



Assessment of Chemical Composition and the Antimicrobial and Antioxidant Activities of *Bassia eriophora* growing in Eastern Province of Saudi Arabia

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ABSTRACT

Medicinal plants are promising source of new therapeutic pharmaceutical agents because of their higher potency and wide diversity in chemical structure as compared to synthetic compounds. The plant characterized chemically by the presence of various bioactive secondary metabolites such as flavonoids, saponins, triterpenoids/steroids, tannins and carbohydrates at different levels in different fractions of plant organs and the absence of cardiac glycosides and alkaloids. The main goals of these investigations were to evaluate the antibacterial and antioxidative effectiveness of different plant organs extracts of *Bassia eriophora*. The antioxidant activity was investigated in accordance to DPPH radical scavenging protocol, using trolox as standard. In addition, the all amounts of phenolic and flavonoid compounds were estimated using Folin-Ciocalteu reagent. Antibacterial assessment was carried out through disk diffusion assay against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) pathogens. The results showed that the extracts of ethyl acetate, butanol and aqueous exhibited a higher antioxidant activity and antioxidant activities as well as higher amounts of phenolics and flavonoids among all the tested different organs extracts. These results reveal that the ethyl acetate, butanol and aqueous extracts of different plant organs of *Bassia eriophora* could be developed as a potential natural antioxidant and antibacterial herbal remedy.

Keywords: *Bassia eriophora*; Chenopodiaceae; Antioxidant; Phytochemical Screening; Antimicrobial

INTRODUCTION

Medicinal plants are promising source of novel therapeutic agents because of their potency and diversity in chemical structure as compared to synthetic compounds. The Kingdom of Saudi Arabia is considered one of the richest biodiversity floras in the area of Arabian Peninsula and contains hundreds of species of medicinal plants. Several of these plants are well known for their therapeutic benefits against infectious diseases [1-4]. *Bassia eriophora* member of family chenopodiaceae, is a sandy herb growing in Saudi Arabia, commonly known as ummulhas, gteena, alguteen. *B. eriophora* is used in folkloric medicine as an herbal remedy to treat renal and rheumatic disorders [5-6]. A literature survey indicated that, only some *Bassia* species were investigated and found to contain saponins glycosides and aglycones of bassic acid skeleton [7]. Two new flavonoid and four known saponins were isolated from *Bassia muricata* [8]. Few species of *Bassia* expressed toxic effects on grazing animals [9]. Also, no data is available on the phytochemical contents, antimicrobial and antioxidant studies have been carried out on *Bassia eriophora*. Therefore the present work is to investigate and study of the phytochemical contents and to evaluate the antimicrobial and antioxidant properties of different plant organs fractions to stand on which extract is active.

EXPERIMENTAL SECTION

Methods

Plant collection

The plant was collected from Al-Hasa, eastern region, Saudi Arabia. Different parts (leaves, stems and roots) of plant were separated and subjected to air-drying according to universal standard herbarium procedures. A voucher sample was kept in Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University [10].

Phytochemical Screening of different plant organs extracts

The air dried powdered plant materials (leaves, stems and roots, 1200 g, 300g and 200 g respectively) were exhaustively extracted twice at room temperature (each for 7 days) using 5 L 70% methyl alcohol, applying cold maceration method to avoid damage of active principals. The solvent mixtures were distilled off under reduced pressure using rotary evaporator and then freeze-dried to yield the total dry extracts of leaves, stems and roots, 150g, 30g and 7g respectively, which were stored in freezer for the next steps.

