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Study of Saponins in Methanol Extract of the Leaves of *Acacia etbaica* subspecies etbaica

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ABSTRACT

The present research was aimed to phytochemical study of saponins present in the leaves extracts of *Acacia etbaica* Schweinf. subspecies etbaica, family: (*Leguminosae*) Fabaceae. Methanol extract of the leaves, its water residue, ethyl acetate and n butanol soluble fractions were tested for phytochemical investigation, the result showed the presence of carbohydrates, flavonoids, saponins, triterpenes and sterols in ethyl acetate; n-butanol fractions and water residue. Tannins were found in ethyl acetate fraction only. The presence of saponins in the studied fractions was confirmed by performing TLC. TLC in n-butanol–acetic acid–water (4:1:5), ethyl acetate fraction reveals 4 spots; n-butanol fraction reveals 5; water residue reveals 3 spots. TLC in ethyl acetate–acetic acid–water (7:2:2), ethyl acetate fraction reveals 3 spots; n-butanol fraction reveals 6 spots; water residue reveals 5 spots. Saponin content of the sample was determined by double solvent extraction gravimetric method. The total saponin content of plant drug was 13.0 ±0.03%. In future this plant can be subjected to isolation of the major constituents and to further pharmacological evaluation.

Keywords: *Acacia etbaica*, Leaves, Phytochemical, Saponins**.**

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INTRODUCTION

Saponins are high-molecular-weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. According to the nature of the aglycone saponins can be classified into steroidal or triterpene groups. Sugars can be attached as one, two, or three sugar chains and the terms monodesmoside, bidesmoside, and tridesmoside have been given to these saponins, respectively (Greek desmos¼chain) [1]. Saponins are extremely widely distributed in the plant kingdom. Even by 1927, Kofler had listed 472 saponin-containing plants [2] and it is now known that over 90 families contain saponins. Gubanov et al. (1970) found, in a systematic investigation of 1730 central Asian plant species, that 76% of the families contained saponins [3]. *Acacia* species contains saponins like Acaciaside A and B [4]. Triterpenoid saponins are widely found in the *Leguminosae* [5,6]. Their biological activities can positively or negatively impact plant traits [5]. Recent studies have illustrated useful pharmacological properties of saponins, including anticholesterolemic and anticancer activities [7,8,9]. Antibacterial antifungal, antiviral, anti-inflammatory and anti-ulcer have been reported about saponins [10-13]. Saponin based adjuvants have the unique ability to enhance immunity [14]. *Acacia etbaica* subspecies etbaica is a one of the most widespread plant in this country; it is common, medium sized tree, locally known as 'Qarad'. Acacia is the most significant genus of family Leguminosae [15]. Traditionally the leaves are crushed and mixed with water and taking orally to reduce stomach pain [16]. The leaves were extracted exhaustively with petroleum ether and then with methanol in a soxhlet apparatus. The aim of the present study was qualitative and quantitative analysis of saponins in methanol extract to establish chemical constituents and to standardize the leaves. In future, this plant can be subjected to isolation of the major constituents and further pharmacological evaluation.

MATERIAL AND METHODS

Plant material

The leaves of A. etbaica subsp. Etbaica were collected in September 2012 from Yaffa, Yemen, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant sample was identified by a taxonomist, Professor Algifri Naser, the department of Botany, of University of Aden, Yemen.

Extraction

The air dried powdered fruits (40 g) were extracted exhaustively with petroleum ether (60- 80 ^{0}C) and then with methanol in a soxhlet apparatus. The methanol extract was concentrated by distilling off the solvent and evaporated to dryness. The residue was suspended in water, extracted successively with ethyl acetate and n butanol (5×40 ml each) and then resulting solutions were concentrated to provide ethyl acetate, n butanol and water soluble parts.

Phytochemical investigation

Methanol extract, its water residue, ethyl acetate and n butanol soluble fractions were tested for the presence of carbohydrates, flavonoids, saponins, tannins, triterpenes and sterols according to standard procedures [17,18].

Thin Layer Chromatography

TLC (silica gel G 60 F254 TLC plates of layer thickness 0.2mm) was established for the ethyl acetate and n butanol soluble fractions and water residue of methanol extract. Various solvent systems such as n-butanol–acetic acid–water (4:1:5), ethyl acetate–acetic acid–water (7:2:2), chloroform– glacial acetic acid - methanol-water (60:32:12:8), chloroform–methanol (2:1), petroleum ether- chloroform - acetic acid (10:4:0.4) were tested to obtain best results. The developed plates were seen under sunlight and UV light at 365 nm before derivatization and then sprayed with Liebermann-Burchard reagent. Number of spots, Rf values were recorded [19,20].

