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In vitro Evaluation of Antioxidant and Antimicrobial Activity of Aqueous Aerial Parts Extracts of *Euphorbia helioscopia* L.

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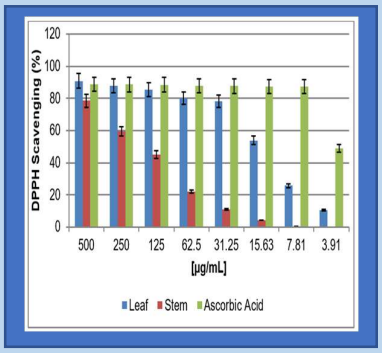
Abstract: Antitumor, antiviral, antimicrobial and antioxidant activities were reported for *Euphorbia helioscopia* usage in traditional medicine. Therefore, minimum inhibitory and bactericidal concentrations (MIC, MBC) of *E. helioscopia* aqueous leaf and stem extracts were evaluated by broth microdilution assay. As well as their antioxidant activity was carried out using DPPH assay. Obtained results showed that MIC values of leaf extract ranged from 0.78±0.0 mg/mL to 12.5±0.0 mg/mL and stem extract ranged from 3.125±0.0 mg/mL to 25±0.0 mg/mL. While, MBC of leaf and stem extracts had a range from 6.25±0.0 mg/mL to 50±0.0 mg/mL and 25±0.0 mg/mL to 50±0.0 mg/mL, respectively. The current study demonstrated that *E. helioscopia* leaf and stem extracts had antioxidant activity. One-way ANOVA test, the *P* values for the leaf and stem aqueous extracts were equal to 0.997 and 0.058, respectively. Consequently, both extracts showed no significant difference relative to the standard ascorbic acid (*P* value > 0.01). In addition, statistical analyses showed no significant differences between the tested concentrations of leaf extract and tested concentrations of ascorbic acid at *P* value > 0.01. The same thing was obtained for tested concentrations of stem aqueous extract, except at concentrations 7.81 and 3.91 µg/mL which showed DPPH inhibition percent 4.30±0.63 and 0.59±0.10, respectively. This indicates that *E. helioscopia* has potential to serve as a natural antioxidant, which makes it a promising candidate for therapeutic applications. Moreover, the positive antibacterial recorded data could potentially be used to develop potent drugs that improve the management and treatment of variety bacterial illnesses.



Keywords: *Euphorbia helioscopia*, antibacterial activity, aqueous leaf extract, aqueous stem extract, antioxidant, MBC, MIC.

Introduction

Plants are described as a rich natural source of medicinal ingredients that have a potential effect against different types of pathogens and diseases (1, 2). In the new era, even though, approximately 25% of the new medications are derived from plant sources (3). In last few decades, wild plants have gained awareness and attention because of their functional food and potential health benefits (4).

Euphorbia is a genus of about 2250 species that belongs to the herbal family Euphorbiaceae. *Euphorbia* is one of the biggest diverse genus in the flowering plants in the plant kingdom. The plants in *Euphorbia* genus, are recognized by a typical milky fluid production. This genus is extensively distributed globally, especially in tropical areas such as Africa and Asia (5). There are 45 species of *Euphorbia* are growing in Palestine (6).

Euphorbia helioscopia is an annual herb that is utilized in traditional, folk or ethnoveterinary herbal medicine in many countries (7-16). It is often used in alternative medicine practices to manage different diseases such as edema, ascites, intestinal parasite infections, pulmonary tuberculosis, cervical tuberculous lymphadenopathy, coughs, laryngeal spasms, colds, emphysema, kidney stones, menstrual issues, infertility, and sexual illnesses. In addition, it is used as Painkiller for treating severe headache, tooth pain, rheumatic diseases, colic, antidote and to relieve pain resulting from scorpion stings and snake bites. Furthermore, this plant is commonly used to cure the skin and mucous membrane infections such as warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, and Guinea-worm (15, 17). In Chinese folk medicine, product of this plant is used lonely or together with other plants to treat several diseases such as bone diseases, cancer of the lung, cervical cancer, esophageal cancer and pulmonary fibrosis (5, 18). In addition, anti-inflammatory activity was reported recently (16). It is also used in combination with other plants or alone to treat several diseases (5).

