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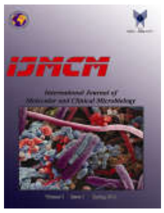

Research Article

Antimicrobial and radical scavenging potentials of *Euphorbia serpens Kunth*

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ABSTRACT

Euphorbia serpens (Euphorbiaceae) is an annual species native to South America and widely distributed as a weed in pantropical regions. It has attracted attention from academic researchers due to its diverse pharmacological properties. In this study, *in vitro* antioxidant capacity and antimicrobial activity of various extracts of the roots, and shoots of *Euphorbia serpens* were investigated using DPPH free radical scavenging assay and disc diffusion method, respectively. Antibacterial activity was tested on three Gram-positive (*Streptococcus pneumoniae*, *Bacillus subtilis* subsp. *Spizizenii,* and *Enterococcus faecalis*) and three Gram-negative (*Shigella flexneri, Proteus vulgaris,* and *Salmonella typhi*) bacteria. The antifungal effect was also examined on three fungi (*Aspergillus fumigatus, Candida albicans,* and *Fusarium oxysporum*). Ethanolic shoots extract showed broad-spectrum antimicrobial effects. The best antioxidant activities were also observed with alcoholic shoot extracts. It is concluded that the shoot of *Euphorbia serpens* is a rich source of bioactive compounds especially if they were extracted by alcohols. They are efficient candidates to treat oxidative stress-related diseases.

1. Introduction

Encompassing over 2000 species, *Euphorbia* is one of the biggest genera of angiosperms in the family Euphorbiaceae s.s., harboring approximately 6300 species in 247 genera (Wurdack et al., 2005; Pahlevani, 2017). The *Euphorbia* species include a broad spectrum of annuals to small trees which cover both tropical and temperate regions (Pahlevani, 2017). *Euphorbia serpens* Kunth is a small, prostrate annual herb with opposite hairless leaves. The inflorescence is a typical cyathium of the genus with appendages and nectary glands. The fruit is a three-lobed capsule with one seed in each locus. *Euphorbia* was named after Euphorbus, a Greek physician who described the plants' medicinal properties and serpens, is Latin for creeping and referring to the habit of the plant. The species is native to South America, but it has been introduced to many countries and is regarded as a pantropical weed. *Euphorbia serpens* was first described in 2006 from Tehran, Iran (Javadi et al., 2006), later it was reported in other parts of the country as well.

More than 5% of the species of *Euphorbia* are considered medicinal plants which indicates the potential of its species as a source of natural products (Ernst et al., 2015). They are also used as ornamental and house plants worldwide. *Euphorbia* species are more frequently used to treat digestive tract disorders, respiratory systems diseases and skin problems (Mavundza et al., 2022). *Euphorbia serpens* is traditionally

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used by people in the Brazilian Pampa to treat urinary infections (Teixeira et al., 2016).

Free radicals, produced as byproducts of normal cellular metabolism, oxidize macromolecules and trigger the development of serious health problems such as hypertension, cancer, multiple sclerosis, Alzheimer's and heart attack (Tan et al., 2018; Yadav et al., 2016). Antioxidants are natural or synthesized substances that scavenge free radicals and prevent, lessen or postpone cell damage and subsequent health problems. The crucial role of antioxidants in organisms is to convert free radicals into less reactive species and prevent oxidation in cells or tissues (Yadav et al., 2016). Phenolic compounds are secondary metabolites produced in plants with one (phenolic acids) or more (polyphenols) aromatic ring(s) and a various number of hydroxyl groups, which are responsible for their antioxidant activities and play important roles in fighting against free radicals (Minatel et al., 2017; Ebrahimnezhad et al., 2022; Al-Mamary and Moussa, 2021). A large number of studies have documented the positive relationship between phenolic content, antioxidant capacity and antimicrobial activity (Abdel-Gawad et al., 2021; Ghafoor et al., 2020). Considering the increasing emergence of drug-resistant pathogens due to the overuse of antibiotics, discovering potent and natural sources of antimicrobial compounds is a necessity (Álvarez-Martínez et al., 2020). It is estimated that more than 200,000 plant secondary metabolites have been isolated and identified so far, which is a small, but certainly growing number considering the 391,000 described plant species (of which around 369,000 (94%) are tracheophytes) (Kessler and Kalske, 2018; Willis, 2017). The growing emergence of multi-drug resistant pathogenic bacteria, as a result of overuse and misuse of antibiotics, has been a big concern during the last decades. Infectious diseases are currently the 2nd leading killer in the world and it is predicted that by 2050, drug-resistant pathogens will cause 300 million premature deaths worldwide (Munita and Arias, 2016; Church and McKillip, 2021). The bacteria can resist or evade antibiotics *via* various biological and biochemical ways including enzymatic alteration of the antibiotics, destroying the antibiotics using *β*-lactamases, decreasing membrane permeability, pumping the antibiotics out of the bacterial cell by efflux pumps and changing the target site for the antibiotic (Church and McKillip, 2021).

