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Evaluation of Antioxidant and Antimicrobial Activities of *Euphorbia terracina* L. from Deltaic Mediterranean Coast, Egypt

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ABSTRACT

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. The main aim of this study is to evaluate antimicrobial and antioxidant activities of *Euphorbia terracina* L. (family Euphorbiaceae) is short-lived perennial herb, up to 80 cm tall. It was collected from northern sector of Nile Delta at the period of May 2015. Different extracts of *Euphorbia terracina*, were screened for their antimicrobial activity against nine pathogenic bacterial and one fungal strain using filter paper disc assay. The antimicrobial activity was significantly variable according to the solvent used. Hexane extract was found to be the most effective against most tested organisms followed by methanol extract showed the inhibitory activities against *Proteus vulgaris* (inhibition zones = 9.3 mm), Petroleum ether and ethyl acetate extracts exhibited most antimicrobial against *Staphylococcus aureus* (inhibition zone = 10 mm, each). Antioxidant activity was screened using DPPH scavenging activity. Ethyl acetate extract was found to be the most effective (IC_{50} = 1.23 mg/mL). Preliminary phytochemical screening on methanol extract showed variations in active secondary compounds. Saponins were found to be the richest one in this regard (39.17±1.3 mg/g D.W.) and total phenolics were estimated 22.11±0.9 mg/g D.W.

1. Introduction

World Health Organization (WHO) has defined medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs [1]. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, the active compounds derived from plant extracts of powerful alternatives that strongly imposed itself on the scene treatment, as these compounds has many therapeutic properties as antioxidants, anti-bacterial and fungi, anti-algae as well as anti-tumor and anti-cancer or proof of cancer and numerous other indications [2,3].

Natural antioxidants have a wide range of biochemical activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals, and alteration of intra cellular redox potential [4]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breakage [5].

Euphorbia terracina (Carnation spurge, family Euphorbiaceae) is a deep-rooted, short-lived perennial herb, up to 80 cm tall. Rapid growth and prolific seeding enable this species to form dense thickets [6]. *E. terracina* is native to Northern Africa, temperate Asia, and some areas of Europe. It has become a serious pest in Western Australia [7, 8].

E. terracina is used as a remedy for fever and paralysis [9]. The occurrence of common triterpenes, flavenoids and coumarins was reported in *E. terracina*. The aerial part of it yielded five new bishomoditerpene lactones named terracinolides C-G, which display the novel C_{22} 17-ethyljatrophane framework [10, 11]. Therefore this study was aimed to evaluate antimicrobial and antioxidant activities of *Euphorbia terracina* and in order to evaluate their medicinal potentiality and their future industrial uses.

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2. Experimental Methods

2.1 Plant Material

Euphorbia terracina L. (Fig. 1) was collected from northern sector of Nile Delta during the period of May 2015. The identification of species was done according to Boulos [6]. It was dried at room temperature and grinded into a powder using a blender.

Fig. 1 General and close-up view of *Euphorbia terracina* L. collected from northern sector of Nile Delta

2.2 Extraction

The dried plant material (50 g) was extracted using 500 mL of different solvents (methanol, petroleum ether, hexane, ethyl acetate) by refluxing for 3 hrs. The obtained extracts were filtered and evaporated to dryness. A stock solution of each extract was prepared in dimethyl sulfoxide (DMSO) and kept at -20 °C for future use [12].

2.3 Phytochemical Analysis

Euphorbia terracina was collected and prepared as previously mentioned. Total phenolics, flavonoids and alkaloids were estimated using spectrophotometric techniques adapted by Harborne [13], Sadasivam and

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Manickam [14] and Bohm and Kocipai-Abyazan [15], respectively. Tannins were determined according to Van Burden and Robinson [16], while Saponin content was estimated by the method adopted by Obdoni and Ochuko [17].

2.4 Determination of Antioxidant Activity

2.4.1 Free Radical Scavenging Activity (DPPH assay)

Antioxidant activity was determined by using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH [18]. Two mL of 0.15 mM DPPH was added to 2 mL of plant extracts in different concentrations (1000, 800, 600, 400, 200 and 100 ppm). A control was prepared by adding 2 mL of DPPH to 2 mL solvent. The mixture was incubated in dark at the room temperature for 30 min. The absorbance was recorded at 517 nm and the IC_{50} was calculated graphically. The antioxidant activity was expressed as:

$$\% \text{ Radical scavenging activity} = [1 - (A_{sample}/A_{control})] \times 100$$

2.5 Antimicrobial Bioassay

2.5.1 Tested Microorganisms

The different extracts of *Euphorbia terracina* were tested against six gram negative bacteria*: Proteus vulgaris, Klebsiella pneumoniae, Shigella, Escherichia coli, Erwinia carotovora, Pseudomonas aeruginosa*, and three gram positive bacteria; *Streptococcus pyogenes, Staphylococcus aureus* and *Bacillus subtilis. Candida albicans* is fungal strain employed in the screening.