Recently, *E. helioscopia* gained great deal of interest and awareness because of their various chemical molecules and a broad spectrum of biological properties and medical values. Phytochemical researches have revealed numerous molecules of terpenoids, flavonoids, lipids and steroids, volatile oils, phenolic compounds, tannins and diterpenoids isolated and identified from *E. helioscopia* plant. Flavonoids and terpenoids are the most important and abundant bioactive molecules

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identified from this plant (5). Extracts of *E. helioscopia* and some of the active chemical molecules showed diverse biological effects, such as antiproliferative, anti-inflammatory, insecticidal, antimicrobial, lipid-lowering, anthelmintic, antioxidative activities, anticancer activity, cytotoxic activity and wound-healing properties (5, 12, 19-23).

Due to *E. helioscopia* folkloric medicinal importance as a popular medical remedy, the current research was performed to assess the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous leaf and stem extracts of *E. helioscopia* growing naturally in Palestine, against different species of bacteria. Furthermore, to investigate the anti-2,2-diphenyl-1-picrylhydrazyl (anti-DPPH, antioxidant) activity of *E. helioscopia* aqueous leaf and stem extracts.

Materials and Methods

Plant collection

The *E. helioscopia* plant was collected in March-April, 2022 from its natural habitat in Tulkarm region, West Bank-Palestine. Dr. Ghadeer Omar, Department of Biology and Biotechnology, An-Najah National University, Palestine, identified the plant species. Representative plant specimen was pressed, at room temperature till complete dryness. Treated chemically for complete preservation with subsequent deposition on herbarium sheets with a voucher number (1896) at An-Najah National University Herbarium, Figure 1. The obtained plants were adequately washed with tap water to eliminate dust and soil, then the plants were placed in a shaded area far from direct sun light to reduce the possibility of active compound loss. For the preparation of the extracts, the air-dried components were finely ground using an electric grinder.

Figure1: Herbarium sheet photo of *E. helioscopia* plant.

Equipments

Microplate reader (Labtech, UK), sterile 96-well microtiter plates (Thermo Fisher Scientific Inc, USA).

Chemicals and reagents

Methanol (Merk), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Sigma-Aldrich, India), Ascorbic acid (Sigma-Aldrich, USA), Muller Hinton broth (HIMEDIA, USA), Nutrient agar (HIMEDIA, USA).

Plant extract preparation

Aqueous leaf and stem extracts preparation

The aqueous extracts were prepared according to method described previously (2, 3).

Determination of MIC and MBC

The MIC values of plants' extracts were measured in sterile 96-well microtiter plates using the broth microdilution method according to the CLSI instructions (24) as also described previously (2). The value of MIC for that extract was identified to be the highest dilution of the plant extract that totally inhibited bacterial growth. The MIC value for the extract was determined by visual examination. The MIC in the current study was conducted on the following bacterial species: *Escherichia coli* (*E. coli* ATCC 25922), *Klebsiella pneumoniae* (*K. pneumoniae* ATCC 13883), *Proteus vulgaris* (*P. vulgaris* ATCC 8427), *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 9027), *Staphylococcus aureus* (*S. aureus* ATCC 6538P), and *Staphylococcus epidermidis (S. epidermidis* ATCC 12228). The minimum bactericidal concentration (MBC) was detected from wells with no visible growth MIC tests, 10 µl was subcultured on plates with nutrient agar and incubated for 18-24 hours at 37ºC. The MBC was defined as the highest dilution of the extract at which specific bacterium species is killed. The average of two replicates ± standard deviations (SD) for each extract was calculated.

DPPH antioxidant assay

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay is considered one of the most frequently proposed method used for evaluating and measuring antioxidant activity. It is simple, efficient, inexpensive method and can be run in the most laboratories, hence, it has been broad utilizations (25, 26). The DPPH assay is a useful indicator of the typical antioxidant profile since most natural antioxidants have reactive hydrogen atoms that act as reductants. The basic idea of this assay is based on the reduction of DPPH, a free stable radical by an antioxidant. During the course of reaction, alcohol-containing solution of DPPH alters from deep violet color to light yellow color (26).