The total extracts of leaves, stems and roots, (25g, 15g and 6g, respectively) were mixed with double distilled deionized water (300 mL) and partitioned to be fractionated with n-hexane (6×500 mL). The resulting n-hexane phases were combined to be concentrated to the least amount using rotary evaporator and then dried to give 12g, 6.5g and 0.5g respectively then stored in a -20 °C freezer in tightly-closed container. The remaining aqueous part was subjected to further fractionation with Chloroform (4×1000 mL). The chloroform fractions were also collected together and its volume was reduced to the minimal amount and then freeze dried to give 3.5g, 3g and 1.5g, respectively, and then all were kept in fridge in a strong-tight container for later use. Similarly, the ethyl acetate as well as n-butanol extracts were also developed using the same previously mentioned protocol to give to give 3g, 3g and 2g respectively for ethyl acetate fraction and to give 1.5g, 1g and 1g respectively for n-butanol fraction. The remaining aqueous fraction was lyophilized to powder to give 2g, 1.5g and 0.5g respectively [11].

The different dried extracts and fractions of leaf, stem and root, respectively were subjected to the phytochemical screening chemical and physical tests using standard methods [12-13].

Determination of the total phenolic constituents

The total phenolic constituent was calculated using the Folin-Ciocalteu index protocol [14]. Stock solutions (1 mg/ml) of different extracts will be prepared in methanol. Half ml of Folin-Ciocalteu reagent and Six milliliters of double distilled deionized water were successively added to 0.1 ml of stock solution of each extracts. In addition, 1.5 ml of a 20% Na₂CO₃ solution and water was added to obtain 10 ml. A reaction will take place within 2 h. at normal room temperature. Then, the absorbance was recorded at 760 nm. Calibration was done using serial dilution of gallic acid as a standard (0.5, 0.4, 0.3, 0.2 and 0.1 mg/ml in distilled water, $y = 0.0156x + 0.0004$, $r^2 = 0.9697$). The amount of phenolic components was demonstrated as the equivalence of milligrams of standard gallic acid per gram of dried plant extract (mg GAE/g).

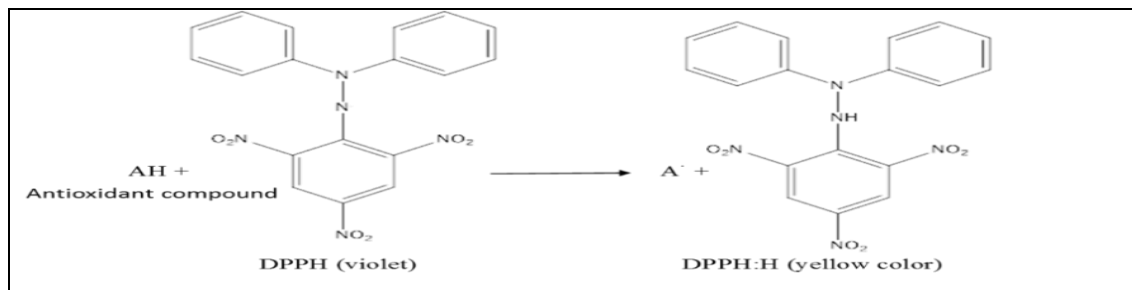
Determination of the total flavonoids constituents

The total flavonoidal constituent was investigated in accordance to Heimler *et al.* [15]. Ten mg of extracts will be diluted in 100 ml of deionized water and acetone with ratio of (1:1 v/v). A solution of 0.25 ml of the serially diluted sample was added to 0.75 µl of a NaNO₂ (5% w/v) solution, as well as 0.15 ml of a recently prepared aluminum chloride (10% w/v) solution, together with 0.5 ml of 1 M NaOH solution. Then the total volume of reactants was completed to 10 ml with deionized double distilled water. The resultant components were kept for 5 min and the absorption was observed at 510 nm against the same components lacking of the sample. Calibration was done using quercetin as reference substance, from that a standard calibration curve got with solutions of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml ($y = 0.0009x + 0.0227$, $r^2 = 0.9771$). The results were shown as the equivalence of milligrams of quercetin per gram of dried plant extract (mg QE/g).

