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Free Radical Scavenging Activity and Phenolic and Flavonoid Contents of *Echinophora Platyloba* DC.

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ABSTRACT: Free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals. The aim of this study is the investigation of antioxidant properties of *Echinophora Platyloba* DC. We evaluates the free radical scavenging activity, total flavonoid and the total phenolic compounds of methanol, hydroalcoholic and water extracts of *Echinophora Platyloba* DC. The antioxidant activity was carried out using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay; total phenol content using the Folin-Ciocalteu method and aluminum chloride colorimetric method was used for flavonoids determination. The total phenol varied from 1.18±07 to 3.15±04 mg g^{-1} in the extracts. Flavonoid contents were between 8.15±22 and 3.16±14 mg g^{-1}. The highest radical scavenging effect belonged to methanol extract. The potential use of *Echinophora platyloba* for their antioxidant and naturalize activities against free radicals is discussed.

KEYWORDS: Antioxidant, free radicals, phenolic contents, flavonoid contents, DPPH

ORIGINAL ARTICLE

INTRODUCION

Phytochemicals are chemicals derived from plant sources. Plants contain hundreds of phytochemicals such as flavonoids and phenolic acids (Fukumoto and Mazza, 2000). Research indicates phytochemicals such as polyphenols have high antioxidant activity (Stojicevic et al., 2008). Free radicals are highly reactive and are generated in the body through normal cellular function. Free radicals are believed to cause lipid oxidation leading to cellular membrane damage. Antioxidant research is a key topic in both the medical and food industry today. Antioxidants protect the body from reactive species (Korkina, 2007; Kumaraswamy et al., 2008). Research investigating bioactive compounds from plant materials has inversely correlated to the intake of bioactive phytochemicals and disease (Cowan, 1999; Babu et al., 2007; Bouayed et al., 2007).

In Iran, fresh and dried aerial parts of some of these species are added to cheese and yoghurt for flavoring. The genus *Echinophora* has been the subject of scant phytochemical and biological investigations (Asghari et al., 2003; Zheng and Wang, S.Y. (2001).

MATERIALS AND METHOD

Chemicals: 2, 2-Diphenyl-1-picryl hydrazyl (DPPH), Aluminium chloride (AlCl3), Potassium acetate, quercetin, Gallic acid, Folin-Ciocalteu phenol reagent, Na2Co3 and methanol were purchased from Merck co. (Germany).

Plant Material

Plant was collected from Hamedan. Plant materials were dried at room temperature, then were grinded and extracted (20g) with methanol, hydroalcoholic and distilled water (200 ml) for 48 h. The extracts were filtered and obtained filtrates were concentrated under the reduced pressure.

Determination of total phenolics

Total phenolic compounds in extracts were determinated spectrophotometrically using the Folin-Ciocalteu reagent. A dilute extract of each plant extract (0.5 ml of 1:10g ml-1) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous Na2Co3 (4ml, 1M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L-1 solutions of gallic acid in methanol:water (50:50,v/v). Total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g dry weight. Samples were analyzed in three replications.

Determination of total flavonoids

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Aluminum chloride colorimetric method was used for flavonoids determination (Pourmorad et al., 2006). Each plant extracts (0.5 ml of 1:10 g ml^{-1}) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100µg ml^{-1} in methanol.

DPPH radical scavenging activity method

The free radical scavenging activity of the plant extracts was evaluated using the stable radical DPPH. A series of extracts with different concentration (1.00-0.01 mg/ml^{-1}) were prepared. Then 2.5 ml of the extract and 1.0 ml of a 3.0×10^{-4} M DPPH solution in methanol were mixed and placed in the dark at the room temperature for 30 min. the absorbance of each sample of the plant extract containing DPPH (A_s) was measured at 517 nm using UV–VIS lambda spectrophotometer. Methanol (1.0 ml) plus the plant extract solution (2.5ml) was used as the blank, while the DPPH solution plus methanol was used as the control. All determinations were performed in triplicate. The DPPH anti-radical scavenging activity, DPPH (%), of each plant extract was determined using the following equation:

$DPPH= 100(1-A_S-A_b/A_c)$

Were A_S is the absorbance in the presence of the plant extract in the DPPH solution, A_c is the absorbance of the control solution (containing only DPPH) and A_b is the absorbance of the sample extract solution without DPPH. Ascorbic acid was used for comparison (EC_{50} = $0.0039\pm0.0007 mg/ml^{-1}$).

RESULTS

Flavonoid and total phenol contents of the extracts

The contents of total phenolic compounds and flavonoids in methanolic, hydroalcoholic and water extracts of *Echinophora platyloba* are presented in Table1.

The obtained results showed that the contents of total phenolic compounds and flavonoids in methanolic extracts of *E. platyloba* were significantly higher than in the corresponding water extracts. The DPPH method was chosen to evaluate the antioxidant activity because it is one of the most effective methods for evaluating the concentration of radical scavenging material effective as a chain breaking mechanism. To calculate the concentration of the extract necessary to decrease DPPH radical concentration by 50% (called EC_{50}). The EC_{50} value was used to measure the antiradical activity of the extract: the lower EC_{50}, the higher is the value of the anti-radical activity. In table 1, the extract obtained by Methanolic extract of *E. platyloba* showed the highest antioxidant activity.

Table 1. Contents of total phenolic compounds and flavonoids in methanolic and water extracts of *E. platyloba*

Extract	Total phenolic compounds (mg/g)	Total flavonoids compounds (mg/g)	DPPH assay (EC_{50})
Methanolic extract of *E. platyloba*	3.15 ± 04^a	8.15 ± 22^a	30.93 ± 1.58^a
Water extract of *E. platyloba*	1.18 ± 07^b	3.16 ± 14^b	73.78 ± 1.36^c
hydroalcoholic extract of *E. platyloba*	1.78 ± 03^b	3.9 ± 09^b	57.20 ± 4.36^b

CONCLUSION

These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Oxidative DNA damage has been implicated to be involved in various degenerative diseases including Alzheimer's disease, Parkinson's disease.

The result of the present study showed that the methanol extracts of *E. platyloba* which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. After this comparative study, our objective will be identification and determination of the amount of individual polyphenolics responsible for the majority of antioxidant activity this plant.

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