A 96-well microplate was utilized for the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical test. In brief, 100 µL of 0.01% methanolic DPPH solution was mixed with 100 µL of varied quantities of each plant extract in methanol (3.906 µg/mL-500µg/mL). The plate was then incubated for 30 minutes in darkness at ambient temperature before reading the absorbance at 540 nm using a microplate reader. (Labtech, UK). Ascorbic acid was employed as a standard at various concentrations (3.906 µg/mL-500µg/mL) (27). All experiments for stem and leaf extracts and standard ascorbic acid were conducted in duplicate.

The radical scavenging activity (%) of DPPH was calculated using to the following formula:

100% DPPH scavenging activity = [(A control - A sample) / A control] × 100%

while the absorbance of the control and sample was (DPPH + Methanol without plant extract or standard) and (DPPH + plant extract or standard), respectively.

Statistical analysis

The results for MIC and MBC values were recorded as the mean values ± SD. For antioxidant analysis, all concentrations of stem and leaf extracts in addition to the standard ascorbic acids were analyzed in duplicate. The results were presented as mean ± SD. The concentration resulting in 50% of inhibition (IC50) was computed using non-linear regression method with Microsoft Excel version 2016. The dose–response curve was established by plotting the inhibition percentages versus concentrations. Moreover, one-way ANOVA was applied to identify if a remarkable distinction could be found among the various studied extract concentrations compared to the ascorbic acid standard. *P* value ≤ 0.01 and *P* value > 0.01 was considered to be significant and not to be significant, respectively. Not significant result means that the antioxidant effect of the plant extract as well as the same the antioxidant effect of the standard (ascorbic acid).

Results and Discussion

Antibacterial activity of *Euphorbia helioscopia* aqueous leaf and stem extracts

The current investigation demonstrated that both aqueous extracts of leaf and stem of *E. helioscopia* were affective against all tested bacteria (Gram-positive and Gram-negative bacteria). The tested bacterial species were susceptible to aqueous leaf extract and aqueous stem extract at a concentration ranged from 0.78±0.0 mg/mL to 12.5±0.0 mg/mL and 3.125±0.0 mg/mL to 25±0.0 mg/mL, respectively. The aqueous leaf extract and aqueous stem extract have MBC effects at a concentration ranged from 6.25±0.0 mg/mL to 50±0.0 mg/mL and 25±0.0 mg/mL to 50±0.0 mg/mL, respectively. Table 1 shows the MIC and MBC profiles of *E. helioscopia* aqueous leaf and stem extracts against various tested bacterial strains.

Table 1: MIC and MBC profiles of *E. helioscopia* aqueous leaf and stem extracts against different bacterial species under study.

Bacterial strain	Aqueous extract of *E. helioscopia*			
	MIC± SD[a] (mg/mL)		MBC ± SD (mg/mL)	
	Leaf	Stem	Leaf	Stem
Escherichia coli (ATCC 25922)	6.25±0.0	12.5±0.0	25±0.0	25±0.0
Klebsiella pneumonia ATCC 13883	6.25±0.0	25±0.0	12.5±0.0	25±0.0
Proteus vulgaris (ATCC 8427)	6.25±0.0	6.25±0.0	25±0.0	50±0.0
Pseudomonas aeruginosa (ATCC 9027)	12.5±0.0	12.5±0.0	50±0.0	50±0.0
Staphylococcus aureus (ATCC 6538P)	0.78±0.0	3.125±0.0	6.25±0.0	25±0.0
Staphylococcus epidermidis (ATCC 12228)	6.25±0.0	12.5±0.0	25±0.0	50±0.0

SD[a]: Standard deviation.

