ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

Study of Triterpenoids in Petroleum Ether Extract of *Acacia etbaica* Subspecies Etbaica Leaves

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Article info

Article History:
Received 4 August 2014
Accepted 23 September 2014

Keywords:
Acacia etbaica, Triterpenes, Sterols, Chromatography.

Abstract

Acacia etbaica Schweinf subspecies etbaica family: Leguminosae is a one of the most widespread plant in Yemen. Very few works has been carried out on the leaves of this plant toward documenting its ethnomedicinal uses and establishing its chemical constituents. The aim of this study is to establish chemical constituents of petroleum ether extract of the leaves toward documenting its ethnomedicinal uses. The principal phytochemical constituents were characterized in petroleum ether extract and its fractions, with colorful reactions and by the establishment of their chromatographic profiles by thin layer chromatography. The results of phytochemical analysis showed the presence of triterpenes and sterols in all studied fractions. Chromatographic profiles were established for the studied fractions. In petroleum ether–chloroform–acetic acid (7:2:1), petroleum ether extract reveals 5 spots; unsaponifiable matter reveals 5 spots; gray amorphous powder reveals 3 spots; orange amorphous powder reveals 3 spots. In Petroleum Ether-acetone (9:1), petroleum ether extract reveals 10 spots; unsaponifiable matter reveals 6; gray amorphous powder reveals 3 spots. In Benzene- chloroform (1:1), petroleum ether extract reveals 5 spots; unsaponifiable matter reveals 4 spots; gray amorphous powder reveals 2 spots. These spots represented sterols and triterpenes. Triterpenes and sterols present in studied leaves along with other constituents may be responsible for valuable pharmacological activities of the drug. This study is a substantial step and it further requires a long term phytochemical and pharmacological studies.

1. INTRODUCTION

The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders. Evidence of use of herbal remedies goes back some 60000 years to a burial site of a Neanderthal man in a cave in northern Iraq, which was uncovered in 1960[1]. Today a great number of modern drugs are still derived from natural sources, and 25% of all prescriptions contain one or more active ingredients from plants[2]. *Acacia etbaica* Schweinf subspecies etbaica family: Leguminosae is a one of the most widespread plant in Yemen; it is common, medium sized tree, locally known as 'Qarad'[3]. Traditionally the leaves are crushed and mixed with water and taking orally to reduce stomach pain[4]. There is no data found before on the phytochemical evaluation of this plant. The aim of this study is to establish chemical constituents of the drug toward documenting its ethnomedicinal uses. In the present study, the petroleum ether extract of the leaves of *Acacia etbaica* subspecies etbaica was subjected to qualitative phytochemical analysis for the presence of triterpenes and sterols. The present study is a substantial step and it further requires a long term phytochemical and pharmacological studies. More detailed study must be done for further isolation leading to the pure compounds and establishment pharmacological activities of this drug.

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2. MATERIALS AND METHODS

2.1 Plant material

The leaves of *Acacia etbaica* subspecies etbaica were collected in September 2012 from Yaffa, Republic of Yemen and were authenticated by a taxonomist, Professor Abdul Nasser Algifri, of the department of biology, faculty of Education, University of Aden. The leaves were stored under the normal environmental conditions for further analysis.

2.2 Extraction and saponification

The dried powdered leaves (50 gm) were extracted with petroleum ether (boiling point 60-80 $^{\circ}$C) in soxhlet extractor. The extract was concentrated to about 30 ml, allowed to stand overnight and the obtained gray amorphous powder (GP) 0.3 g with melting point 59-60^{0}C was filtered. The petroleum ether filtrate confirms the presence of sterols and triterpenes[5], and then subjected to saponification, to remove fatty material and yield unsaponified matter. For saponification petroleum ether extract was stirred overnight at room temperature with 1 M ethanolic potassium hydroxide (30 ml). The mixture was diluted with water and extracted with three portions of diethyl ether (each of 30 ml). The combined ether extract was saponified again with 1 M ethanolic potassium hydroxide, washed with several batches of distilled water until neutral to pH paper and then dried sequentially with short columns of anhydrous sodium sulfate, deactivated alumina and anhydrous sodium sulfate. Chlorophylls and oils were saponified and separated as a layer in the bottom of the bottle; the upper ethereal layer was contain the yellow pigments, sterols and triterpenes. Removal of solvent yields of 0.20 g an unsaponifiable matter (UM) suitable for farther study[6]. The gray amorphous powder was confirmed the presence of sterols and triterpenes qualitatively by phytochemical test and thin layer chromatography and then subjected to saponification as prescribed above to yield unsaponified matter. Removal of solvent yields of 0.1mg an orange amorphous powder (OP) with a melting point (159-160°C) suitable

for further study.

2.3 Phytochemical investigation of triterpenoids

The principal phytochemical constituents were characterized in petroleum ether extract, unsaponified matter (UM), gray amorphous powder (GP) and orange amorphous powder (OP), with colorful reactions.

2.3.1 Colorful reaction for triterpenoids

The usual reagents of characterization that we used, allowed us to put in evidence of the presence of triterpenes and sterols are Salkowski and Liebermann-Burchard[7, 8, 9]. A small portion of petroleum ether extract, unsaponified matter, gray amorphous powder (GP) and orange amorphous powder (OP) separately were dissolved in chloroform and solutions were subjected to qualitative phytochemical investigation.