DPPH radical scavenging activity

This protocol based mainly on the reduction of methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent in case of existence of a proton donating free radical scavenger substance, because of that interaction the non-radical form DPPH-H will be formed. The decrease in DPPH radical will be monitored by the reduction in its absorbance at 515 nm caused by antioxidant moiety present in reaction media. As, a result of that the color was changed to yellow instead of violet. This change in color is a simple yet effective qualitative and quantitative parameter for the

presence of an antioxidant. Therefore, DPPH moiety is an important substance to investigate the free radical scavenger effects of antioxidants [16].



The absorbance of different amounts of the test extracts were dissolved in Methanol (100 μl) in a 96-well micro-titration plate, was recorded at 515 nm at zero time as A blank. Then, 200 μM DPPH solution (100 μl) was add to every well, then was kept at room temperature for half an hour. Followed by, measurement of the absorbance again as Ab sample. The percentage of inhibition was measured using this equation:

$$\% \text{ of inhibition} = (1 - [\text{Ab sample} - \text{Ab blank}] / \{\text{Ab control} - \text{Ab blank}\}) \times 100$$

Where Ab control is the absorbance of mixture (with DMSO and all other reactant without test extracts) [17]. IC_{50} was recorded as the sample concentration that is essential to cause inhibition of DPPH radical to be formed by 50%.

Antimicrobial Assay

Staphylococcus aureus (gram-positive) and *Escherichia coli* (gram-negative) were tested for their susceptibilities to the 15 fractions dissolved in DMSO with concentration of 1mg/ml by disk diffusion protocol applying guidelines of the Clinical Laboratory Standards Institute. An inoculum used and placed on Muller Hinton agar plates, contained the used isolates grew on Muller Hinton broth with a turbidity of a 0.5 McFarland standard. Discs with diameter of 6mm, having one drop of each fraction were placed on Muller Hinton agar plates. Diameters of zone of growth inhibition were observed and taken 24 h later. The incubation was kept at 37°C [18].

Infra-red spectroscopy screening of different plant organs extracts

Infra-red spectra of the different plant organs extracts were recorded to detect the presence of various functional groups of different constituents as a confirmatory tool for their occurrence. One μl of different plant organs extracts was loaded on KBr disc and placed in the sample chamber of FT-IR spectrophotometer and the spectra were recorded in the range of 4000–500 cm^{-1} on Shimadzu 330 FTIR spectrometer. The most important absorption frequencies appeared in functional group region as well as fingerprint region of the spectra[19].

RESULTS AND DISCUSSION

Phyto-chemical screening of different plant organs extracts

The preliminary phytochemical screening of extracts of different plant organs showed the existence of various important principals such as flavonoids, saponins, triterpenoids/steroids, , carbohydrates and tannins at different levels in different extracts of plant organs and the absence of cardiac glycosides, anthraquinones and alkaloids as shown in Table 1.

Determination of total phenolic constituents

Determination of total phenolic constituents showed that the quantity of total phenolic components varies from 0.314 ± 0.032 to 64.359 ± 21.967 mg GAE/g of dry extract (Figure 1). Ethyl acetate fraction contains the highest percentage of total phenolic components, followed by butanol fraction then the aqueous fraction. Chloroform fraction contains the least amount while n. hexane extract hardly contains any phenolic contents.

Table1: Preliminary phytochemical screening of different extracts from different plant organs.

Chemical test/Plant organ	Leaves					Stems					Roots				
	nH	CH	EA	BT	Aq.	nH	CH	EA	BT	Aq.	nH	CH	EA	BT	Aq.
Flavonoids Alkaline solution test	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Saponins Foam test	-	+	+	+	+	-	+	+	+	+	-	-	+	+	-
Triterpenoids/steroids Liebermann-Burchard test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids Dragendorff's reagent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones Borntrager's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins 10% FeCl ₃	-	-	+	+	+	-	-	+	+	-	-	-	+	+	-
Cardiac glycosides Keller Killiani test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates Molisch's test	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+

nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, AQ; Aqueous fraction.