2.5.2 Antimicrobial Activity

Filter paper discs (5 mm in diameter) were prepared before use and sterilized in an autoclave for 20-30 min. A sterile paper disc was socked in crude extract of the studied plant and then placed over the surface of the inoculated nutrient agar in antibacterial assay and on potato dextrose agar in antifungal assay [19]. All Petri dishes were incubated at 37 °C for 24 hrs. After incubation, the diameter of inhibition zone (cm) was measured for recording the clear zone and compared with the DMSO as control.

3. Results and Discussion

3.1 Phytochemical Constituents

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants [20]. The phytochemical analyses of the air dried aerial parts of *Euphorbia terracina* are presented in Table 1. *E. terracina* exhibited the highest content of saponins and phenolics (39.17±1.3 mg/g D.W. and 22.11±0.9 mg/g D.W., respectively).

Table 1 The concentration of the active constituents in mg/g dry weight for the *Euphorbia terracina*

Plant species	Phenolics	Saponins	Tannins	Alkaloids	Flavonoids
	mg/g Dry Weight				
Euphorbia terracina	22.11±0.9	39.17±1.3	4.87±0.3	8.25±0.6	10.24±0.5

3.2 Antioxidant Activity

The evaluation of the antioxidant activity of the four plant extracts is showed in Table 2. By increasing the plant extract concentration there was a corresponding continuous increase in scavenging activity. In case of ethyl acetate, hexane, petroleum ether and methanol extracts the increase was up to 1000 µg/mL where the scavenging activity was 50.25%, 23.85%, 11.35% and 10.98, respectively.

Table 2 Percent scavenging activity of different extracts of *Euphorbia terracina*

Concentration	% of scavenging activity				
µg/mL	Methanol	Hexane	Petroleum ether	Ethyl acetate	Catechol
1000	10.98	23.85	11.35	50.25	89.54
800	9.28	21.87	6.57	25.04	82.69
600	6.57	16.21	3.57	21.71	72.68
400	5.24	14.83	2.00	18.80	61.54
200	3.54	6.73	2.00	12.23	51.24
100	1.96	3.67	1.86	10.23	31.49
IC_{50} (mg/mL)	4.98	2.09	5.00	1.23	0.29

The IC_{50} values of the extracts of *E. terracina* were presented in Table 2 and Fig. 2. Ethyl acetate extract had the highest scavenging activity (1.23 mg/mL). The radical scavenging activity of the other extracts and standard decreased in the following order: catechol, hexane, methanol and petroleum ether.

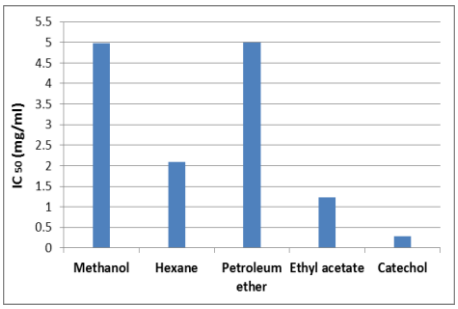

Fig. 2 IC_{50} values (mg/mL) of *E. terracina* extracts and natural antioxidant catechol (standard)

3.3 Antimicrobial Activity Assessment

The development of microbial resistance to presently available antibiotics led the search for new antimicrobial agents [21]. Due to the problem of microbial resistance to antibiotics, attention is given toward biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antimicrobial activities [22]. The antibacterial activity of *E. terracina* was assayed in vitro by agar well diffusion method against nine different bacterial strains. The part used for the study was shoot system, while the solvents used were methanol, petroleum ether, hexane and ethyl acetate. Therefore, in all, four extracts were evaluated for antibacterial activity as shown in Table 3 and Fig. 3.

Table 3 The inhibitory activity of *E. terracina* extract against the tested organisms as demonstrated by diameters of the inhibition zone (mm)

Test microorganisms	Plant extracts				Standard antibiotic	
	Methanol	Hexane	Pet. ether	Ethyl acetate	Ampicillin	Amphotericin B
Gram positive bacteria						
Staphylococcus aureus	-	9.5	10	9	16	-
Bacillis subtillis	7	10	6.5	7		-
Streptococcus pyogenes	7	7	-	-	25	-
Gram negative bacteria						
Pseudomonas aeruginosa	7	-	-	-	10	-
Escherichia coli	-	7.5	6.5	-	14	-
Proteus vulgaris	9.3	-	6.5	-	16	-
Klebsiella pneumoniae	7	10.5	7	-	27	-
Shigella sp.	-	-	-	7	-	-
Erwinia carotovora	-	10	6.5	8	-	-
Fungi						
Candida albicans	7	9	-	8	-	3

