Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 2 pp. 341–345, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF THE EXTRACT OF *EPHEDRA PROCERA* FISCH. ET MEY

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Abstract: *Ephedra procera* belonging to the family Ephedraceae is a poison and medicinal plant. The main aim of present study was to determine total phenolic content and antioxidant and antibacterial activities of ethanolic extract from the aerial parts of *E. procera* collected from a natural habitat in Chaharmahal va Bakhtiari province, Southwestern Iran. The total phenolic content of the extract by Folin-Ciocalteu method and the antioxidant activity using DPPH assay were determined. The antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the extract were evaluated against five bacteria, including *Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes, Bacillus cereus* and *Staphylococcus aureus*. Total phenolic content in the extract of *E. procera* was 0.718 mg tannic acid/g dry weight extract. The results indicated that the ethanolic extract of *E. procera* exhibited radical scavenging activity. In addition, the results of this study confirmed that the ethanolic extract of *E. procera* exhibited antibacterial activity. In conclusion, the extract of *E. procera* could be an important source of phenolic components with antioxidant capacity and antibacterial activity.

Keywords: *Ephedra procera*, total phenolic content, biological activity

Ephedra L. (Ephedraceae) is a genus belonging to the Gnetales, the closest living relatives of the Angiosperms (1, 2). The family Ephedraceae has the only genus *Ephedra* L., which consists of about 50 species of perennials and shrubs in the world. *Ephedra* L. generally grows wild in arid and semiarid climates and is distributed mainly in the temperate zones of Europe, Asia and North America (3). The *Ephedra* consists of a group of perennial, evergreen, and dioecious sub-shrub species growing up to four feet tall, with slender and joined stems (4). The *Ephedra* species are called "joint-pine", "joint fir", "sea grape", "mormon-tea" or "shrubby horsetails" in English, and "Ormak", "Rish-boz" or "Ali-jonak" in Persian (5). In the flora of Iran, 12 species of *Ephedra* has been reported (5). This genus is commonly used by the Chinese people as a folk medicine for treatment of allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches, and nasal congestion (6). Results of previous studies on biological activity of the plant indicted that the extract from aerial parts of the *Ephedra* species have antimicrobial and antioxidant activities (7-12) and anti-ulcer properties (3). The main chemical compounds have been identified and isolated from *Ephedra* extract as alkaloids group such as ephedrine, pseudoephedrine, and norpseudoephedrine (4, 13, 14).

Medicinal plants can contain a wide variety of free radical scavenging molecules, including phenolic compounds, terpenoids etc., which are rich in antioxidant activity (15-17). Phenolic compounds are an integral part of the human diet and could be helpful against cancers, arteriosclerosis, ischemia, and inflammatory disease, which are caused by exposure to oxidative stress (18).

To our knowledge, there are no published reports on total phenolic content, antibacterial and antioxidant activities of *Ephedra procerea* belonging to the family Ephedraceae. The main objective of this study was to evaluate the content of phenolic compounds, antioxidants and antibacterial activities of the extract from *E. procera*.

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EXPERIMENTAL

Plant material

The aerial parts of *E. procera* were collected from a natural habitat in Chaharmahal va Bakhtiari province, Southwestern Iran (latitude 31°N; longitude 50°E; altitude 2250 m above sea level) in April 2012 (Fig. 1). Identifications were consequently confirmed with the help of the authentic specimens deposited at the Herbarium, Research Center for Agriculture & Natural Resources, Chaharmahal va Bakhtiari province, Shahrekord, Iran (No. 3025). Soil physical and chemical characteristics of natural habitat, including pH, electrical conductivity (EC), organic carbon (OC%) and soil texture were determined (Table 1).

Chemicals and reagents

Tannic acid, Na_2CO_3, and ethanol used in this study were purchased from Merck Co. (Darmstadt, Germany). The Folin-Ciocalteu reagent, and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (Steinheim, Germany).

Extract preparation

The aerial parts of the plant were shade dried and ground into a powder (100 g), macerated in 200 mL of ethanol 70% and filtered and then were dried at 35°C under rotary vacuum (Model Zirbus 302w, Italy). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

Determination of total phenolic content

The total phenolic content in each extract was determined using the Folin-Ciocalteu method following procedure of Singleton and Rossi (19) with some modifications. Briefly, 0.5 mL of the sample were mixed with 2.5 mL of Folin-Ciocalteu's phenol reagent and kept for 5 min at 37°C. Then, 2 mL of saturated Na_2CO_3 (7.5%) was added and the mixture was brought to 10 mL with the addition of deionized-distilled water. The mixture was maintained at room temperature in the dark for 120 min and then the absorbance of the mixture was measured at 765 nm against a reagent blank using a UV–Vis spectrophotometer (Shimadzu Corp., Japan). Tannic acid equivalent (TAE) was used as the reference standard and the TPC was expressed as mg of TAE equivalents per gram of each extract on dry basis.

