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Antioxidant Activity of Arial Parts of *Borago officinalis*

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Abstract: *Borago officinalis* (Boraginaceae) is a medicinal plant that is cultivated in different parts of Iran. It has different components such as Phenolic compounds, flavonoides, rosmarinic acid, pyrrolizidine alkaloids, etc. It is used as anticonvulsant, bronchodilator and vasodilator. The antioxidant activity of different fractions of this plant such as Chloro Form (CF), Ethyl Ecetate (EF) and n-butanol (NF) were studied by DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay. Total phenolic content was determined spectrophotometrically using Folin-Ciocalteau reagent. This study showed that ethyl acetate and n-butanol fractions were more effective than the others in antioxidant purposes.

INTRODUCTION

*Borago officinalis*is a medicinal plant that is originally from West Mediterranean areas, Central Europe, Spain and North Africa and it was cultivated in different city of Iran in recent years[1]. Historical articles shows that people from North Africa tribes have transferred it to Spain and then to other areas and have distributed this plant in these areas[2-4]. It is biennial plant that is belonging to the family Boraginaceae. The whole plant is covered with white, prickly hairs. The stems, about 1-1/5 feet high are branched and hollow. It has alternate leaves with wavy edge, wrinkled, green, oval and pointed. They are 3-7 cm long and about 2-5 cm broad. The flowers are bright blue and star-shaped. They are distinguished by their prominent black anthers. The fruit consists of four brownish-black nutlets. The plant arises during January-June. The medicinal parts of plant are leaves, flowers, arial parts, seeds[1]. The borage contains different constituents such as Phenolic compounds, flavonoides[5], rosmarinic acid, anthocyanins, pyrrolizidine alkaloids[6], fatty acids (Linoleic acid, Oleic acid, palmitic acid, Stearic acid, Eicosenoic acid, Erusic acid), Saponins, unsaturated terpenoides, sterol and hydrolysable mucilage[7]. It is useful as anticonvulsant, bronchodilator and vasodilator. Several plants in *Boraginaceae* family, individually *Borage* and its Iranian variety (*Echiumamoenum*) have been used in Iranian traditional medicineas sedative[7]. Borage increases urine excretion, decreases blood pressure and[8, 9]. The term antioxidant refers to free radical scavengers, inhibitors of lipid peroxidation and chelating agent[10]. The total antioxidant capacity of plants depends on the synergistic action of different constituents of them[11]. Natural antioxidants are effective in different kinds of complications such as cancer[12, 6], burn[13], diabetes[14], hyperlipidemia[15]. They can prevent the side effects of other compounds[16]. In this study, total phenolic content was determined spectrophotometrically using Folin-Ciocalteau reagent and the antioxidant activity of arial parts of *Borago officinalis* extracts elucidated by DPPH assay.

MATERIALS AND METHODS

Plants materials: Fresh arial parts of *Borago Officinalis* (BO) were collected in June, 2015 from Mazandaran, Iran. The aerial parts of plants were dried in the shade at room temperature. They were stored in the dark at 25°C.

Chemicals: All chemicals and reagents were analytical grade or purest qualitypurchased from Sigma, Merck, Aldrich and Fluka.

Extraction methods: About 100 gp owdered plant was extracted with 80% ethanol by percolation at room temperature (3×48 h). The solvent was evaporated under vacuum in a rotary evaporator until dryness. The dried extract was fractionated based on increasing polarity by Choloro form, Ethylacetate and n-buthanol. All fractions were stored at 4°C until use.

