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Spectrophotometric Determination of Total Phenol and Flavonoid Contents of Aerial Parts of *Blepharis edulis* (Pers.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SA and ME managed the analyses of the study. Author ME managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Quantitative determination of phenols and flavonoids of *Blepharis edulis* aerial parts was carried out using spectrophotometery techniques. Gallic acid and quercetin were used as the standards for calibration for the phenols and flavonoids respectively. *B. edulis* commonly known as Elsihaa, it has wide range of folkloric medicinal uses. In the current study the presences of useful secondary metabolites were observed, also high amount of phenolic and flavonoid contents with 34.85±2.1 mg/g and 59.56±0.29 mg/g respectively. These findings support its medicinal uses.

Keywords: Phenols; flavonoids; phytochemical screening; Blepharis edulis.

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1. INTRODUCTION

Phenolics are aromatic compounds with hydroxyl substitutions. Plant phenolics arise from two main biosynthetic pathways: (1) via shikimic acid (benzoic acid derivatives, lignans, coumarins, etc.) and (2) through acetate, leading to polyketides, which afford by cyclization to products such as xanthones and quinines [1,2,3]. All phenols inhibit oxidation and they play an important role in the natural host defence mechanism of plants against infectious diseases and inhibit multiplication of plant pathogenic bacteria, viruses, and fungi. Stress conditions such as excessive UV light, wounding, thus environmental factors may have a significant contribution to the content of phenolic acids in plants [4,5,6,7].

Flavonoids constitute one of the largest and recently very popular group of phytochemicals. They are mixed biosynthesis, consisting of units derived from both shikimic acid and polyketide pathways. Until now, more than 4500 flavonoids have been identified and this number is constantly growing because of the great structural diversity arising from the various hydroxylation, metoxylation, glycosylation, and acylation patterns. Most frequently encountered groups of flavonoid aglycones include flavones, flavonols, anthocyanidins, isoflavones, flavanones, dihydroflavonols, biflavonoids, calchones, and aurones. Flavonoid aglycones possess the chemical properties of phenolics, and thus they are slightly acidic. Those possessing a number of unsubstituted hydroxyl groups, or sugar moieties, are polar substances and soluble in polar organic solvents. Flavonoids have important roles in plant physiology and are components of the diet of numerous herbivores and omnivores, including humans. This group of compounds exhibit an extraordinary array of biochemical and pharmacological activities in mammalian systems, such as anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, antimicrobic, and antiviral [8,9,10]. With the increased popularity and use of herbal medicines containing flavonoids, the question of composition determination and standardization arises. It is almost impossible to compare any kind of biological activity without the chemical characterization of the plant extract. For this reason, it is important that scientists working in this field are aware of the importance of the content analysis and for that purpose use the appropriate analytical tools.

Blepharis edulis (Pers.). Synonyms: *Blepharis persica*. Commonly known as “Elsihaa”. Family: (*Acanthaceae*). It is an erect, annual herb up to 250-600 mm tall; much branched and sometimes becomes woody with age. The stem is sticky with glandular hairs and marked with longitudinal parallel lines. Found in Pakistan, Iran, India, Afghanistan, Sudan and Egypt [11]. It is used in folk medicine to treat asthma, cough, fever, inflammation of throat [12] festering wounds and ulcers. It is appetizer, astringent to bowels [13].

In the current study we assessed the total phenol and flavonoid contents of the ethanolic extract and fractions of *B. edulis*, Sudanese origin, phytochemical screening is also conducted.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Solvents and chemicals

Folin- Ciocalteau were obtained from (Sigma-Aldrich, USA), all solvents and reagents used were of analytical grade including ethanol, n-hexane, chloroform, ethyl acetate, n-butanol, methanol, ferric chloride ($FeC1_3$), potassium hydroxide KOH, sodium hydroxide NaOH, hydrochloric acid HCl, lead acetate (PbAc), sulphuric acid (H_2SO_4), copper acetate, gallic acid, sodium carbonate, $NaNO_2$, $AlCl_3$.

