chloride (0.1 mL, 10%), and distilled water (2.8 mL). Then, extracts were placed for 30 min at room temperature; afterward, the absorbance of the reactions was evaluated using spectrophotometer (Biochrom WPA Biowave II UV/Visible) at 415 nm. The calibration curve was created through making quercetin solutions (12.5 to 100 µg mL⁻¹) in methanol. Finally, the total flavonoid content was stated as mg of quercetin equivalents g⁻¹ of dry sample.

Antibacterial screening

Microorganisms *Staphylococcus aureus* PTCC 1431, *Bacillus subtilis* PTCC 1023, *Pseudomonas aeruginosa* PTCC 1074, *Escherichia coli* PTCC 1330, *Pseudomonas syringae* subsp. *Syringae* ICMP 5089, *Pseudomonas viridiflava* ICMP 2848 and *Xanthomonas campestris* pv. *Campestris* ICMP 13 were obtained from the Sari Agricultural and Natural Resources University (SANRU) microbiology laboratory.

Antibacterial effect of the methanol and chloroform extracts of samples was assessed with the disk diffusion method utilizing Mueller-Hinton agar [17], and investigation of inhibition zones of the extracts. The filter paper discs of 6 mm diameter (Padtan, Iran) were sterilized then impregnated with 25 µL of methanol and chloroform extracts, separately. The sterile impregnated discs were put on the agar surface by flamed forceps and softly compressed down to ensure perfect contact of the discs with the agar surface. The incubation condition was 37 °C for quality control strains and 27 °C for plant bacteria for 24 h. Whole trials were performed in triplicate and the consequences were stated as mean ± SD.

Antifungal effect

The following microorganisms were utilized: *Fusarium oxysporum*, *Pyricularia oryzae*, and *Botrytis cinerea*.

The antifungal property of the methanol and chloroform extracts was examined with the agar-well diffusion method [16]. Potato Dextrose Agar (PDA) was seeded by tested fungus. Sterile paper discs of 6 mm diameter (Padtan, Iran) were impregnated by 25 µL of the methanol and chloroform extracts of samples, separately. The sterile impregnated discs were put on the level of the seeded agar plate. The incubation conditions utilized were 28 °C and 70% RH for 12-14 days for *Pyricularia oryzae* and 7-9 days for *Botrytis cinerea*, and *Fusarium oxysporum*. The antifungal effect was imagined as a zone of inhibition of fungal growth around the paper disc and the consequences were stated as mean ± SD after three repetitions. Pathogen grown on PDA without plant extract was utilized as control.

Statistical analysis

Methanol and chloroform extracts examined in a triple for chemical analysis and bioassays. Data were exposed to Analysis of Variance (ANOVA), following a completely randomized design to determine the

Least Significant Difference (LSD) at $P < 0.05$ by SPSS statistical software package (SPSS v. 11.5, IBM Corporation, Armonk, NY, USA). All consequences were stated as mean \pm SD.

Results And Discussion

Essential oils compounds

S. flavescens and *P. harmala* plants had shown the best antimicrobial activities. Therefore were selected for GC/MS analysis to identify the effective compounds. The results are shown below, separately.

S. flavescens

Thirty three constituents were known in essential oil of *S. flavescens* aerial parts, representing 93.70% of the total essential oil. The essential oil combinations are listed in order of their elution on the HP-5MS column. Decane (0.44%), p-Cymene (0.31%), γ -Terpinene (0.39%), α -Terpinolene (0.26%), Terpinen-4-ol (0.35%), 4-isopropyl-2-cyclohexenone (0.46%), 1,6- cyclodecadiene (4.59%), Benzaldehyde, 4-(1-methylethyl)- (1.12%), Thymol (1.70%), Carvacrol (0.26%), β -Damascenone (0.91%), Caryophyllene (1.09%), Nerylacetone (0.44%), 2,6,10,14-Tetramethylheptadecane (0.49%), Alloaromadendrene (6.59%), α -curcumene (0.55%), β -Ionone (0.55%), 3,5-Di-tert-butylphenol (0.48%), Germacrene D (0.35%), Dodecanoic acid (3.37%), (+)-spathulenol (15.39%), Caryophyllene oxide (1.43%), Ledene (0.67%), Tetradecanoic acid (1.13%), 6,10,14-trimethylpentadecan-2-one (5.15%), Diisobutyl phthalate (0.65%), methyl 14-methylpentadecanoate (1.99%), *n*-Hexadecanoic acid (8.86%), Butyl 2-ethyl hexyl phthalate (1.20%), Squalene (8.87%), Ethyl linoleolate (4.99%), Neophytadiene (17.61%), and Linoleic acid (1.06%).

