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Determination of Phenolic and Flavonoid Contents of Roots and Shoots of *Euphorbia serpens* Kunth Using Different Solvents

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Abstract

Euphorbia serpens Kunth (Euphorbiaceae) is an exotic annual plant species native to South America but is regarded as a pantropical weed. In this paper, the total phenolic and flavonoid contents of ethanolic, methanolic, dichloromethane, and petroleum ether extracts of shoots and roots of *Euphorbia serpens* were assessed *in vitro*. The plant materials were collected from Zabol, Sistan and Baluchestan in June 2022. The Folin-Ciocalteu and aluminum chloride colorimetric instructions were followed to evaluate the total phenolic and flavonoid contents of the extracts, respectively. The methanolic extract of aerial parts contained the highest amount of phenolic compounds (59.205 mg GAE/g), while the lowest content of phenols was found in the dichloromethane extract of roots (29.794 mg GAE/g). Also, the greatest amount of flavonoids was recorded for methanol extracts of aerial parts (34 mg RE/g), whereas the ethanol extract of roots contained the lowest amount of flavonoids (1.204 mg q/g). The aerial parts of *Euphorbia serpens*, in general, contain higher amounts of polyphenols as compared with the underground parts. The results also showed that phenolic and flavonoid contents vary significantly with the employed solvent. It can be concluded that the aerial parts of *Euphorbia serpens* are rich sources of polyphenolic compounds, signaling their potential for high antioxidant activity and nutritional and pharmaceutical importance.

1. Introduction

Euphorbia, belonging to the family Euphorbiaceae s.s., is one of the largest genera of flowering plants with over 2000 species of annual, perennial, succulent, shrub, and even small trees in temperate and tropical areas [1-2]. Over 5% of *Euphorbia* species are used for medicinal purposes, often for skin problems, digestive disorders, and respiratory diseases [3-4]. *Euphorbia serpens* Kunth is a small creeping annual plant native to South America, growing as a pantropical weed in Iran and many other countries [5]. The species can grow densely and cover the ground completely in warm places where water supply is available. *Euphorbia serpens* is regarded as a medicinal plant by locals in the Brazilian Pampa, where it is used against urinary infections [6].

Plants produce a wide range of primary and secondary compounds to fulfill their physiological processes that are vital in their life cycle. Phenolic compounds are a group of secondary compounds, largely biosynthesized through shikimate, pentose phosphate, and phenylpropanoid pathways in plants. They help plants to adapt to various environmental stresses such as heavy metals, temperature, drought, pathogenic agents, and ultraviolet radiation [7-8]. Natural phenolic compounds are substantial in lowering the risks of several dreadful diseases, such as cardiovascular and pulmonary disease, diabetes, neurodegenerative diseases, etc., by preventing oxidative stress [8-9]. Over 8000 phenolic compounds have already been described, which are responsible for multifarious therapeutic activities in humans, with low toxicity [9].

The secondary compounds' biosynthesis and accumulation are affected by both genetic and environmental factors as well as the plant age and specific organs [10]. Various limiting factors, including temperature, carbon dioxide, salinity, soil properties, circadian rhythm, geography, as well as gene and enzyme regulation, impact on physiological and biochemical processes and subsequently on secondary compounds synthesis and accumulation, affecting the quality of therapeutic ingredients [10-12].

There is growing evidence that total phenolic and flavonoid contents are positively correlated with the antioxidant capacity of various organs of plants. Antioxidants are molecules that scavenge free radicals, inhibit oxidation reactions, and repair damaged cells. They play crucial roles in reducing the risk of developing various chronic diseases, neurodegenerative disorders, and cancer in the human body [13-14]. Antioxidants also retard the onset of lipid oxidation, caused by the reaction of molecular oxygen with fats, thereby delaying the oxidative rancidity of foods [15]. The antioxidant and antimicrobial properties of phenolic compounds candidate them as promising eco-friendly substances in the food industry. Nowadays, there is a growing concern about the risks of synthesized additives and preservatives in the food industry for human health. Plant-derived antioxidant and antimicrobial compounds can open new horizons in safety, shelf life, and quality of foods as substitutes for chemically manufactured additives and preservatives, as safer and risk-free components in appropriate doses [16-18].

In the present research, the attempt has been made to evaluate the quantity of the flavonoid and phenolic compounds in shoots and roots of the exotic weed *Euphorbia serpens* and deal with the influence of solvent characteristics on the amount of extracted metabolites. Also, this study aims to investigate the potential of this plant for pharmaceutical and nutritional purposes.