2.1.2 Plants materials collection and identification

Aerial parts of *B. edulis* were gathered from River Nile state in February 2015. Samples were identified and authenticated by plant taxonomists at the herbarium of Medicinal and Aromatic Plants Research Institute, National Center for Research. Khartoum, Sudan.

2.2 Methods

2.2.1 Extraction

The fresh samples were dried in shades for 7 days, powdered then used for extraction. Extraction was carried out according to published method of Osama, 2015 [14]. The shade-dried samples were socked in 80% ethanol in the ratio of (1:10) at room temperature for 3 days then filtered and they were dried by using rotary evaporator, this process was repeated till the solvent at the last time returned to colourless.

The weight of the solid residues was recorded and taken as yield of crude extracts. The yield a percentage was calculated as follows:

yield % = (weigh of extract/weigh of sample) X 100

2.2.2 Fractionation

The crude extracts were fractionated using liquid-liquid extraction method, which were carried by dissolving the samples in dist. H_2O and were then partitioned between n-hexane chloroform, ethyl acetate, and n-butanol using separation funnel apparatus.

2.2.3 Phytochemical screening

Phytochemical screening was conducted to determine the presence of natural products in the fractions of *B. edulis* using standard methods of Trease and Evans, 1989; Odebiyi and Sofowora, 1978 [15,16] as following.

2.2.4 Phenols (ferric chloride test)

To 1 mL of extract 2 mL of distilled water were added followed by few drops of 10% ferric chloride ($FeC1_3$). Appearance of blue or green colour indicates presence of phenols.

2.2.5 Flavonoids

Three different tests were used for the identification of flavonoids.

2.2.6 Potassium hydroxide (KOH) test

About mL of extracts were treated with few drops of 10% potassium hydroxide solution. Formation of intense yellow colour indicates the presence of flavonoids.

2.2.7 Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.2.8 Tannins (ferric chloride test)

About 0.5 mL of the extract was boiled with 10 mL of distilled water in a test tube and then, few drops of 5% Ferric Chloride solution was added and the reaction mixture was observed for blue, greenish black colour change.

2.2.9 Coumarins

To 1 mL of extract, 1 mL of 10% NaOH was added formation of yellow colour presents a positive.

2.2.10 Quinones

To 1 mL of extract, 1 mL of concentrated sulphuric acid (H_2SO_4) was added formation of red colour shows a positive result.

2.2.11 Alkaloids

Two different tests were used for the identification of alkaloids.

2.2.12 Dragendroff's test

Filtrates were treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

2.2.13 Wagner's test

To 0.5 mL of the extract 2 mL of Wagner's reagent was added and the reaction mixture is observed for the formation of reddish brown precipitate.

2.2.14 Triterpenes and steroids (Salkowski test)

Salkowski test was used to identification steroid and terpenoid. To 0.5 mL of each of the extract, 2 mL of chloroform was added and then 3 mL of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids and steroids.

2.2.15 Diterpenes (copper acetate test)

Extracts were dissolved in water and treated with 3 to 4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

2.2.16 Test for Saponins (frothing test)

About 0.5 mL of the extract was added to 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for the stable persistent froth.

2.2.17 Determination of total phenolics contents

Total phenolic content was assessed approximately by using Folin-Ciocalteau Phenol reagent through the method of Wolfe et al. [17] with slight modification using standard curve generated with gallic acid. To determine the total

phenolic content. 0.5 mL of extract (1 mg/mL) mixed with 0.4 mL Folin-Ciocalteu reagent (diluted with water 1:10 v/v) and 0 .910 mL (75%) sodium carbonate. The mixture was allowed to stand for 90 min at room temperature for colour development. Absorbance was measured at 765 nm using multiplat reader spectrophotometer. Total phenolic content is expressed as mg/g gallic acid equivalent (GAE) and was determined using the equation based on the calibration curve: y = 0.0017x -0.0029, where y is the absorbance and x is the gallic acid equivalent (mg/g).