Antioxidant test

The DPPH radical scavenging activity of extract was determined using the method proposed by Hung et al. (20). The extracts at concentrations of 16 to 500 µg/mL were mixed with an equal volume of 0.2 mM ethanol solution of DPPH. The disap-

Table 1. Geographical and climate of natural habitats of *E. procera*.

Region	P	T	pH	E.C.	O. C.	Sand %	Silt %	Clay %
Rig mountain	13.4	37.5	7.58	1.291	1.872	18.5	42	39.5

E.C.: electrical conductivity (dS/m), O.C.: organic carbon (%), and Sand, Silt and Clay in %. Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value in the mean of 10 to 15 year data. Soil characteristics are based on average of samples taken from three farms in each region.

Figure 1. Aerial parts of *E. procera*

pearance of the DPPH after 30 min of incubation at room temperature was determined spectrophotometrically at 515 nm. Ethanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant served as the control and was measured daily. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically and the percentage inhibition was calculated according to the equation:

$$\% \text{ inhibition} = \left[\frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}}\right] \times 100$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 30 min. The food preservative butylhydroxyanisole (BHA) was used as positive control.

Antibacterial test

Five strains of bacteria, including *Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes, Bacillus cereus*, and *Staphylococcus aureus* were obtained from Food Microbiology Laboratory, Veterinary Medicine Faculty, (I.A.U.) Iran. The density of bacteria culture for the test was adjusted to 0.5 McFarland standards (1.0×10^7 c.f.u./mL) and measured using a spectrophotometer (Eppendorf, AG, Germany). The MIC values were evaluated using the broth serial dilution method according to standard methods (21). Bacterial strains were cultured overnight at 37°C in Mueller Hinton broth (MHB). The extract dissolved in 5% DMSO was first diluted to the highest concentration (250 µg/mL) to be tested, and then a series of twofold dilutions were made in a concentration range from 16 to 250 µg/mL in 10 mL sterile test tubes containing nutrient broth. After incubation at 37°C for 24 h, absorbance at 630 nm was used as a measurement of bacterial growth using a spectrophotometer (22).

The MBC of extracts were determined according to the MIC values, i.e., 5 µL from MIC tubes were transferred to agar plates and incubated at 37°C for 24 h. The MBC was referred to the minimum concentration of extracts with no viable bacteria. Experiments were performed in three different times.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic content was determined spectrometrically according to the Folin-Ciocalteu method and calculated as tannic acid equivalents (TAE). Total phenolics content for the extract of *E. procera* was 718 mg TAE/g DWE. Total phenol content (TPC) was determined in comparison with standard tannic acid. Rustaiyan et al. (23) reported that a total phenolic content of *Ephedra sarcocarpa* growing in Iran was 709.18 mg catechin equivalent/g extract. Moreover, in other study, Rustaiyan et al. (23) reported that total phenolic content of *E. laristanica* growing in Iran was 513.03 µmol gallic acid/g extract. Results of a study of Ghasemi Pirbalouti et al. (3) indicated that total phenolic content in the extract of *Ephedra pachyclada* collected from Kerman, Iran was 45 mg of GAE/g dry weight.

Antioxidant test

Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems. The potential antioxidant activity of the extract was determined by the scavenging activity of the stable free radical DPPH. This is a quick, reliable and reproducible method to assess the *in vitro* antioxidant activity of pure compounds as well as plant extracts (24). The DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (25-27). The effect of antioxidants on DPPH is based on their ability to donate a hydrogen atom to DPPH, thus converting the radical into a stable molecule (28). The lower IC_{50} value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC_{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. In our study, the antioxidant activity of the ethanol extract from *E. procera* was expressed as IC_{50} with value 0.056 mg/mL, indicating that the extract acts as good DPPH scavenger. Rustaiyan et al. (29) reported that IC_{50} value in the DPPH assay of the methanolic extract of *Ephedra laristanica* growing in Iran was 4.6 mg/mL. Phenolics or polyphenols have received considerable attention, because of their physiological function, including antioxidant, antimutagenic, and antitumor activities (30). Plants phenolics present in herbs, because of their potential antioxidant activity, have been received considerable attention (31). Phenolic compounds, due to their antioxidant activities and free radical scavenging abilities, are widely distributed in plants (32), which have gained much attention and potentially have beneficial implications for human health (33). Therefore, phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of phenolics is mainly due to their redox properties, which make them acting as reducing agents, hydrogen donors, and singlet oxygen quenchers (34).