2.4 Chromatography

Petroleum ether extract, unsaponified matter (UM), gray amorphous powder (GP) and orange amorphous powder (OP) were subjected to thin layer chromatographic studies, to find out the probable number of triterpenes and sterols present in them. A small portion of each fraction separately was dissolved in chloroform and spotted on silica gel 60 F254 pre-coated TLC plates. Various solvent systems were tested to obtain best results. The best resolutions were obtained by using petroleum ether-acetone (9:1), benzene- chloroform (1:1) and petroleum ether–chloroform–acetic acid (7:2:1). Chromatograms were evaluated under UV light at 254 nm and 365 nm before and after derivatization with Liebermann-Burchard reagent. The Rf values were calculated as well as the colour of spots were observed[5, 10].

3. RESULTS AND DISCUSSION

The principal phytochemical constituents were characterized in petroleum ether extract, (UM), (GP) and (OP), with colorful reactions and by the establishment of their chromatographic profiles by TLC. The results of phytochemical screening showed the presence of triterpenes and sterols in all studied fractions.

Chromatographic studies on TLC plates coated with silica gel G60 F254, were performed for the petroleum ether extract, UM, GP and OP. The chromatograms showed the presence of several zones that corresponded to triterpenes and sterols. They were detected by quenching fluorescence under UV-254 nm and by pink, purple, blue and green fluorescence under UV light at 365 nm before and after derivatization with Liebermann-Burchard reagent. The Rf values were calculated as well as the colour of spots were observed. TLC profiling in Petroleum ether–chloroform–acetic acid (7:2:1), petroleum ether extract reveals 5 spots; UM reveals 5 spots; GP reveals 3 spots; OP reveals 3 spots (Table 1). TLC profiling in Petroleum Ether-aceton (9:1), petroleum ether extract reveals 10 spots; UM reveals 6 spots; GP reveals 3 spots (Table 2). TLC profiling in Benzene- chloroform (1:1), petroleum ether extract reveals 5 spots; UM reveals 4 spots; GP reveals 2 spots (Table3).

The revelation of different TLC of the fractions with the reagent of Liebermann-Büchard, confirmed the presence of sterols and triterpens. Unsaturated and hydroxylated triterpenes and steroids give a red, blue or green coloration with acetic anhydride and sulphuric acid[9, 10]. 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[8]. 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[11].

Table 1: TLC profiling in Petroleum ether–chloroform–acetic acid (7:2:1)

S. No.	Rf	Petroleum Ether extract	UM	GP	OP	Spot Colour At 254 nm	Spot Colour At 365 nm	Spot Colour At 365 nm after derivatization
1	0.62	+++	++	-	-	Black spot	Pink	Purple
2	0.67	+++	++	++	++	Black spot	Purple	Purple
3	0.74	+++	++	-	-	Black spot	Purple	Purple
4	0.81	+++	++	++	++	Black spot	Purple	Deep Red
5	0.87	+++	++	++	++	Black spot	Pink	Purple

+++ = Most intense, ++ = moderately intense, + = Least intense, - =absent.

Table 2: TLC profiling in Petroleum Ether-aceton (9:1)

S. No.	Rf	Petroleum Ether extract	UM	GP	OP	Spot Colour At 254 nm	Spot Colour At 365 nm	Spot Colour At 365 nm after derivatization
1	0.12	+++	+	-	-	Dark Green	Deep Green	Deep Green
2	0.16	+	-	-	-	Brown	Brown	Purple
3	0.21	++	+	-	-	Dark Green	Brown	Brown
4	0.26	+	-	-	-	Dark brown	Pink	Not visible
5	0.28	+++	+++	+	-	Not visible	Not visible	Deep Brown
6	0.38	++	-	-	-	Dark brown	Pink	Not visible
7	0.42	+++	+++	-	-	Dark	Pink	Deep Pink
8	0.45	+	+	+	-	Dark	Pink	Brown
9	0.50	+++	-	-	-	Dark	Pink	brown
10	0.55	++	-	-	-	Dark	Pink	Light Brown
11	0.61	++	+	-	-	Dark	Pink	Not visible
12	0.80	+	+	-	-	Not visible	Not visible	Brown
13	0.84	+	-	-	-	Dark	Deep Pink	Not visible
14	0.88	+	-	+	-	Not visible	Not visible	Brown

+++ = Most intense, ++ = moderately intense, + = Least intense, - =absent

Table 3: TLC profiling in Benzene- chloroform (1:1)

S. No.	Rf	Petroleum Ether extract	UM	GP	OP	Spot Colour At 254 nm	Spot Colour At 365 nm	Spot Colour At 365 nm after derivatization
1	0.15	+	-	-	-	Black spot	Purple	Blue-green
2	0.16	-	-	+	-	Black spot	Not visible	Not visible
3	0.23	+	+	-	-	Black spot	Purple	Blue-green
4	0.32	-	-	+	-	Not visible	Not visible	Light Yellow
5	0.35	+	+	-	-	Black spot	Pink	Deep Purple
6	0.45	+	+	-	-	Black spot	Purple	Purple
7	0.60	+	+	-	-	Black spot	Red	Deep Red
8	0.76	-	-	+	-	Not visible	Not visible	Light Yellow

+++ = Most intense, ++ = moderately intense, + = Least intense, - =absent.

4. CONCLUSIONS

The present study indicates that the leaves of *Acacia etbaica* subspecies etbaica might serve as important medicinal plant. The present study shows that this plant has triterpenes and sterols compounds which have proven medicinal activity, so in this context this plant might serve as a plant based remedy for many ailments such as cancer, ulcer, depression, antioxidant, etc. Further phytochemical and pharmacological investigation is very essential to prove the efficacy of this medicinal herb. Moreover isolation and bioassay guided studies of triterpene compounds from this plant is critical.

5. ACKNOWLEDGEMENT

The authors are grateful to Professor Abdul Nasser Algifri, of the department of Biology, of University of Aden, for his help to identify and authenticated the plant specimen.

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