GC/MS analysis showed that the main components of the essential oil were Neophytadiene (17.61%), Spathulenol (15.39%), and Squalene (8.87%).

P. harmala

Eighteen components were identified in essential oil of *P. harmala* fruits representing 91.76% of the total essential oil. The essential oil compounds are listed in order of their elution on the HP-5MS column. Decane (1.05%), m-Cymene (0.78%), γ -Terpinene (0.74%), 4- carvomenthenol (1.52%), 4-isopropyl-2-cyclohexenone (0.81%), Cuminaldehyde (2.58%), Thymol (2.46%), β -caryophyllene (1.44%), 6,10-dimethyl-5,9-undecadiene-2-one (0.88%), Alloaromadendrene (5.00%), (-)-Spathulenol (37.83%), (+)-Aromadendrene (1.07%), β -oplophenone (0.39%), Methyl palmitate (1.14%), *n*-Hexadecanoic acid (13.21%), Methyl linoleate (1.04%), Linoleic acid (11.08%), and Elaidic acid (8.72%).

GC/MS analysis showed that the main components of the essential oil were Spathulenol (37.83%), *n*-Hexadecanoic acid (13.21%), and Linoleic acid (11.08%).

Protein content and enzymes activity

Plants have evolved antioxidant pathways that are common enough to maintain them from oxidative injury during times of natural growth and moderate stress. Both enzymatic and non-enzymatic systems protect tissue from activated oxygen species, produced as the consequence of exterior environmental stresses, such as dryness, chilling and air pollution. Certain of the enzymatic antioxidant defense systems contain Super Oxide Dismutase (SOD), Catalase (CAT), and Guaiacol Peroxidase (GPX) [27]. In this research, the activity of 2 enzymes (CAT and GPX) was evaluated. Moreover, protein content was measured by bovine serum albumin as a standard. The outcomes are exhibited in Figure 1. As shown, the maximum and minimum activities of catalase were found in *J. conglomeratus* and *S. flavescens* plants, respectively. Also, guaiacol peroxidase activity assay indicated that *J. conglomeratus* plant had the most activity. Furthermore, the minimum guaiacol peroxidase activity was related to *R. repens* plant. Moreover, the maximum and minimum protein content was seen in *M. azedarach* fruit and *J. conglomeratus* plant, respectively.

DPPH radical scavenging assess

The effect of antioxidants on DPPH is assumed to be because of their hydrogen donating capability [28]. Table 2 shows the DPPH radical scavenging effect of tested plants. As presented, the most free radical scavenging capacity of the plants was determined in *P. harmala* extract with an IC_{50} value of $0.46 \pm 0.12 \mu\text{g mL}^{-1}$.