Antibacterial test

The antibacterial activity of the ethanol extract of *E. procera* was tested against the five pathogenic bacteria, including *Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes, Bacillus cereus* and *Staphylococcus aureus* by using the serial dilution method. The extract demonstrated relative inhibitory activities against the pathogenic bacteria tested. The growth inhibiting activity varied according to the dose and bacterial strain. The MICs and MBCs of the tested samples are presented in Table 2. MBC values were mostly higher than MIC values. The results indicated that the different bacteria species demonstrated different levels of sensitivity to the extract. The MICs of the extract were within concentration ranges from 250 to 500 µg/mL, and the respective MBCs were 500 and > 500 µg/mL. Generally, the ethanol extract from *E. procera* indicated moderate to good inhibitory activities against five bacteria investigated. Antimicrobial activity of some *Ephedra* species, including *Ephedra altissima* Desf. (35), *Ephedra transitorai* (36), *Ephedra nebrodensis* (37), and *Ephedra breana* (38) has been noticed in recent years. Kwon et al. (39) reported the antimicrobial activity of *Ephedra sinica* extracts against bacteria, such as *Vibrio parahaemolyticus, Clostridium perfringens, Bacillus subtilis* and *Staphylococcus aureus*. In addition, Rustaiyan et al. (23) studied the antimicrobial capacity of the methanolic extract of *E. sarcocarpa* growing in Iran against Gram-positive and Gram-negative bacteria and fungi. Results of their study indicated that the extract of *E. sarcocarpa* inhibited the growth of Gram negative bacteria, being *Pseudomonas aeruginosa* (MIC = 16 µg/mL). Results of other study by Rustaiyan et al. (29) indicated the antimicrobial activity of the methanolic extract of *E. laristanica* growing in Iran. They reported that the extract of *E. laristanica* inhibited the growth of Gram negative bacteria, especially *Escherichia coli* (MIC = 32 µg/mL). Lee and Lee (10) reported that quinaldic acid isolated from the stems of *E. pachyclada* had antibacterial activity against *Clostridium difficile* and *C. perfringens,* while had no effect on the growth of *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *L. casei*.

Results from this study suggest that phenolic compounds are responsible of the antibacterial activity of extract of *E. procera*. Numerous works have reported the antibacterial effects of these metabolites against a wide range of bacteria (40-42). Phenolic compounds can act at two different levels: the cell membrane and cell wall of the microorganisms (43). They can interact with the membrane proteins of bacteria by means of hydrogen bonding through their hydroxyl groups, which can result in changes in membrane permeability and cause cell destruction. They can also penetrate into bacterial cells and coagulate cell content (44).

CONCLUSIONS

The present study is apparently the first report of quantitative total phenol profile, antioxidant and antibacterial activities of the ethanol extract from the aerial parts of *E. procera*. Results of this study indicated that the extract from *E. procera* had the highest antibacterial properties. Phenolic compounds present in the plant are responsible for its effective free radical scavenging, antioxidant and antibacterial activities. With regard to the results of this present study the extract of *E. procera* could be an important source of phenolic compounds with antioxidant capacity and antibacterial activity. Nonetheless, in order to gain better views on the antioxidant levels and activities in *Ephedra* species, further studies on purification, identification and quantification of each phenolic compound and other non-phenolic compounds are necessary in the future.

Acknowledgments

This study was supported by Research Center for Medicinal Plants & Ethnoveterinary, I.A.U., Shahrekord Branch, Iran.

Table 2. Antibacterial activity of extract of *E. procera*.

Pathogens	Gram	MIC (µg/mL)	MBC (µg/mL)
Proteus vulgaris	Negative	250	500
Pseudomonas aeruginosa	Negative	500	> 500
Enterobacter aerogenes	Negative	250	500
Bacillus cereus	Positive	250	500
Staphylococcus aureus	Positive	500	> 500

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Received: 23. 06. 2014