Antibacterial activity of *E. helioscopia* has previously been demonstrated utilizing various types of extracts, various plant parts and different methods against different species of microbial pathogens (15, 19, 20, 28-30). Variation in results of antimicrobial activities among studies, may be due to different methods, different types of bacteria (Gram-positive and Gram-negative), different species of bacteria, different types of extracts and parts of the plant. Extracts of this plant have antimicrobial effect due to the diverse phytochemical compounds that are considered as a bioactive ingredient (28). According to previously published studies, the family of Euphorbaceae has adverse spectrum of phytochemical compounds, such as tannins, phenolic, alkaloids, anthraquinones, saponins, flavonoids, diterpenes, glycosides, lipids and volatile oils. These ingredients have a pivotal application against different types of diseases and human pathogens (5, 15, 28, 31).

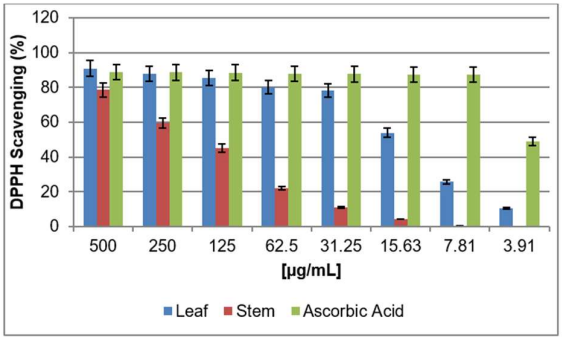

Figure 2: The antioxidant effect of different concentrations of *E. helioscopia* aqueous leaf and stem extracts on DPPH radicals, ascorbic acid was used aa a standard.

DPPH Antioxidant Assay

The DPPH free radical scavenging assay is an antioxidant assay that is commonly utilized in laboratories to find out the ability of natural products to scavenge free radicals. The findings of this study demonstrated that *E. helioscopia* aqueous leaf and stem extracts had a high antioxidant activity (Figure 2 and Table 2).

Table 2: The DPPH inhibition % with the statistical *P* values under the studied two extract *of Euphorbia helioscopia*.

Extract Type	[Extract] µg/mL	DPPH inhibition %	*P* value
Leaf	500	90.80 ±0.92	0.10*
	250	87.88±0.90	0.11*
	125	85.53±0.91	0.21*
	62.50	80.10±1.23	0.08*
	31.25	78.30±1.13	0.31*
	15.63	53.91±0.63	0.08*
	7.81	25.70±.0.30	0.07*
	3.91	10.40±0.12	0.10*
Stem	500	80.97±0.51	0.31*
	250	78.50±1.12	0.26*
	125	59.48±1.14	0.13*
	62.50	45.20±0.92	0.04*
	31.25	22.10±0.98	0.13*
	15.63	10.87±0.71	0.02*
	7.81	4.30±0.63	0.005**
	3.91	0.59±0.10	0.001**

*P value > 0.01 was not significant compared to ascorbic acid (The antioxidant effect of the plant extract as well as the same the antioxidant effect of the standard (ascorbic acid).
**P value ≤ 0.01 was significant compared to ascorbic acid.

After applying one-way ANOVA test, the *P* values for the leaf and stem aqueous extracts were equal to 0.997 and 0.058, respectively. Consequently, both extracts showed no significant difference relative to the standard ascorbic acid (*P* value > 0.01). Moreover, according to the calculated *P* values (Table 2), results showed no significant differences between the tested concentrations of leaf extract and tested concentrations of ascorbic acid at *P* value > 0.01. The same thing was obtained for tested concentrations of stem aqueous extract, except at concentrations 7.81 and 3.91 µg/mL which showed DPPH inhibition percent 4.30±0.63 and 0.59±0.10, respectively.

The activity of aqueous leaf extract had a range from 10.4%-90.8% (SD: ±0.12 - ±0.92) at concentrations ranged from 3.91 µg/mL-500 µg/mL with half maximal inhibitory concentration (IC50) value 12.64 µg/mL ±0.67. Ascorbic acid activity ranged from 48.8% to 88.8% (SD: ±0.14 - ±0.42) at concentrations ranged from 3.91 µg/mL to 500 µg/mL with an IC50 value of 4.067 µg/mL ± 0.22. In addition, the activity of aqueous stem extract ranged from 0.59% to 80.97% (SD: ±0.10 - ±0.51) at concentrations ranged from 3.91 µg/mL-500 µg/mL with IC50 value 62.56 µg/mL ± 0.93 (Table 2 and 3).