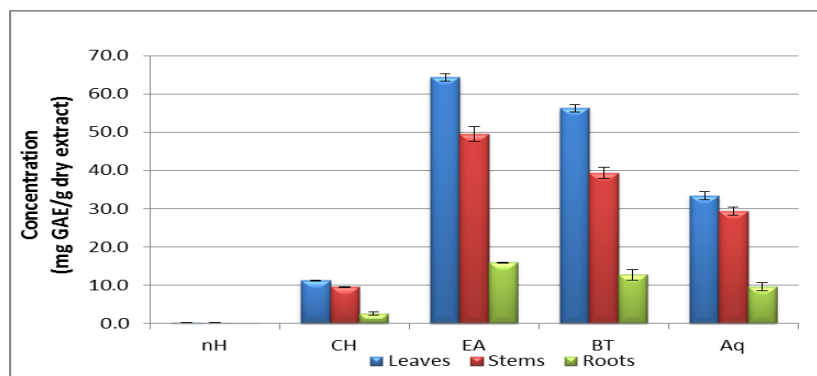


Figure 1: Total flavonoid contents of different extracts of *Bassia eriophora*, nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq., Aqueous fraction, GAE; Gallic Acid Equivalent. Data are the means \pm standard deviation of three replicate

Determination of total flavonoid constituents

Determination of total flavonoids content depicted that the amount of total flavonoid constituents differed from organ to organ and varies from 0.068 ± 0.020 to 11.573 ± 0.215 mg QE/g of dry extract (Figure 2). Ethyl acetate fraction is the richest fraction in flavonoid components, next to it the butanol fraction then the aqueous fraction. Similarly, to phenolic contents, the chloroform fraction contains the least amount compared to other fractions, while n-hexane fraction contains very less flavonoid constituents.

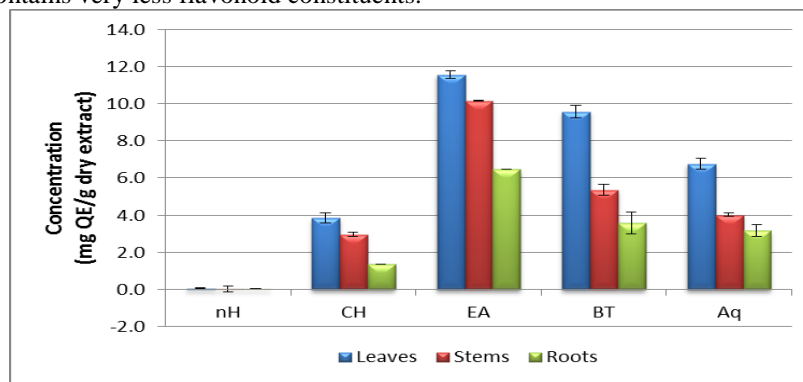


Figure 2: Total flavonoid contents of different extracts of *Bassia eriophora*, nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq., Aqueous fraction, QE; Quercetin Equivalent. Data are the means \pm standard deviation of three replicate

DPPH radical scavenging activity

On the other hand, all fractions were examined for their radical scavenger activity using the DPPH free radical scavenging assay (Figure 3). Ethyl acetate and butanol fractions showed marked scavenging activities (IC_{50} : 19.2 to 46.9 $\mu\text{g/ml}$, respectively), aqueous showed moderate activity (IC_{50} : 39.9 to 73.2 $\mu\text{g/ml}$) and hexane and chloroform fractions demonstrated much weaker effects with IC_{50} above 100 $\mu\text{g/ml}$, comparable with the standard trolox (IC_{50} : 21.8 μM).

