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Full Length Research Paper

Antioxidant and antibacterial activity of the extracts of *Echinophora platyloba* D.C

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Hydroalcholic and aqueous extracts of *Echinophora platyloba* D.C were screened for their possible antioxidant activity by β-carotene-linoleic acid and antibacterial effects using the microdilution method for *Alcaligenes faecalis*, *Serratia marcescens*, *Providencia rettgeri* and *Listeria monocytogenes*. Total phenols, flavonols and flavonoids were determined colorimetrically. In the β-carotene-linoleic acid system, oxidation was effectively inhibited by aqueous extract. The Minimum Inhibitory Concentration (MIC) for ethanolic and aqueous extracts was between the range of 31.25 and 62.5 mg/ml. Minimum Bactericidal Concentrations (MBC) was between 31.25 and 250 mg/ml for ethanolic and aqueous extracts. Total phenols were 138±3.25 mg/g and 230±3.26 mg/g Gallic acid equivalent, total flavonols were 132±3.87 mg/g and 88±3.3 mg/g and flavonoids were 127±1.63 mg/g and 92±3.26 mg/g Rutin equivalent, respectively. These findings indicate that different plant extracts can be used in food protection conservation and alternative for synthetic preservatives.

Key words: *Echinophora platyloba*, antioxidant activity, antibacterial activity, extract.

INTRODUCTION

In recent years, there has been an interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents in order to restrict the use of synthetic preservatives and antioxidants in the food industry (Genena et al., 2008). On the other hand, pathogenic and spoilage microorganisms are still a problem for public health and regulatory agencies as well as economic losses for food industries in the world, despite the use of range of synthetic preservatives for many years as sodium benzoate, sodium nitrite, sulfites, lactic, propionic and sorbic acids (Amensour et al., 2010; Stanojević et al., 2010). However, there have been discussions about the safety aspects of chemical preservatives to foods as regards toxicity, carcinogenic and teratogenic attributes (Moreira et al., 2005).

Antioxidants from plant origin are compounds that demonstrated biological effects and can inhibit oxidation by scavenging free radicals (Ani et al., 2006). Therefore, addition of natural sources compounds as antioxidants and antimicrobials could increase the shelf life of foods by protecting food items against oxidation and microbial invasion (Sulaiman et al., 2011). Also the consumption of natural antioxidant added to food could be transmitted to the body and may inhibit reactive oxygen species (ROS) formed in human cell and oxidative damage (Chandini et al., 2008).

Echinophora platyloba D.C species from *Echinophora* genus is (Umbelliferae, subfamily Apioideae, tribe Echinophoreae) an aromatic, mid-summer plant that wildly grows and mainly used for imparting flavor and taste to the food in Iran (Chaharmahal va Bakhteyari

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province) (Avijgan et al., 2010). In a study the major constituents of *E. platyloba* essential oil was identified as trans-β-ocimene (Asghari et al., 2003). Few earlier studies have reported antifungal and antibacterial effect of the extracts of *E. platyloba*. The aim of this study was to investigate antioxidant and antibacterial properties of hydroalcholic and aqueous extracts of *E. platyloba* D.C on food spoilage bacteria.

MATERIALS AND METHODS

Chemical

Folin-Ciocalteu reagent, Gallic acid, Rutin, *β*-carotene and Butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, USA) and other chemicals and solvents with analytical reagent grade were obtained from Merck Company, Germany.

Collection plant and preparation of extracts

The *E. platyloba* D.C was collected in September 2011 from Charmahal va Bakhtiari province of Iran and identified by the standard botanic work in Medical Plants Research Center in Shahre-kord University of Medical Sciences, Iran.

Aqueous extracts were obtained by maceration of dry plant stems (100 g) with 500 cc distilled water for 48 h. Also ethanolic extracts of plant samples were made by soaking in 80% denatured ethanol using a ratio of 100 g (samples): 500 ml (ethanol) for 48 h.

Extracts were then filtered and the filtrate was concentrated using a rotatory-evaporator, dried and then powdered (40°C). Stock concentrations of 500 mg/ml of dry extract in DMSO were prepared, sterile filtered (0.22 µm) and stored in the dark at 4°C (Mahmood et al., 2012).