Total phenol and flavonoid content of the extracts

It has been recognized that the flavonoids demonstration antioxidant effect and their effectiveness on human health and nutrition are considerable. Chelating or scavenging procedures are the mechanism of action of flavonoids [29]. The evaluation of total flavonoid content was based on the absorbance amount of tested plant solutions that react with aluminum chloride reagent, followed using comparing by the standard solution of quercetin equivalents. The standard curve of quercetin was performed utilizing quercetin concentration ranging from 12.5 to 100 $\mu\text{g mL}^{-1}$. The following equation stated the absorbance of the standard solution of quercetin as a function of concentration:

$$Y = 0.0056x + 0.1764, R^2 = 0.9878$$

Where x is the absorbance and Y is the quercetin equivalent (mg g^{-1}). The flavonoid content of samples is shown in Table 3. As shown, the most phenol content was determined in *A. maurorum*, *P. harmala* and *S. flavescens* extracts with a value of 45.43, 39.3 and 39.07 mg of quercetin equivalents g^{-1} of dry matter, respectively.

Phenolic compounds gained from plants are a class of secondary metabolites which act as an antioxidant or free radical terminators. Therefore, it is needed to evaluate the total content of phenols in the tested plants [30]. The designation of the total phenolic amount was based on the absorbance amount of sample solutions ($100 \mu\text{g mL}^{-1}$) that react by Folin-Ciocalteu reagent, followed with comparing by the standard solution of gallic acid equivalents. The standard curve of gallic acid was performed

utilizing gallic acid concentration ranging from 12.5 to 100 $\mu\text{g mL}^{-1}$. The following equation stated the absorbance of the gallic acid standard solution as a function of concentration:

$$Y = 0.0954x + 0.196, R^2 = 0.9973$$

Where x is the absorbance and Y is the gallic acid equivalent (mg g^{-1}). The phenol content of the samples is presented in Table 3. As shown, the most phenol content was determined in *P. harmala* and *A. maurorum* extracts with a value of 155.29 ± 0.20 and 146.71 ± 0.02 mg Gallic Acid Equivalents (GAE) g^{-1} dry matters, respectively.

Antibacterial screening

Antibacterial activity of methanolic and chloroformic extracts including *A. maurorum*, *S. flavescens*, *R. repens*, *M. azedarach*, *P. harmala* and *J. conglomeratus* in different concentrations (0.01, 0.03, 0.06, 0.12, 0.25 and 0.5 ppm) were tested versus 3 gram-positive (*B. subtilis*, *S. aureus*, *R. toxicus*) and 5 gram-negative (*P. aeruginosa*, *E. coli*, *X. campestris*, *P. viridiflava*, *P. syringae*) bacteria. The results at 0.5 ppm are shown in Figures 2 and 3. Also, in other concentrations, similar results were observed that for simplifying the discussion we considered only 0.5 ppm concentration. As shown in Figure 2, methanolic extracts of *S. flavescens*, *P. harmala* fruit and *J. conglomeratus* and chloroformic extracts of *P. harmala* fruit, *S. flavescens*, and *P. harmala* showed the maximum antibacterial activity on *P. aeruginosa*, respectively. Also, methanolic extract of *J. conglomeratus* fruits and chloroformic extracts of *M. azedarach* and *J. conglomeratus* fruit had no antibacterial effect on *P. aeruginosa* (Figure 2a). Also, methanolic extract of *P. harmala* and chloroformic extracts of *P. harmala* fruit, *R. repens*, and *M. azedarach* had the maximum antibacterial activity against *B. subtilis*, respectively. Also, chloroformic extract of *A. maurorum* extract had no antibacterial activity on *B. subtilis* (Figure 2b). Furthermore, methanolic extracts of *P. harmala* fruit, *P. harmala*, and *J. conglomeratus* and chloroformic extracts of *M. azedarach* and *P. harmala* fruit indicated the maximum antibacterial activity on *E. coli*, respectively (Figure 2c). Moreover, methanolic extracts of *P. harmala* fruit and aerial part and chloroformic extracts of *S. flavescens* and *P. harmala* fruit had the maximum antibacterial activity on *S. aureus*, respectively (Figure 2d). Also, the antibacterial activity of tested plants on plant bacteria strains is shown in Figure 3. As indicated, methanolic extracts of *P. harmala* fruit and *S. flavescens* and chloroformic extracts of *R. repens* and *M. azedarach* showed the maximum antibacterial activity against *R. toxicus*, respectively (Figure 3a). Furthermore, methanolic extracts of *R. repens* and *P. harmala* fruit and chloroformic extracts of *P. harmala* fruit, *J. conglomeratus* fruit and, *A. maurorum* indicated the maximum antibacterial activity against *X. campestris*, respectively (Figure 3b). Moreover, methanolic extract of *P. harmala* fruit and chloroformic extracts of *P. harmala* and *J. conglomeratus* displayed the maximum antibacterial activity on *P. viridiflava* (Figure 3c). Also, methanolic extracts of *S. flavescens*, *P. harmala* fruit and *R. repens* and chloroformic extracts of *R. repens* represented the maximum antibacterial activity on *P. syringae*, respectively. But, methanolic extract of *J. conglomeratus* fruit showed no antibacterial activity (Figure 3d).