Table 3: The half maximum inhibitory concentration values (IC50) of *E. helioscopia* aqueous leaf and stem extracts, ascorbic acid was used as standard.

Extract	IC50 (µg/mL) ± SD
Leaf aqueous extract	12.64±0.67
Stem aqueous extract	62.56±0.93
Ascorbic acid (Standard)	4.067±0.22

SD[a]: Standard deviation.

The antioxidant property of *E. helioscopia* aqueous leaf and stem extracts was detected and represented in terms of IC50 which indicates the sample concentration that required to block 50% of DPPH in the reaction mixture. Ascorbic acid was utilized as standard in these reactions. After 30 min incubation both aqueous leaf and stem extracts exhibited an antioxidant effect that elevated as the concentrations of the tested extract increases. Nevertheless, aqueous leaf extract showed higher DPPH inhibition percentage compared to aqueous stem extract. In current study, the IC50 values 12.64±0.67 µg/mL and 62.56±0.93 µg/mL for aqueous leaf extract and stem extract, respectively. However, in another research the antioxidant activity of different parts extracts of *E. helioscopia* showed flower, leaf and stem methanolic extracts with IC50 value of 26.66 ± 0.000 µg/mL, 65.25 ± 0.004 and 80.17± 0.012 µg/mL, respectively, indicating flower one being higher antioxidant agent. Also the IC50 values in flower, leaf and stem ethanol extracts ranged from 27.55 ± 0.005 to 179.02 ± 0.957µg/mL (12). Furthermore, the antioxidant activity of the whole *E. helioscopia* plant parts variable extracts types were examined. In their study, the methanolic extract showed maximum antioxidant activity with lowest IC50 value (600 ± 20 µg/mL) followed by the ethanolic one (1600 ± 200 µg/mL) and then the aqueous extract (2800 ± 300 µg/mL) (23). The overall observed variation in obtained results of the antioxidant bioactivity in all previously mentioned studies including this study can be referred to the differences in the methodology, extraction type and plant parts.

Conclusion

Current study outcomes have shown that *E. helioscopia* aqueous leaf and stem extracts possess antioxidant as well as antibacterial activity. The former activity exhibited that leaf and stem extracts had notable efficacy as in DPPH free radical scavenging assay comparable to that of ascorbic acid. One-way ANOVA test, the *P* values for the leaf and stem aqueous extracts were equal to 0.997 and 0.058, respectively. Consequently, both extracts showed no significant difference relative to the standard ascorbic acid (*P* value > 0.01). In addition, statistical analyses showed no significant differences between the tested concentrations of leaf extract and tested concentrations of ascorbic acid at *P* value > 0.01. The same thing was obtained for tested concentrations of stem aqueous extract, except at concentrations 7.81 and 3.91 µg/mL which showed DPPH inhibition percent 4.30±0.63 and 0.59±0.10, respectively. This indicates that *E. helioscopia* has potential to serve as a natural antioxidant, which makes it a promising candidate for therapeutic applications in biological systems. Moreover, the positive antibacterial recorded data could potentially be used to develop potent drugs that improve the management and treatment of variety bacterial illnesses. Regarding all of this, additional phytochemical searches, purification and characterization of the biologically active elements present in the plant species extracts under consideration still proposed. Nevertheless, in vivo research will be required to evaluate their real physiological activity along with cytotoxicity detection.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The raw data that's needed to reproduce these findings might be found in the manuscript's body and illustrations.

Author's contribution

Study conception and design: GA and GO; plant collection GA; plant identification: GO; experimental part: GO and LA; data analyses: LA; draft manuscript preparation GA, GO and LA. The findings of this research were assessed by all authors, who subsequently approved the final version of the manuscript.

Conflicts of interest

All authors state that there are no potential conflicts of interest associated with the publication of this manuscript.

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