Determination of saponins

Saponin content of the sample was determined by double solvent extraction gravimetric method [18]. 2g of the powdered sample was mixed with 50mls of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 minutes at 55 ^{0}C. It was filtered through filter paper through Whatman filter paper. The residue was extracted with 50mls of the 20% ethanol and both extracts were pooled together. The combined extract was reduced to about 40mls at 90 ^{0}C and transferred to a separating funnel where 40mls of diethyl ether was added and shaken vigorously. Separation was done by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partition was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted with 60mls of normal butanol. The combined extracts were washed twice with 10ml of 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. It was dried at 60 ^{0}C in the oven and reweighed. The experiment was repeated two more times to get an average.

$$\% \text{ Saponins} = \frac{W2 - W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where W1 = Weight of evaporating dish;
W2 = Weight of dish + sample

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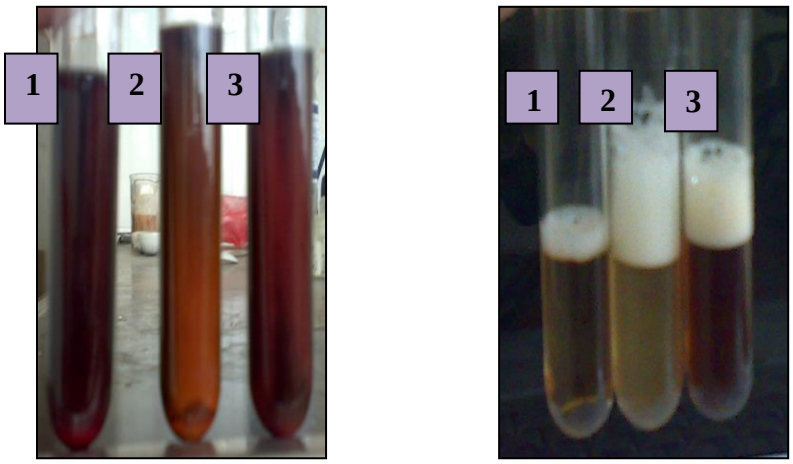
RESULTS

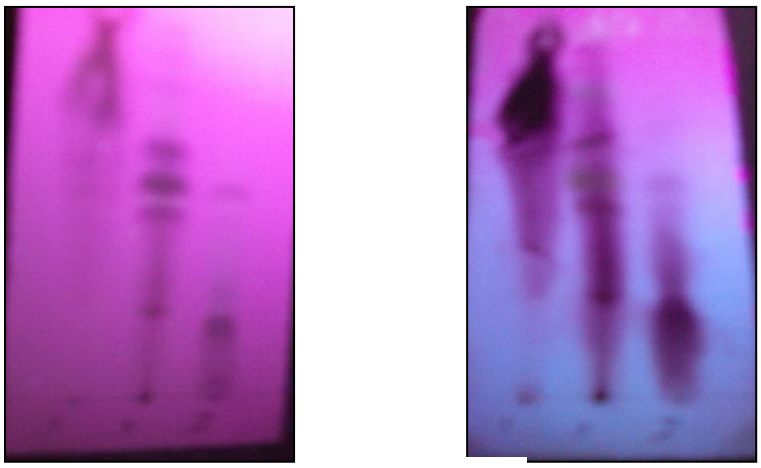
Phytochemical investigation

The result of phytochemical investigation showed the presence of carbohydrates, flavonoids, saponins, triterpenes and sterols in ethyl acetate; n butanol soluble fractions and water residue of methanol extract of the leaves of A. etbaica subsp. Tannins were found in ethyl acetate fraction only. Etbaica. Formation of 2.5 cm layer of foam in test tube with n butanol fraction for 10 minutes according to 0.5 cm and 1cm layer of foam in test tubes with ethyl acetate, and water fractions respectively indicate the presence of saponins abundantly in n-butanol fraction. Photos of the test tubes were taken by using digital camera (figures 1,2).



a b

Figure 2.Test tubes with carbohydrates identification (A) and saponins identification (B) in ethyl acetate faction (1), n-Butanol faction (2) and water residue (3) of methanol extract of the leaves of Acacia etbaic subspecies etbaica.



a b

Figure 3: TLC plate of ethyl acetate faction (1), n-Butanol faction (2) and water residue (3) obtained in n-butanol–acetic acid–water (4:1:5) under UV 365 nm A- before derivatization, and B- after derivatization with Liebermann-Burchard reagent.

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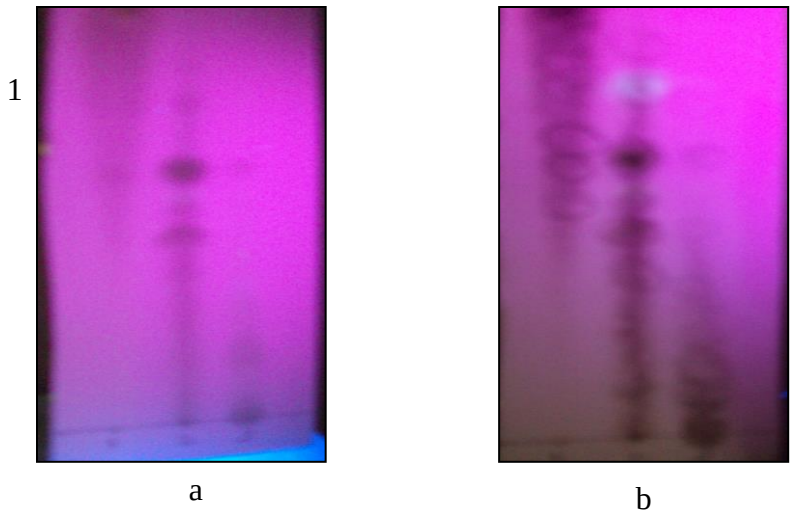