2. Materials and Methods

2.1 *Plant material collection*

The plant's shoot and root were collected in June 2022 from the countryside of Zabol (coordinates 31° 01' 50.16" N 61° 29' 41.64" E), Sistan and Baluchistan (Figure 1). The sampled species was authenticated by the first author, a plant taxonomist at the University of Zabol, where the voucher specimen (*E. serpens* N0: 1502*)* has been stored. The fruit-bearing shoots and roots were separated, cleaned, air-dried in the shade for a few days, and converted to powder using a blender.

2.2 *Preparation of the plant extracts*

The ethanolic, methanolic, dichloromethane, and petroleum ether extracts of shoots and roots of *Euphorbia serpens* were obtained by soaking 3 g of the corresponding powder in 30 mL of each solvent. The mixtures were continuously shaken at room temperature in the dark for 24 h to get the maximum possible amount of compounds. The extracts were then filtered through Whatman No. 2 papers, and the filtrates were lyophilized to obtain dried extracts.

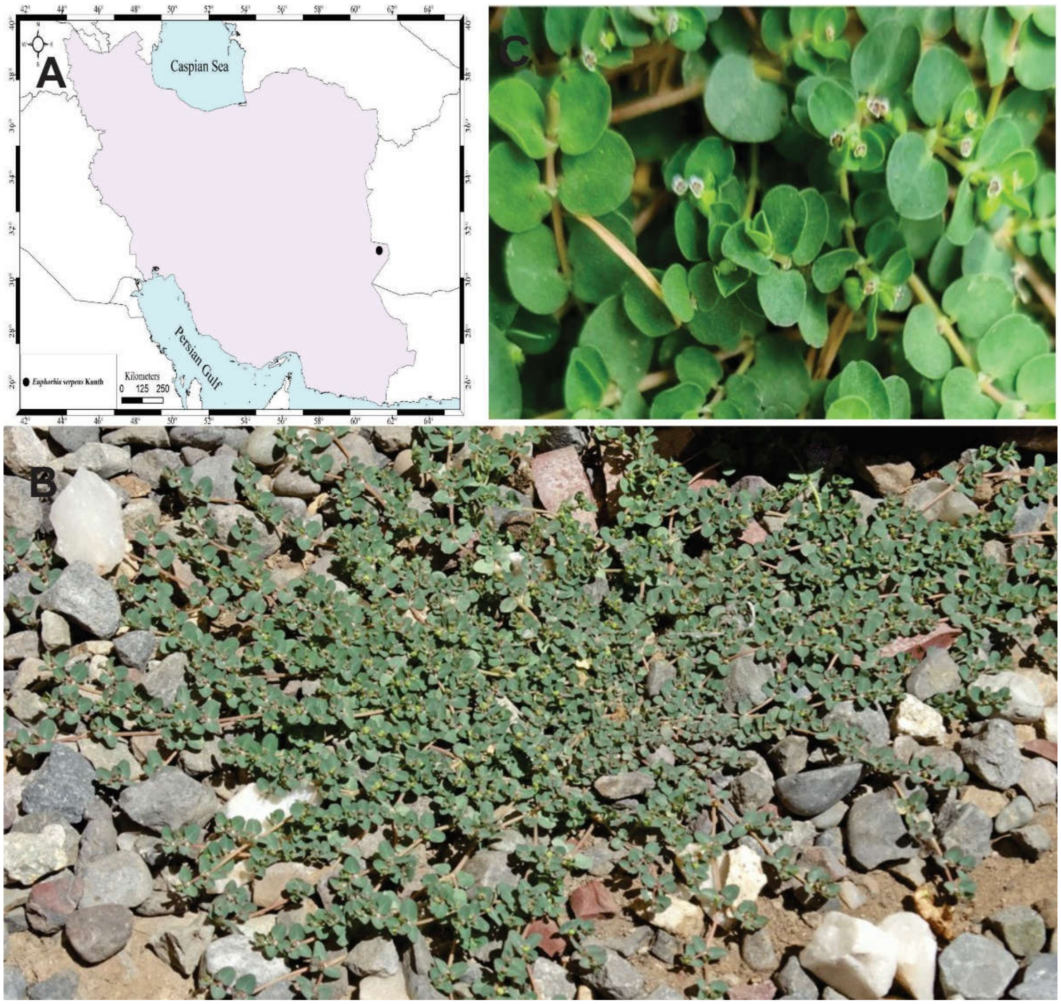

Figure 1. A) Geographic locations of the studied population of *Euphorbia serpens*. B) the habit and habitat of the studied population. C) morphological details of a fruit-bearing branch of *E. serpens*.