Obviously, extracts from the same plant organ contain different chemical compositions depending on the extractive solvent. This study evaluates the antioxidant capacity and antimicrobial activity of the roots and shoots of *Euphorbia serpens* using four solvents with different polarities to obtain a more detailed understanding of the pharmaceutical and nutritional potentials of this folk medicinal plant.

2. Materials and Methods

2.1 Preparation of the plant material

A population of *Euphorbia serpens* was collected from the city of Zabol, (coordinates 31°02′39″N 61°31′37″E, 480m.), Sistan and Baluchistan, in June 2022 (Figure 1). The plant name was authenticated by the second author, a plant systematist at the University of Zabol, where the voucher specimen (*E. serpens N0: 1502)* is deposited. The roots and aerial parts of the samples were segregated and dried in shade at room temperature for several days. The air-dried material was then milled using an electrical blender.

2.2. Extract preparation

To prepare the ethanolic, methanolic, dichloromethane and petroleum ether extracts of shoots and roots of *Euphorbia serpens*, 3 g of powdered roots and shoots were weighed into eight separate beakers. A volume of 30 ml of each solvent was added to them and the mixtures were placed on a rotary for 24 hours and shaken unceasingly in the dark at room temperature to maximize the compound extraction. Then, the mixtures were passed through a Whatman paper No 2 and the filtrates were allowed to evaporate at 37 ºC to obtain the dried extracts.

2.3 Antioxidant activity

The antioxidant activity of the extracts was tested using the DPPH (2,2-Diphenyl-1-picrylhydrazil) protocol and the IC_{50} (Half-maximal inhibitory concentration) values of plant extracts were achieved, following the previously published papers (Beyzaei et al., 2018; Beyzaei et al., 2021). A volume of 1 ml of each plant extract (concentrations of 25, 50, and 100 µg.ml^{-1}) was mixed with 4 ml of freshly prepared methanolic solution of DPPH (0.004% w/v) in separate test tubes. The mixtures were shaken and then rested in the dark for 30 min at room temperature. Then, the absorbance of the remaining DPPH radical was measured at 517 nm.

The same procedure was followed for blank sample. The equation: I% = [(A blank - A sample) / (A blank)] × 100 was applied to calculate the inhibition percentage of samples, where A is the absorbance at 517 nm. Finally, the equation of the straight line was obtained and the IC_{50} was quantified where y equals 50.

Figure 1. A) The locality of the studied population of *Euphorbia serpens*. B) The habit of the plant. C) Details on leaves, inflorescences and fruit of *E. serpens*.

2.4 Antimicrobial activity

The ethanolic, methanolic, dichloromethane and petroleum ether dried extracts of roots and shoots of *Euphorbia serpens* were dissolved in 10% DMSO (dimethyl sulfoxide) to stock concentrations of 100 $mg.ml^{-1}$. The antimicrobial activity of all eight solutions was tested against three Gram-negative bacterial strains of *Shigella flexneri* (PTCC 1234), *Proteus vulgaris* (PTCC 1079) and *Salmonella typhi* (PTCC 1609), three Gram-positive bacterial strains of *Streptococcus pneumoniae* (PTCC 1240), *Bacillus subtilis* subsp. *Spizizenii* (PTCC 1023) and *Enterococcus faecalis* (PTCC 1778), and three fungal strains including *Aspergillus fumigatus* (PTCC 5009), *Candida albicans* (PTCC 5027) and *Fusarium oxysporum* (PTCC 5115). All examined microorganisms were purchased from the Persian Type Culture Collection (PTCC), Tehran, Iran. The disk diffusion susceptibility test was used to determine the sensitivity or resistance of examined pathogens by quantifying the IZDs (Beyzaei et al., 2017).