2.2.18 Evaluation of total flavonoid content

The total flavonoid content was measured using a modified colorimetric method Kim et al. [18]. 1 mL of extract (1 mg/mL in methanol) was added to a test-tube. Then 0.3 mL of 5% $NaNO_2$, after 5 min 0.3 mL of 10% $AlCl_3$ and after another 5 min 2 mL of 1 M NaOH. The absorbance was measured against the blank at 510 nm after 15 min. The standard curve was prepared using different concentration of Quercetin. The flavonoid content was expressed as mg Quercetin equivalents (QE) per g of dry weight.

2.3 Statistical Analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program. Student t test was used to determine significant difference between control and plant extracts at level of $P < 0.05$.

3. RESULTS

In the current study the (80%) ethanol used for extraction was able to dissolve 34.18% of the plant weigh, the water fraction was the most abundant fraction with 41.82%, the other fraction showed variable yield % as shown in Fig. 1.

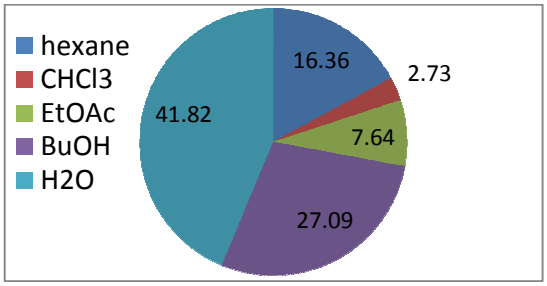

Fig. 1. Yield percentage of *B. edulis* fractions

As shown in Table 1 phytochemical screening of the plant samples revealed the presence of valuable secondary metabolites in all fractions.

As one of the most important antioxidant plant components, phenolic compounds were widely investigated in many medicinal plant and vegetables [19]. Flavonoids as it is one of major phenolic compounds it was therefore quantification of its presence was established among all extracts of selected plant, the highest value was notes with 54.33 ± 0.74 which belong to chloroform fraction. The amounts of total phenolic and flavonoid compounds of the ethanol extract and its fractions were shown in Table 2.

Table 1. Preliminary screening of secondary metabolites in the fractions of *B. edulis*

Family of compound	Type of test	Interference				
		n-hexane	$CHCl_3$	EtOAc	n-BuOH	H_2O
Phenols	$FeCl_3$	+v	+v	+v	+v	+v
Tannins	$FeCl_3$	-v	+v	+v	+v	+v
Flavonoids	KOH	+v	+v	+v	+v	+v
	Lead acetate	+v	+v	+v	+v	+v
Coumarins	NaOH	+v	+v	+v	+v	+v
Quninous	H_2SO_4	+v	+v	+v	+v	+v
Alkloids	Dragendorff's	-v	+v	+v	+v	+v
	Wagner's	-v	+v	+v	+v	+v
Triterpenes	Salkowski	+v	+v	+v	+v	+v
diterpenes	Copper acetate	+v	+v	+v	+v	+v
Steroids	Salkowski	+v	+v	+v	+v	+v
Saponins	Forth	-v	+v	+v	+v	+v

+ve positive

Table 2. Total phenol and flavonoid contents of ethanolic extract fractions of *B. edulis*

Type of test	Crude	Hexane	$CHCl_3$	EtOAc	BuOH	H_2O
GAE	34.85±2.1	36.32±0.9	65.06±1.3	55.75±3.3	31.27±1.1	23.46±0.9
QE	59.56±0.29	33.73±0.95	54.33±0.74	52.67±0.36	32.30±0.39	34.55±0.30