Antifungal activity

Antifungal property of the methanolic and chloroformic extracts was tested using the agar well diffusion method. The results of the experiments showed that none of the tested plants had antifungal activity.

Conclusion

In conclusion, the consequences validate the traditional use of the herb against antimicrobial diseases. Also, the results indicated that these plants can act as a potential antimicrobial agent and deserves further studies to be safely used in the control of disease and pests.

Declarations

Acknowledgements

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Competing interest

The authors have no conflicts of interest.

Author's contributions

Gh. N. and S. Gh designed the experiment and revised the manuscript with co-author. Z. H. conducted the experimental work. Gh. N., S. Gh. and Z. H. analyzed the data and wrote the manuscript.

Availability of data and materials

All data and materials are all provided.

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Tables

Table 1. Characteristics of investigated plants

Scientific name	Family	Parts of sample
<i>Sophora flavescens</i>	Fabaceae	Aerial
<i>Rhaponticum repens</i>	Asteraceae	Aerial
<i>Alhagi maurorum</i>	Fabaceae	Aerial
<i>Melia azedarach</i>	Meliaceae	Fruit
<i>Peganum harmala</i>	Nitrariaceae	Fruit and Aerial
<i>Juncus conglomeratus</i>	Juncaceae	Fruit and Aerial

Table 2. DPPH radical scavenging activity of tested plants

Scientific name	Parts of sample	IC ₅₀ (µg mL ⁻¹)
<i>S. flavescens</i>	Aerial	6.12 ± 0.77
<i>R. repens</i>	Aerial	6.94 ± 1.12
<i>A. maurorum</i>	Aerial	7.87 ± 1.09
<i>M. azedarach</i>	Fruit	11.02 ± 1.36
<i>P. harmala</i>	Fruit	0.46 ± 0.12
<i>J. conglomeratus</i>	Fruit	7.19 ± 0.89

Table 3. Total phenol and flavonoid content of the extracts

Scientific name	Parts of sample	Total phenol content	Total flavonoid content
<i>S. flavescens</i>	Aerial	39.07 ± 0.01	69.39 ± 0.01
<i>R. repens</i>	Aerial	24.72 ± 0.03	68.86 ± 0.03
<i>A. maurorum</i>	Aerial	45.43 ± 0.02	146.71 ± 0.02
<i>M. azedarach</i>	Fruit	21.96 ± 0.00	48.68 ± 0.00
<i>P. harmala</i>	Fruit	39.30 ± 0.20	155.29 ± 0.20
<i>J. conglomeratus</i>	Fruit	45.66 ± 0.10	46.54 ± 0.10