Total phenolics

The amount of total phenolic content in the obtained extracts was estimated by Folin Ciocalteu method of Akhlaghi et al. (2011), with some modifications. 0.01 to 0.02 g of dried extracts was dissolved in 10 mL of 60% methanol. Then 0.1 ml of extract solution was added followed by 0.5 ml of 10% Folin Ciocalteu reagent and after 4 to 8 min were mixed with 0.4 ml of 7.5% aqueous sodium bicarbonate. The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetry at 765 nm with a spectrophotometer (Unico UV-2100, USA). The results are expressed as mg of Gallic acid equivalents/g of extract (GAEs).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination with some modifications (Chang et al., 2002). 0.01 to 0.02 g of dried extracts was dissolved in 10 mL of 60% methanol. 1 ml of extract solution was mixed with 1 mL of 2% aluminum chloride and 6 mL of 5% potassium acetate. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. The amount of 2% aluminum chloride was substituted by the same amount of distilled water in the blank. Total flavonoids were expressed in terms of Rutin equivalent (RE) (mg/g), which is a common reference compound.

Total flavonols

Similarly, Aluminum chloride colorimetric method was used for flavonols determination as described above, with differences being that incubation period at room temperature was 150 min and the absorbance of the reaction mixture was measured at 440 nm (Miliauskas et al., 2004). Total flavonols were expressed in terms of Rutin equivalent (mg/g).

Antioxidant activity determination

Total antioxidant capacity of *E. platyloba* D.C extracts was assayed using the β-carotene and linoleic acid (Kamkar et al., 2010). A stock solution of *β*-carotene (0.5 mg) in 1 mL of chloroform, linoleic acid (25 µl), and Tween-40 (200 mg) was prepared and chloroform completely evaporated using a vacuum evaporator at 50ºC. Then, 100 mL of oxygenated water (30 min 100 ml/min) were added under vigorous shaking. 2500 µl aliquots of this emulsion were added to test tubes and 350 µl of the extracts prepared at 2g/l concentrations were added and incubated for 48 h at room temperature. The same procedure was repeated with BHT (as positive control) and a blank. The absorbance of the solutions was measured at 490 nm. Antioxidant capacities of the extracts were compared with those of BHT and blank.

Microorganisms and antimicrobial activity

Cultures of *Alcaligenes faecalis* PTCC (Persian type culture collection) 1624, *Serratia marcescens* PTCC 1621, *Providencia rettgeri* PTCC 1512 and *Listeria monocytogenes* PTCC 1163 were obtained from Iranian Research Organization for Science and Technology (IROST). Bacteria were cultured on nutrient agar at 37°C. A 0.5 McFarland Standard was used to create inoculum densities of 1.5×10^8 cfu/ml in Phosphate-Buffered Saline (PBS) using the direct suspension method for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (Isenberg, 2004). MIC was determined by microtiter broth method in sterile flat-bottom 96-well polystyrene plates (Quave et al., 2008). Extracts were dissolved in dimethylsulfoxide (DMSO). Further serial dilution techniques were performed to determine the MIC of extracts at concentrations of 7.8 to 500 mg/ml after 18 h growth (5 µl bacterial suspension plus 95 µl Mueller Hinton broth plus 100 µl serial 2-fold dilution extracts). Negative controls (195 µl Mueller-Hinton broth plus 5 µl bacterial suspension, without antimicrobial substances) and positive controls were also similarly processed (95 µl Mueller Hinton broth plus 5 µl bacterial suspension with 100 µl appropriate antibiotic). Bacterial growth was detected by optical density readings using an ELISA reader (State fax 2100: Made in USA) at 450 nm at 0 and 18 h post-inoculation. The MIC was defined as the first well, without turbidity for growth at 18 h post-inoculation. All wells that showed no visible growth were transferred to Mueller Hinton agar and incubated at 37°C for 24h. The lowest concentration that could not produce a single bacterial colony was the MBC. All experiments were performed in triplicates.

RESULTS

The total contents of phenolic, flavonoid and flavonol are shown in Table 1 and antioxidant capacity in Figure 1.

Table 1. Total phenolic, flavonoid, flavonol of ethanolic and aqueous extracts of *Echinophora platyloba* D.C.

Extract	Phenolic (mg/g)	Flavonoid (mg/g)	Flavonol (mg/g)
Ethanolic	138±3.25	127±1.63	132±3.87
Aqueous	230±3.26	92±3.26	88±3.3

Table 2. Minimum Inhibitory concentration and minimum bactericidal concentration (mg/ml) of ethanolic and aqueous extracts of *Echinophora platyloba* D.C.