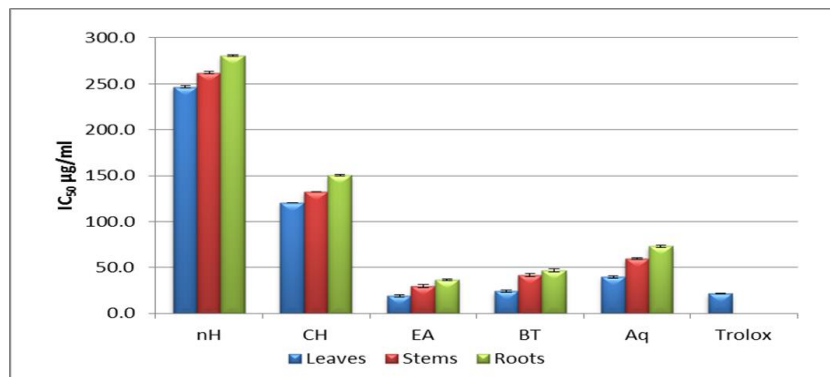


Figure 3: IC_{50} values of different extracts of *Bassia eriophora*, nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq., Aqueous fraction, QE; Quercetin Equivalent. Data are the means \pm standard deviation of three replicate

Antimicrobial activity

The results of antimicrobial screening of different plant organs extracts are recorded in Table 2. It was observed that *E. coli* exhibited a moderate inhibition zone ranging from 9 to 20 mm for all of the examined fractions. n-Hexane and chloroform fractions of stem extract did not exhibit any activity against *E. Coli*. On other hand, all extracts showed very good activity against *S. aureus* with inhibition zones measuring between 27 and 40 mm (Table 2). The Presence of high amounts of flavonoids and phenolics in these extracts may be the reason for the marked antibacterial activity.

Table2: Antibacterial activities of different extracts from different plant organs

Bacterial Strains	Extracts/Standards Inhibition zone diameter in(mm)																			
	nHL	CHL	EAL	BTL	AQL	nHS	CHS	EAS	BTS	AQS	nHR	CHR	EAR	BTR	AQR	Er.	Ox.	Am.	Ak.	DMSO
<i>S. aureus</i>	30	30	29	32	28	29	27	35	27	31	30	30	34	29	40	22	19	-	-	18
<i>E. coli</i>	13	10	9	12	9	0	0	14	17	24	19	19	20	12	22	-	-	8	19	0

nHL; n-Hexane leaves fraction, CHL; Chloroform leaves fraction, EAL; Ethyl acetate leaves fraction, BTL; Butanol leaves fraction, AQL; Aqueous leaves fraction, nHS; n-Hexane stems fraction, CHS; Chloroform stems fraction, EAS; Ethyl acetate stems fraction, BTS; Butanol stems fraction, AQS; Aqueous stems fraction, nHR; n-Hexane roots fraction, CHR; Chloroform roots fraction, EAR; Ethyl acetate roots fraction, BTR; Butanol roots fraction, AQR; Aqueous roots fraction, Am; ampicillin, Er; erythromycin, Ox; oxacillin, Ak; amikacin

Infra-red spectroscopy screening of different plant organs extracts

FT-IR spectral analyses data of different plant organs extracts revealed the presence of multiple functional groups. Spectral data of most of the extracts confirmed the presence of bioactive functional groups such as alcoholic, aldehydic, acidic and aromatic groups at different frequencies. These results also confirm the results of phytochemical screening.

CONCLUSION

The value of herbal extracts as antimicrobial and antioxidant agents gained two great merits; being it is of natural origin and it is of low risks to replace synthetic ones. The present work showed that *Bassia eriophora* different organs extracts exhibited good antioxidant properties specially the ethyl acetate and butanol fractions. This could be attributed to the higher content of phenolic and flavonoid in these fractions, which was proven from the higher values of their corresponding mg GAE/g and mg QE/g per dry powdered extracts. In addition, the different extracts showed noticeable antimicrobial effectiveness against gram-positive and gram-negative bacterial strains. These observed results would serve as scientific evidence based suggestion to further developing of some of Saudi plants into natural antioxidant and antimicrobial herbal remedy.

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