2.3 *Total phenolic content assessment*

The total phenolic contents of the roots and aerial parts of *Euphorbia serpens* were evaluated using the Folin-Ciocalteu reagent, with slight modifications [19]. On this account, 0.5 mL of each previously prepared extract was dissolved in methanol (100 µg/mL) in separate tubes. Then, 5 mL of Folin-Ciocalteu reagent (0.2 N) was added to them, and the solutions were shaken attentively and kept at room temperature for 5 min. Each tube received 2 mL of sodium carbonate solution (75 g/L) and was incubated in the dark for two h. Finally, the absorbance was read at 760 nm against a water blank *via* a spectrophotometer (model 6405 UV/Vis Jenway). This protocol was followed for gallic acid concentrations of 50, 100, and 200 μg/mL for drawing the calibration curve of absorbance against concentration. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dried extract (mg GAE/g DM) based on three replications.

2.4 ***Total flavonoid content assessment***

The total flavonoid contents of ethanolic, methanolic, dichloromethane, and petroleum ether extracts of roots and shoots of *Euphorbia serpens* were determined using the aluminum chloride colorimetric technique as modified by Richard et al. [20]. According to the mentioned procedure, 2 mL of aluminum trichloride ($AlCl_3$) in methanol (2%) was added to 2 mL of each extract (100 μg/mL). The mixture was well homogenized and incubated for 10 min at room temperature. A UV-Vis spectrophotometer was used to read the absorbance at 415 nm against a blank sample containing 2 mL of methanol, and 2 mL of each plant extract without $AlCl_3$. Quercetin was used in concentrations of 25, 50, and 100 μg/mL to draw the calibration curve of absorbance against concentration, and the results were expressed as mg QE/g DM. For all tests, triplicate measurements were made and averaged.

3. Results and Discussion

3.1 ***Determination of total phenolic content***

The total phenolic contents, based on the Folin-Ciocalteu method, in ethanolic, methanolic dichloromethane, and petroleum ether extracts of shoots and roots of *Euphorbia serpens* are shown in Table 1 and Figure 2. The standard curve was produced using different concentrations of gallic acid (Y= 0.0051 X+0.03575 R^2=0.8306), based on which the total phenolic contents of shoots and roots of *Euphorbia serpens* in various solvents were determined (Figure 3A). The values of total phenolic contents ranged from 29.79 to 59.21 mg gallic acid equivalents/g dried mass. The aerial parts of *Euphorbia serpens* showed higher phenolic content compared to the roots, irrespective of solvents employed (Table 1 and Figure 2). The highest and lowest amounts of phenolic compounds were found in methanolic extract of aerial parts and dichloromethane extract of the roots, respectively. The highest phenolic compound yield in the most polar solvent, i.e., methanol, indicates that it is the most efficient solvent in extracting phenolic compounds from *Euphorbia serpens* organs. The results of this study showed that the total phenolic content of an extract depends on the employed solvent.

3.2 ***Determination of total flavonoid content***

The total flavonoid contents, according to the aluminum chloride colorimetric method, in ethanolic, methanolic dichloromethane, and petroleum ether extracts of shoots and roots of *Euphorbia serpens,* are presented in Table 1 and Figure 4. To calculate the total phenolic contents of examined organs, the reference curve equation was obtained as y=0.0044x+0.0444 (R^2=0.973), using various concentrations of quercetin (Figure 3B). The values of total flavonoids varied from 1.205 to 34 mg quercetin equivalents/g dry mass. The results showed that similar to phenolic content, the amount of flavonoids in aerial parts of *Euphorbia serpens* is remarkably higher compared to those of the underground parts (Table 1 and Figure 2). Among the examined solvents, the greatest amount of flavonoid content was found in methanol, followed by ethanol.

Table 1. Total phenolic and flavonoid contents of the shoot and root extracts of *Euphorbia serpens* using different solvents

Samples	Aerial parts extract				Root extract			
Solvents	ethanol	methanol	dichloromethane	petroleum ether	ethanol	methanol	dichloromethane	petroleum ether
TPC	44.69608	59.20588	29.79412	30.77451	36.85294	46.26471	30.57843	30.38235
TFC	25.454	34	6.47727	18	1.20455	5.22727	3.79545	7.34091

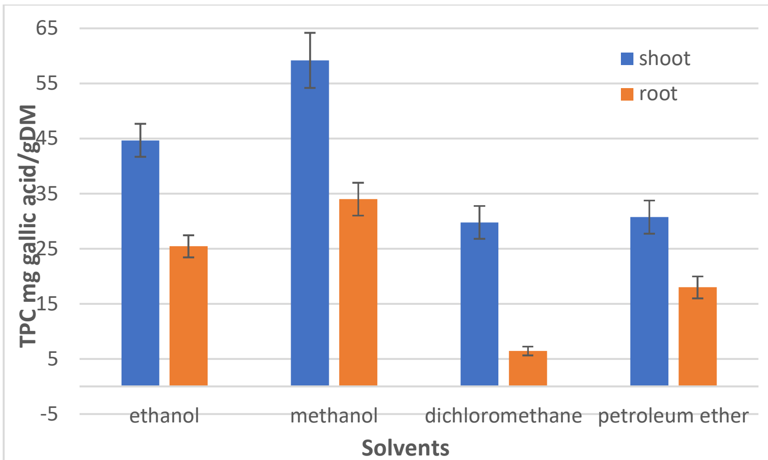

Figure 2. Total phenolic content (mg GAE/g dry m.) of aerial parts and roots of *Euphorbia serpens* extracted *via* different solvents