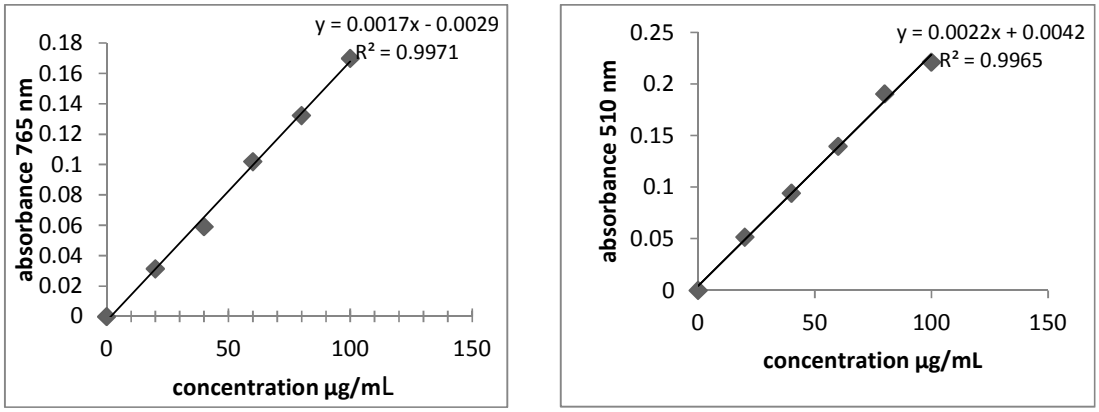

Fig. 2. Standard curve of (A) Gallic acid and (B) Quercetin shows the absorbance against concentration

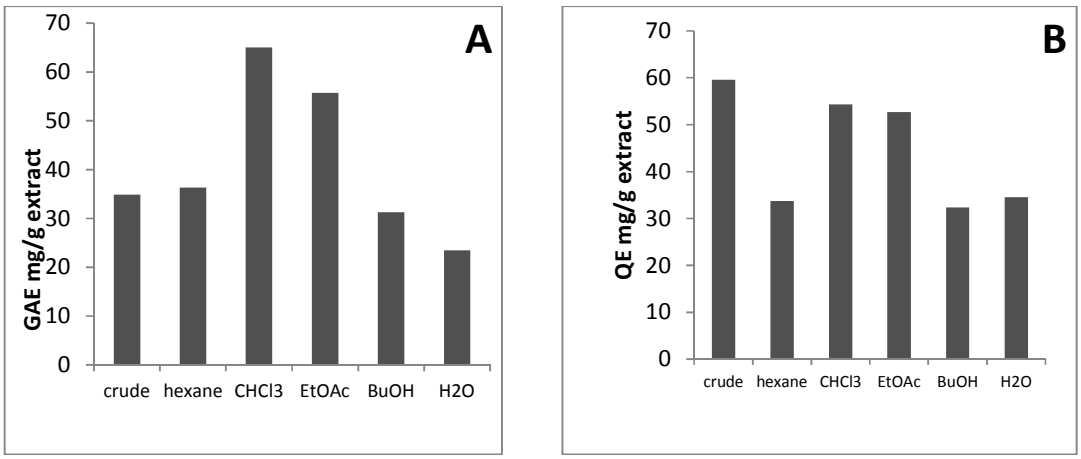

Fig. 3. (A) Total phenolic and (B) flavonoid contents of ethanolic extract fractions of *B. edulis*

4. DISCUSSION

Plants extracts are used in traditional medical practices to treat different types of ailments since long. Crude extracts from nature and compounds purified from these extracts can serve as better drug sources as herbal medicines and have less side effects, biofriendly and of nutritional value [20]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. high concentrations of more bioactive flavonoid compounds were detected with ethanol 80% which has used in the current study [21,22,23]. Phytochemical screening showed the presence of tested secondary metabolites in all fractions except the tannins, alkaloids and Saponins in n-hexane fraction which may indicates the high diversity and concentration of these secondary metabolites, however the aerial parts containing different plant organs e.g. leaves, stem, bark, flowers etc. all these parts have a different chemical composition with variable concentrations.

Plant phenolic compounds especially flavonoids are currently of growing interest owning to their supposed properties in promoting health (anti-oxidants) [24]. Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity and also may exert protection against heart disease [25]. In the current study the chloroform fraction showed the highest phenolic and flavonoid contents which may indicate the presence of high amount of non-polar phenolic substances such as less polar flavonoids (aglycones) [26]. These results could

give an obvious clue for the wide range of bioactivities of this plants.

5. CONCLUSION

In conclusion all fractions of *B. edulis* showed the presence of valuable secondary metabolites except the tannins, alkaloids and Saponins in n- hexane fraction and also containing high amount of phenols and flavonoids constituents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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