Extract	*Alcaligenes faecalis*		*Serratia marcescens*		*Providencia rettgeri*		*Listeria monocytogenes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ethanolic	62.5	250	31.25	125	62.5	125	31.25	31.25
Aqueous	31.25	31.25	62.5	125	62.5	250	31.25	62.5

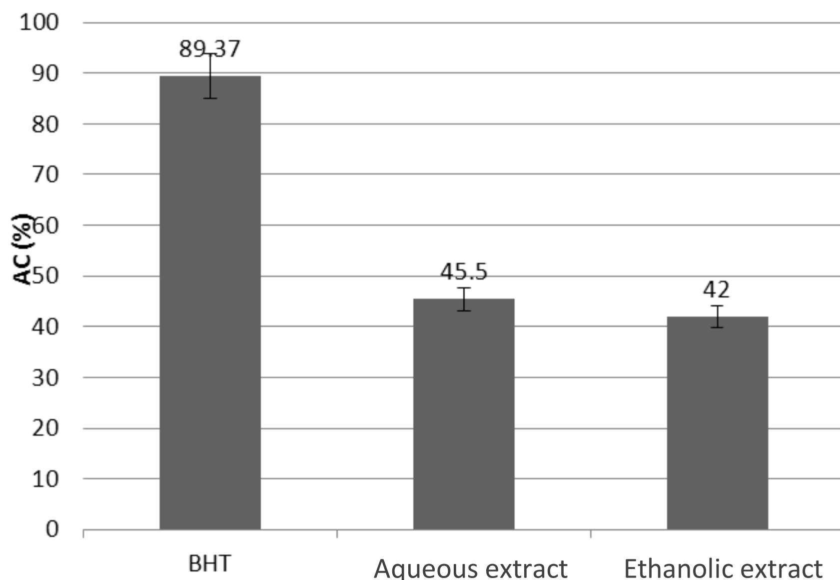

Figure 1. Antioxidant capacity (AC) ethanolic and aqueous extracts of *Echinophora platyloba* D.C.

The highest values of antioxidant capacity and phenolic compounds were observed in aqueous extracts of *E. platyloba* D.C ($P<0.05$). However antioxidant capacity of two extracts was lower than BHT as reference antioxidant. The ethanolic and aqueous extracts of *E. platyloba* D.C. were found to be effective against all the strains of tested bacteria. *Alcaligenes faecalis* was the most sensitive bacterium against aqueous extract and *Listeria monocytogenes* was the most sensitive bacterium against ethanolic extract. MIC and MBC were the same. The inhibitory effect of different concentrations of two extracts of the plant on the growth of various strains of tested bacteria by microdilution method was shown in Table 2.

DISCUSSION

Nowadays, food processors and consumers focus more on replacement of synthetic preservatives with natural additives such as different extract and essential oils of plants (Sulaiman et al., 2011). *E. platyloba* is a traditional herb of the plant native to Iran. According to this study two extract of the plant have relatively high level of phenolic compounds. Previous surveys demonstrated that *E. platyloba* is a rich source of saponin, alkaloid and

flavonoid (Avijgan et al., 2010).

The different antioxidant capacity of the ethanolic and aqueous extracts can be ascribed to their total phenolic content (Karadeniz et al., 2005) and the types of solvent used due to different polarity of compounds. None of the polar solvents as methanol were found to be suitable for extraction of the polyphenols like water as polar solvent (Khalid et al., 2011: Ismail and Tan, 2002). However, results of this study showed that the water extract of *E. platyloba* had higher amounts of phenolic compounds compared to ethanolic extract that was in line with the review by Moller et al. (1999). Also the bleaching of β-carotene as free radical mediated in the absence of antioxidant due to oxidation of this compound by hydroperoxides formed by linoleic acid. Then this oxidized compound loses its orange color that can be measured by spectrophotometer (Jayaprakasha et al., 2001). The presence of antioxidants as ethanolic and aqueous extracts of *E. platyloba* can protect the bleaching of β-carotene.

Several researchers have reported the antibacterial properties of plants. Previous reports show that *E. platyloba* has significant antifungal activity (Avijgan et al., 2010; Cox et al. 2010; Chandini et al., 2008). Also Avijgan et al. (2010) showed that ethanolic extract of *E. platyloba* had anticandida properties but did not exhibit any inhibitory activity on Gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogones*). In this study, we found that the ethanolic and aqueous extracts of *E. platyloba* have antibacterial activity on three gram negative and one gram positive bacteria listed above. However, the gram negative bacteria are more resistant than gram positive, due to restricted diffusion of the hydrophobic compounds by the hydrophilic cell wall structure as lipo-polysaccharide (LPS) (Sulaiman et al., 2011: Amensour et al., 2010).

Therefore, with attention to results obtained, the present study provides the basic information for the use of *E. platyloba* as the new antimicrobial and antioxidant agents are equal for some types of food. It is suggested that future studies on the properties of this plant occur in food models.

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