Figures

Figure 1. Enzymes activity and protein content

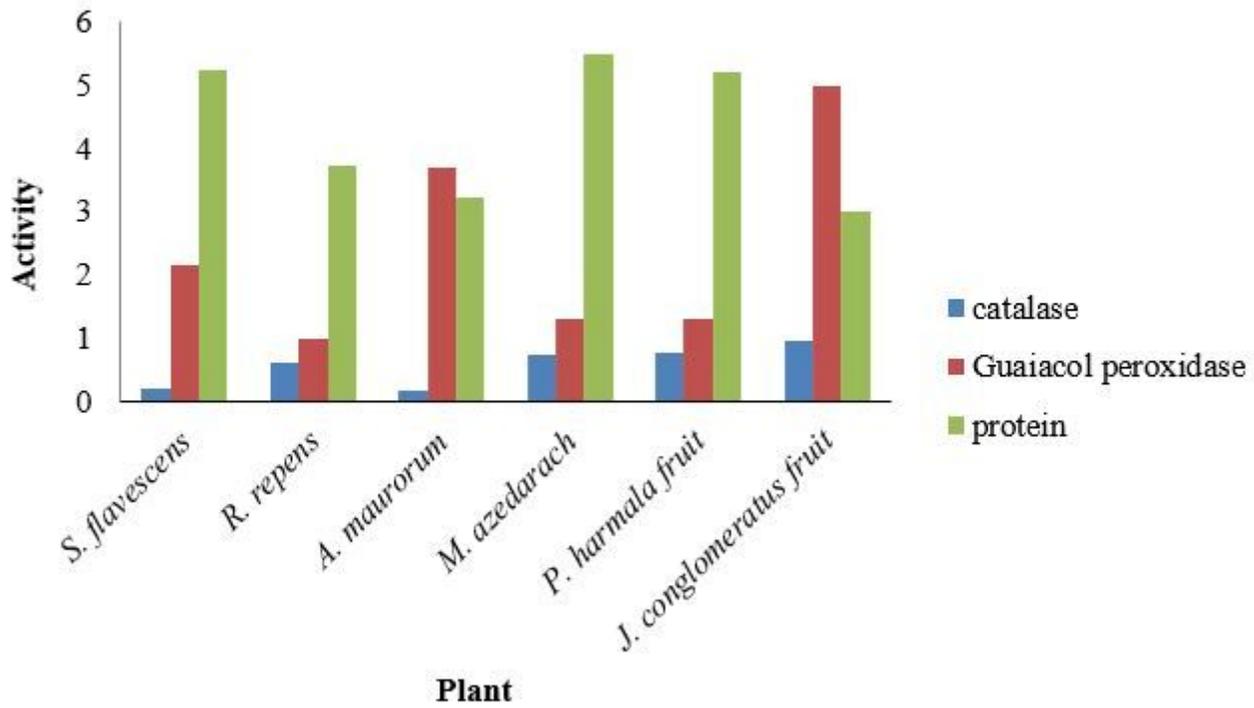


Figure 1

Enzymes activity and protein content

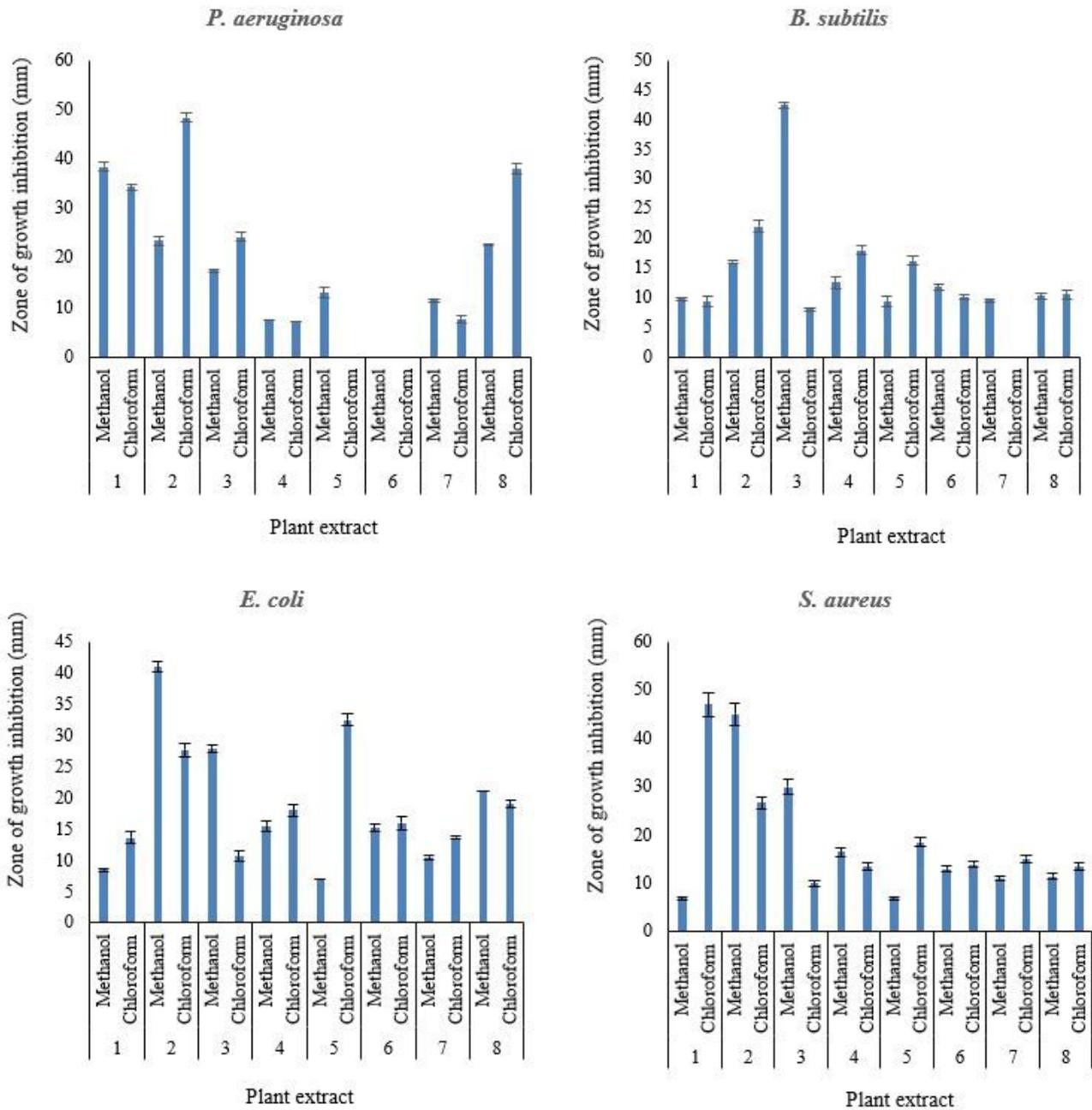


Figure 2

Antibacterial activity of methanolic and chloroformic extracts including 1: *S. flavescens*; 2: *P. harmala* fruit; 3: *P. harmala*; 4: *R. repens*; 5: *M. azedarach*; 6: *J. conglomeratus* fruit; 7: *A. maurorum*; 8: *J. conglomeratus* on standard bacteria strains. Data were exposed to Analysis of Variance (ANOVA), following a completely randomized design to determine the Least Significant Difference (LSD) at $P < 0.05$ by SPSS statistical software package (SPSS v. 11.5, IBM Corporation, Armonk, NY, USA). All consequences were stated as mean \pm SD.