2.5 Statistical data analysis

All tests were performed in triplicate and reported as average ± SD (Standard Division). Data were analyzed statistically by ANOVA and Tukey’s tests at a significance level of P-Value < 0.05 using the SPSS statistical software (version 22).

3. Results

Antioxidant effects of extracts were studied *via* DPPH free radical scavenging method. The results were reported as IC_{50} values in Table 1.

The best antioxidant properties were recorded with methanolic and ethanolic shoots extracts, respectively. Unlike petroleum ether, methanol was able to effectively extract the antioxidant factors of both roots and shoots. The results indicated that the shoots possess higher antioxidant contents compared to the roots.

Inhibitory potentials of all extracts were evaluated against some important pathogenic bacterial and fungal strains from different genera. The IZD values are shown in Table 2.

Table 1. IC_{50} values as $\mu g.ml^{-1}$±SD determined by DPPH free radical scavenging method

Extracts	EtOH	MeOH	DCM	PET
Shoot	20.41±1.78	13.17±1.53	553.26±4.16	172.57±3.62
Root	101.56±3.45	27.09±2.69	48.35±2.47	247.45±3.94

Table 2. The inhibition zone diameters (as mm±SD) of *Euphorbia serpens* extracts

Strains	Extracts	EtOH	MeOH	DCM	PET
S. flexneri	Shoot	11.62±0.38	0	0	11.28±0.52
	Root	0	8.11±0.25	13.74±0.71	9.57±0.66
P. vulngaris	Shoot	11.51±0.22	13.42±0.17	0	0
	Root	8.65±0.48	9.41±0.83	0	0
S. typhi	Shoot	10.52±0.32	0	12.88±0.71	0
	Root	0	0	0	0
S. pneumoniae	Shoot	0	0	0	0
	Root	0	0	0	9.04±0.29
B. subtilis	Shoot	11.15±0.67	0	9.08±0.83	0
	Root	0	0	13.44±0.08	0
E. faecalis	Shoot	0	0	0	0
	Root	0	0	0	0
A. fumigatus	Shoot	9.94±0.46	0	9.61±0.23	0
	Root	0	11.32±0.26	0	0
C. albicans	Shoot	13.73±0.69	12.48±0.53	8.44±0.65	0
	Root	11.02±0.27	10.79±0.17	0	9.04±0.29
F. oxysporum	Shoot	0	0	0	0
	Root	0	0	0	0

EtOH = Ethanol; MeOH = Methanol; DCM = Dichloromethane; PET = Petroleum ether.

Broad-spectrum antimicrobial activity was observed with ethanolic shoot extract. Petroleum ether shoot extract only inhibited the growth of *S. flexneri* strains. Petroleum ether root extract was the only effective agent against *S. pneumoniae*. No inhibitory effects were observed with all extracts on *E. faecalis* and *C. albicans* strains. It seems that ethanol and dichloromethane are suitable solvents for the extraction of biologically active compounds from the shoots of *Euphorbia serpens*. Methanol could extract the maximum bioactive compounds from the plant roots.

4. Discussion

From the past to the present, the use of plants in the treatment or prevention of diseases has been widespread (Jamshidi-Kia et al., 2018). Advances in the pharmaceutical industry have led to the design of new drugs based on active plant ingredients (Mathur and Hoskins, 2017). Increased levels of free radicals can damage body organs, tissues, and cells by attacking biomolecules such as carbohydrates, lipids, proteins, RNA, and DNA and ultimately cause oxidative stress (Engwa et al., 2022). Many plants are rich sources of minerals, vitamins, proteins, fibers and enzymes to prevent oxidative stress (Alagawany et al., 2020; Patel and Patel, 2016). They are considered good antioxidant agents in dealing with free radicals and oxidative stress-related diseases.