* The recorded value is mean value of 3 replicates

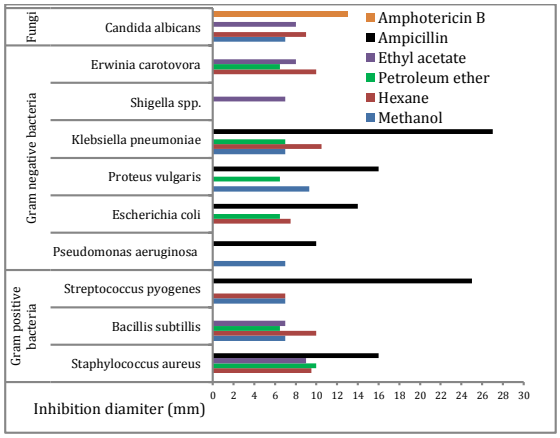

Fig. 3 Antimicrobial activity of different extract of *E. terracina* and standard antibiotic

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In the present study hexane extract of *E. terracina* exhibited appreciable broad spectrum (70%) against both Gram-positive bacteria and Gram-negative bacteria (Fig. 4), followed by petroleum ether and methanol extracts (60%, each) then ethyl acetate (50%). Hexane extract showed the inhibitory activities against *Bacillis subtillis*, *Erwinia carotovora* (10 mm, each) and *Klebsiella pneumoniae* (10.5 mm).

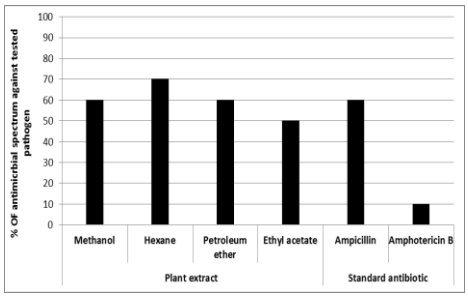

Fig. 4 % of antimicrobial spectrum of *E. terracina* extracts and standard antibiotic

The pathogen *S. aureus* was the most sensitive bacteria in case of petroleum ether and ethyl acetate extracts (10 mm and 9 mm, respectively), whereas *Proteus vulgaris* was the most sensitive in case of methanol extract. This might be due to the high polarity of methanol [23].

Many plant species present inhibition zones of differing diameters; however, size difference of the inhibition zone depends primarily upon many factors for e.g. diffusion capacity of substances (present in the extracts) in the agar medium, antimicrobial activity of diffused substances, growth and metabolic activity of microorganisms in the medium [24].

The most susceptible bacterium was *B. subtillis*, *S. aureus*, *K. pneumoniae* and *E. carotovora* while the most resistant bacteria were *Pseudomonas aeruginosa* and *Shigella* sp. The antifungal activity of hexane extract showed the highest activity against *C. albicans* (9 mm), followed by ethyl acetate (8 mm) then methanol extract (7 mm).

Although secondary products can have a variety of functions in plants, it is likely that their ecological function may have some bearing on potential medicinal effects for humans. For example, secondary products involved in plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic [25].

Most of the known medicinal plants exert antimicrobial potential. Tannins bind with proteins rich with proline and can interfere with protein synthesis [26]. Antimicrobial effect of flavonoids could be attributed to form complex with soluble proteins and cell walls of bacteria [27]. Saponin have been ability to cause protein leakage as well as certain enzymes from the cell [28]. Alkaloids have been suggested to function as antibacterial agents [29].

From the above results, it can be concluded that Gram-positive bacteria are susceptible to plant extracts more as compared to Gram-negative bacteria. Various workers have already reported similar results [30-33]. Abdallah [34] found similar results that the methanol extract of *E. terracina* from Saudi Arabia was active against *E. coli, K. pneumoniae, P. aeruginosa, B. subtilus, S. aureus* and *C. albicans.* El-Amier et al. [35, 36] also reported that *Senecio glaucus* and *Urospermum picroides* of (Asteraceae, from coastal desert of Nile Delta) extract showed an inhibition zone against same investigated microorganisms.

4. Conclusion

Efforts must be geared towards measures that will enhance the effectiveness, efficiency and rational use of medicinal plants, especially through the integration into national, regional and local health policies and programmes. The present results emphasize the validity of using *E. terracina* extracts in traditional medicine and contains substances that can be used for therapeutic purposes. Generally, the use of particular herb in medicine is quite safe compared to the chemically synthesized drug, but further studies should be carried out for enhancing the activity of plant extracts. It is also recommended to test safety and toxicity of any plant extract before its pharmaceutical application.

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