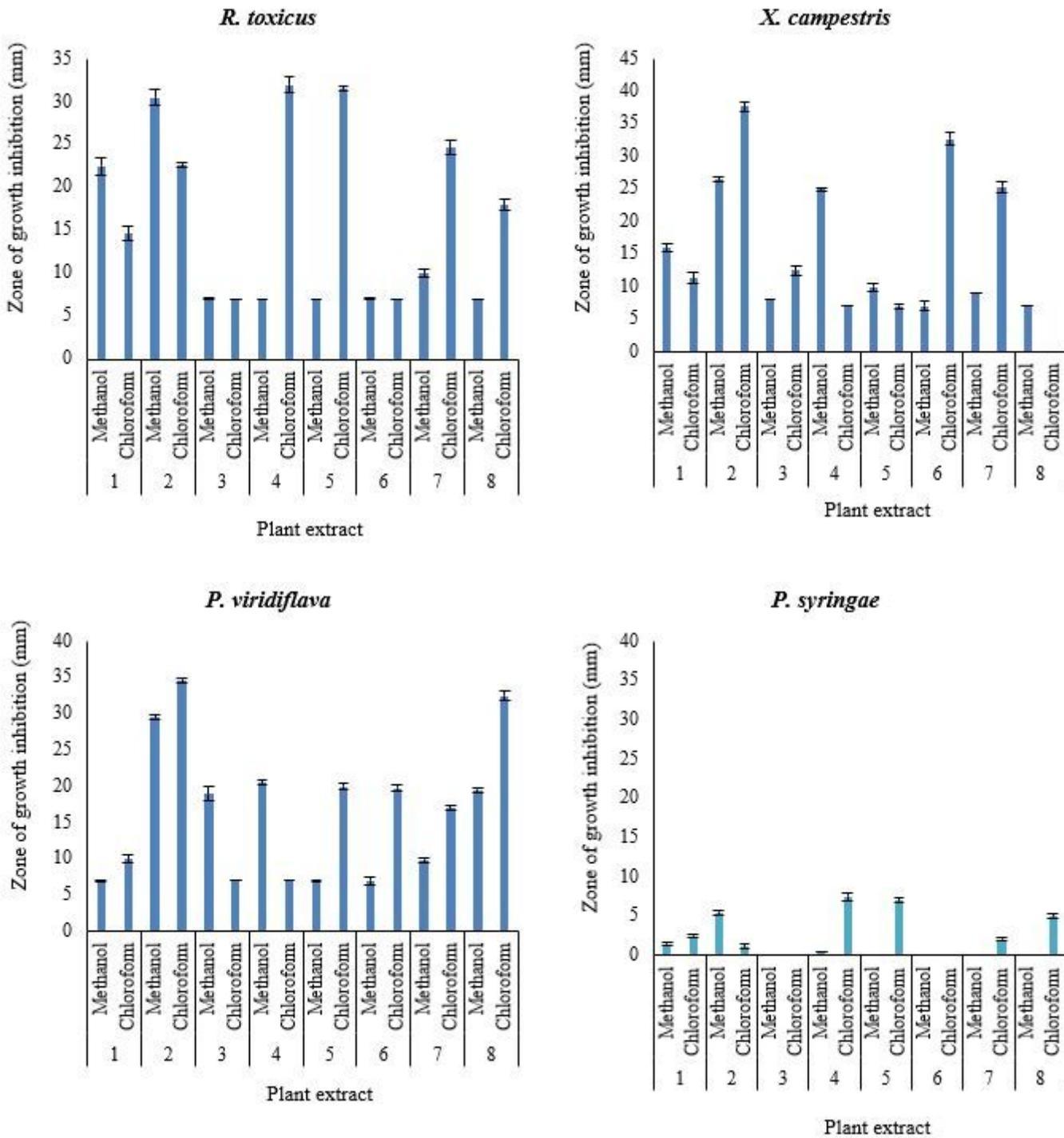


Figure 3

Antibacterial activity of methanolic and chloroformic extracts including 1: *S. flavescens*; 2: *P. harmala* fruit; 3: *P. harmala*; 4: *R. repens*; 5: *M. azedarach*; 6: *J. conglomeratus* fruit; 7: *A. maurorum*; 8: *J. conglomeratus* on plant bacteria strains. Data were exposed to Analysis of Variance (ANOVA), following a completely randomized design to determine the Least Significant Difference (LSD) at $P < 0.05$ by SPSS statistical software package (SPSS v. 11.5, IBM Corporation, Armonk, NY, USA). All consequences were stated as mean \pm SD.