Species of the genus *Euphorbia*, the third largest genus of flowering plants, are prescribed to treat a wide range of human and animal diseases such as *actinic keratosis*, skin and eye cancers, disorders of digestive, respiratory, nervous, endocrine, blood and muscular-skeletal systems, mental complaints, infections and inflammation (Kemboi et al., 2020). *Ingenol mebutate* is one of the active plant ingredients of *Euphorbia* sap and used for the treatment of *actinic keratosis (*Ernst et al., 2015). Some species of the genus *Euphorbia* have been effective against pathogenic microorganisms. For example, *methanolic extracts* of leaves and seeds of *E. dendroides*, *E. biumbellata* and *E. terracina* collected from Algeria were tested against some pathogenic bacteria and fungi in which antimicrobial effects were observed with all extracts (IZDs of 7 to 22 mm) and the results showed that antimicrobial effects of leaf extracts were more than those of seed extracts (Zeghad et al., 2016). Their broader antimicrobial effects compared to our extracts are due to the higher concentration of the initial solutions (150 vs 100 $mg.ml^{-1}$), even though genetic differences are also involved. Methanolic extracts of aerial parts were also evacuated for their antioxidant capacities against DPPH where the IC_{50} values ranged from 49 to 84 $mg.ml^{-1}$. IC_{50} values of 0.013-.0553 $mg.ml^{-1}$ were observed with *Euphorbia serpens* extracts tested in this study showing higher antioxidant capacities compared with the extracts of above mentioned three species. Inhibitory activity of ethanolic crude extract of *Euphorbia serpens* Kunth leaves (collected from India) and their fractions from ethanol, *n*-butanol, *n*-hexane, and chloroform were assessed on *Candida spp.*, Gram-positive *S. aureus* and Gram-negative *E. coli* and *Klebsiella spp.*; high, intermediate and null activates were observed with all extracts against Gram-positive bacterium, fungus, and Gram-negative bacteria, respectively (*Ravi Kantha Reddy* and *Venkateswara Rao, 2019)*. The IZDs of 6.67-23 and 0-7 mm were recorded by extracts against *S. aureus* and *Candida spp.,* respectively. But our tested extracts were more effective in inhibiting the growth of Gram-negative bacteria than Gram-positive bacteria. Antibacterial effects of extracts of Indian *Euphorbia serpens* in water, ethanol, ethyl acetate, dichloromethane, and hexane were studied against 10 antibiotic-resistant and 5 non-resistant *Vibrio cholera* strains (Payne et al., 2015). The maximum anti-*Vibrio* activity was observed with aqueous extract against all tested strains. The MIC values of 3.92 and 12.32 $mg.ml^{-1}$ were recorded against *V. cholerae* strain by bioactive components separated by column chromatography from *E. serpens* and Amaranthus *viridis*, respectively. In addition, their high antioxidant capacities were proved based on TRP (Total reducing power), FRAP (Ferric reducing ability of plasma) and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assessments. *E. serpens* extracts showed much higher antioxidant activity than that of *A. viridis.*

5. Conclusion

The presence of a wide range of biologically active ingredients in extracts of shoots and roots of *Euphorbia serpens* Kunth prompted us to investigate their antimicrobial and antioxidant properties. The ethanolic and dichloromethane shoot extracts and methanolic root extract were able to block the growth of more tested bacterial and fungal pathogens. According to the results obtained from the DPPH free radical scavenging test, alcoholic extracts showed high antioxidant potentials. Oxidative stress caused due to an imbalance between free radicals and antioxidant plays a key role in the pathogenesis of several diseases such as cancer, Alzheimer's, atherosclerosis, arthritis, Parkinson's, diabetes, cataract, hypertension, asthma, respiratory and cardiovascular diseases and inflammatory disorders. The high antioxidant potential of *Euphorbia serpens* Kunth is a suggested solution in the treatment or prevention of these diseases.

Author contributions

HB supervised the data collection and prepared the final version of the manuscript. MD contributed to the plant sampling and identification as well as manuscript preparation. ZE collected the laboratory data. All authors have approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest associated with this manuscript. The authors alone are responsible for the content of the paper.

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