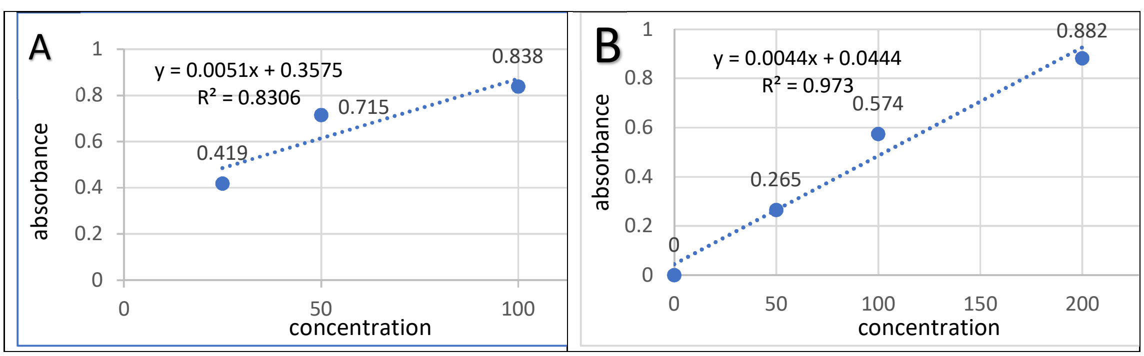

Figure 3. A. Reference curve produced based on various concentrations of Gallic acid, B. Calibration curve produced based on different concentrations of quercetin

The findings of this paper revealed that the alcoholic extracts of *Euphorbia serpens*, particularly those of the shoots, contain good amounts of phenolic as well as flavonoid compounds. There is only scarce knowledge of *Euphorbia serpens* chemical composition in the existing literature. The presence of tannins, saponins and flavonoids, as well as significant antioxidant activity, have been reported for crude ethanol extract of *Euphorbia serpens* and its ethylacetate and *n*-butanol fractions [21]. However, an amazingly high content of total phenolic (369,8 mg EGA/g sample) and a considerably low content of flavonoids (4.5 mg Q/g sample) were reported for the hydroalcoholic extract of *E. serpens* from Argentina [22], which, at least for phenolic content is not consistent with the results of this study.

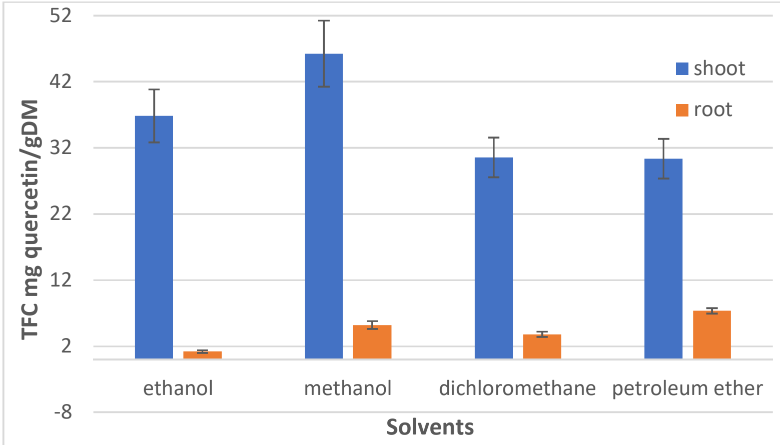

Figure 4. Total flavonoid content (mg QE/g dry mass) of aerial parts and roots of *Euphorbia serpens* extracted *via* different solvents

4. Conclusions

The determination of phenolic and flavonoid contents of roots and aerial parts of *Euphorbia serpens,* using various solvents, indicated several characteristics: 1- the amount of polyphenolic compounds in various extracts is solvent dependent, 2- the phenolic and flavonoid contents in aerial parts of the plant are higher than those of underground parts, 3- aerial parts of *Euphorbia serpens* are rich sources of phenolic and flavonoid compounds, signaling high potential antioxidant activity. The quantification of polyphenolics in medicinal plants can be considered a guide for their potential use in delaying or preventing numerous diseases associated with oxidative stress in human beings and animals, as well as in food preservation, since medicines and food products derived from plant sources are readily accessible, less expensive and nontoxic in proper doses. Further investigation into the biological activities of *Euphorbia serpens* and their mechanism of action is recommended for understanding its medicinal or nutritional values in human life quality.

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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