Figure 4: TLC plate of ethyl acetate faction (1), n-Butanol faction (2) and water residue (3) obtained in ethyl acetate–acetic acid–water (7:2:2) under UV 365 nm, A- before derivatization, and B- after derivatization with Liebermann-Burchard reagent.

Thin Layer Chromatography

The presence of saponins in the studied fractions was confirmed by performing TLC (silica gel G 60 F254 TLC plates of layer thickness 0.2mm) separation technique. Various solvent systems were tested to obtain best results. The best resolution was obtained by using the following systems: n-butanol–acetic acid–water (4:1:5), ethyl acetate–acetic acid–water (7:2:2). Photos of the plates were taken at 365 nm before derivatization with Liebermann-Burchard reagent and after derivatization. The Rf values were calculated as well as the colour of spots were observed, which is mentioned in Table 1 and figures 3, 4 and 5.

Determination of saponins

The quantitative phytochemical analysis of the plant drug was studied. The total saponin content of plant drug was 13.0 ±0.03%.

DISCUSSION

The leaves of *Acacia etbaica* subspecies etbaica are an important drug used in traditional medicine of Yemen to reduce stomach pain [16]. The presence of carbohydrates, flavonoids, saponins, triterpenes and sterols in all tested fractions may conform the pharmacological properties of the drug. The above phytochemical investigation and TLC profile have significance in this regard.

The thin layer chromatographic analysis as presented in table 1 reveals the number of constituent compounds present in each extract fraction. A substance can be directly identified by its Rf. value by comparison to the Rf of a standard or pure compound [21]. However, in the

present study, no comparison has been made to ascertain which compound they may be/are, but it indicates the number of possible compounds present in each extract fraction, since there was separation resulting in discrete spots showing the distances traveled by each spot in centimeters and thus Rf values calculated as shown. Thus the number of spots only has been used to indicate the number of constituent compounds suspected to be present in studied fractions.

Table 1: Rf Values of different solvent system of different faction of methanol extract of the leaves of Acacia etbaic subspecies etbaica.

solvent systems	Factions	No. of spots	Rf values	Spot Colour At 365 nm before derivatization	Spot Colour At 365 nm after derivatization
n-butanol–acetic acid–water (4:1:5)	Ethyl acetate faction	4	0,22 0,30 0.38 0.46	Pink Purple Purple Purple	Purple Purple Purple Purple
	n-Butanol faction	5	0.39 0.47 0.56 0.67 0.8	Purple Purple Purple Pink Light Pink	Purple Green Purple Blue Pink
	water residue	3	0.39 0.44 0.58	Pink Purple Purple	Purple Purple Purple
ethyl acetate–acetic acid–water (7:2:2)	Ethyl acetate faction	3	0,43 0,51 0.56	Purple Purple Purple	Purple Purple Purple
	n-Butanol faction	6	0.31 0.39 0.45 0.52 0.66 0.74	Purple Purple Pink Purple Pink Pink	Purple Purple Purple Deep Purple Blue Pink
	water residue	5	0.07 0.12 0.28 0.40 0.54	Brown Brown Pink Pink Pink	Brown Brown Pink Pink Pink

The results of the phytochemical tests and TLC showed the presence of triterpene and sterol saponins in all studied fractions. Unsaturated and hydroxylated triterpenes and steroids give a red, blue or green coloration with acetic anhydride and sulphuric acid [22]. Since triterpenoid saponins tend to produce a pink or purple shade and steroid saponins a blue-green coloration, differentiation of the two classes is possible [1]. Indeed, the extracts which showed under UV 366 nm yellow stains, contain some sterols whereas those that presented red stains contain triterpenes of type oleanane and ursane [23].

The saponin content of the sample was 13.0 ±0.03%. Saponins are known to have various pharmacological activities such as anti-ulcer, antimicrobial, cytotoxic and antitumour [1].

CONCLUSIONS

In the present investigation, methanol extract of the leaves of *Acacia etbaica* subspecies etbaica and its different fractions were subjected to phytochemical screening. Phytochemical screening showed the presence of variety of primary and secondary metabolites. TLC findings were in agreement with the data of qualitative chemical tests and the spots characteristic of saponins were observed. TLC fingerprint profile was established for the varied fractions of bioactive methanol extract. The high content of sapinins in the studied leaves and the presence of phytochemicals which have proven medicinal activity, so in this context this plant might serve as a plant based remedy for many ailments such as ulcer, cancer, antioxidant, etc. Moreover, isolation and bioassay guided studies of saponins from this plant is critical.

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