Total phenolic contents: Total phenolic content was determined spectrophotometrically using Folin-Ciocalteau reagent[17]. Folin-Ciocalteau reagent (0.75 mL), previously diluted 10-fold with distilled water were mixed thoroughly with 200 µL of appropriate dilutions of the extract or fraction solutions and allowed to stand at room temperature for 5 min. Then 0.75 mL of sodium bicarbonate solution (60 g L^{-1}) was added to the mixture followed by storing at room temperature for 90 min. Subsequently, absorbance was measured at 760 nm using a UV-visible spectrophotometer. Total phenolics were quantified by calibration standard curve obtained using various known concentrations of gallic acid (50-200 µg mL^{-1} in methanol). The concentrations expressed as µg of Gallic Acid mg^{-1} of extract or fraction. All determinations were carried out in triplicate and the mean values were presented.

Antioxidant activity

Diphenyl-1-Picryl Hydrazyl (DPPH) assay: DPPH antioxidant assay is based on the measurement of the reducing ability of antioxidants toward DPPH radical. The ability can be evaluated by measuring the decrease of its absorbance and the reaction is monitored by a spectrometer at 517 nm. This assay was carried out according to Amanzadeh *et al*.[18]. Inhibition of DPPH free radical in percent is calculated as: Inhibition % = 100-[(control absorption-Sample absorption)/control absorption]×100

The concentration that causes 50% decrease in the initial DPPH radical concentration is defined as IC_{50}. BHA and Vit C were used as used as positive control and all experiments were carried out at least 3 times[18].

RESULTS AND DISCUSSION

Total phenolics contents: Phenolic compounds are powerful chain breaking antioxidants. The concentration of phenolics in the fractions expressed as µg of pyrocatechol per mg of the fractions is shown in Table 1. Ethyl acetate fraction was found as the highest phenolics content fraction.

Diphenyl-1-Picryl Hydrazyl (DPPH) assay: In radical scavenging activity when DPPH expose to antioxidant constituents, its purplecolor changes to yellow. The degree of discoloration of DPPH indicates the scavenging potential of the antioxidant extracts and essential oils[19]. The results of DPPHscavenging are highly comparable to other free radical scavenging methods[20]. IC50 of fractions is shown in Table 1. IC50 of BHA and ascorbic acid as positive control were 30.5± 0.82 and 4.09± 0.26 µg mL^{-1}, respectively. The DPPH radical scavenging activity of the tested samples were in the order: Ascorbic acid >BHA>ethyl acetate fraction>n-butanol fraction>Choloro form fraction (Table 1). The results from this study confirmed ethyl acetate and n-butanol as theextraction solvent has an important effect on total phenolicand flavonoid contents.The high amount of concentration of polyphenolics in the ethyl acetate fraction may be due to high free radical scavenging activity. Presence of different constituents in plants was caused different pharmacological effects of them therefore Presence of hydroxyl groups in the polyphenolics and flavonoidsca uses free radical scavenging of phenolic compounds[21]. According to the results non polar solvent are not efficient enough for extraction of *Borago officinalis*. Zemmouri *et al*.[22] illustrated the differences between aqueous and hydroalcoholic extracts of Algerian *Borago officinalis* leaves. These extracts evaluated for their antioxidant properties by DPPH radical scavenging. The ethanolic extract showed the lower IC50 values and the highest amount of phenolic contents (94.09±1.72 mg g^{-1} dry extract). Phytochemistry analysis, identified the presence of phenolic acids, flavonoids[22], sterol[23] in the extracts of this plant. Therefore, this investigation is the first study about antioxidant activities of *Borago officinalis* as a cultivated plant.

Table 1: Total phenolic contents and antioxidant activity of *Borago officinalis* fractions

Samples	Phenolic contents	IC_{50} (µg mL^{-1})
ethyl acetate fraction	72.21±0.64	88.85 ±1.07
n-butanol fraction	69.80±0.86	93.04±0.87
Choloro form fraction	18.45±0.38	135.69 ±0.70

CONCLUSION

In this study, an antioxidant screening of fractions of *Borago officinalis* was carried out for the first time. The phenolic compounds and flavonoids was responsible for the antioxidant activity of plants. Based on these observations, ethyl acetate and n-butanol fractions can be good candidates for further *in vivo* biological studies and phytochemical investigations.

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