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Research Article

PHARMACOGNOSTICAL STUDIES OF LEAVES OF *ACACIA ETBAICA* SUBSPECIES UNCINATA

SALEH KASSEM ALGFRI*

Department of Pharmacognosy, Faculty of Pharmacy, University of Aden, Aden, Yemen. Email: algfri_s25@yahoo.com

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ABSTRACT

Objective: The objective of the study is to analyze the microscopic, macroscopic, and physicochemical standards of the leaves of the *Acacia etbaica* subspecies uncinata.

Methods: Pharamacognostic studies (macroscopic, microscopic, and powder microscopy) were carried out. Physicochemical standards (ash values, extractives values, and moisture content by loss on drying) were determined. Fluorescence analysis of powdered drug was also performed.

Results: The macroscopic study showed that the leaves were bipinnate with 3–11 pairs of pinnae, each containing 7–25 pairs of leaflets. The leaflet was linear with parallel margins and a rounded at the apex, color was dark green, odor was characteristic and the taste was astringent. The characteristic microscopy of leaves showed the presence of polygonal and rectangular epidermal cells in the center of the lamina and rectangular at the edges, paracytic stomata, non-glandular trichomes, and reticulate venations. The microscopic study of petiole, rachis, and rachilla revealed the presence of elongate, swollen conical-shaped, flagelliform, and wavy trichomes. The powder microscopy also revealed paracytic stomata, trichomes with pedestals, and annular vessels. Physiochemical analysis of dried leaf powder showed total ash, acid insoluble ash, water-soluble ash, water extractive value, ethanol extractive value, and moisture content as 6.11%, 2.50%, 4.57%, 32.50%, 14.10%, and 7.26% w/w respectively. The fluorescence analysis of leaf powder was established.

Conclusion: Various pharmacognostic, physicochemical, and fluorescence parameters observed in this study will help in the identification and standardization of the leaves of *A. etbaica* subspecies uncinata.

Keywords: *Acacia etbaica* subspecies uncinata, Pharmacognostic, Physicochemical, fluorescence.

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INTRODUCTION

Medicinal plants are widely used all over the world. According to the latest WHO researches, 11% of the 252 basic medicines are in fact herbal preparations [1], and 70–80% of world's population relies on traditional healthcare. In addition acceptance of traditional medicines, especially herbal medicines in the developed world is sharply increasing [2-4]. All medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective [5,6]. Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine [7]. Pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of a crude drug used in traditional medicine [8]. In Yemen, the use of medicinal and aromatic plant species goes back thousands of years and form an important part of the culture [9]. Nevertheless, little scientific research was done to investigate the plants of Yemen used in herbal medicine [10]. *Acacia etbaica* subspecies uncinata, family leguminosae is widespread in Yemen [11], its leaves have been used to treat various ailments including stomach ache [12]. However, no much scientific validation has been made for this plant; so the current study was aimed to determine the pharmacognostic, physicochemical, and fluorescence standards for the leaves of *A. etbaica* subspecies uncinata.

METHODS

Plant material

A. etbaica subspecies uncinata leaves were collected in October 2013 from Dhala, 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 Abdul Nasser Algifri, of the Department of Biology, of the University of Aden, Yemen.

Chemicals and reagents

All chemicals and reagents used for the study were analytical grade.

Pharmacognostic study

Macroscopic evaluation

Morphology of studied leaves was observed with the help of the magnifying lens. Parameters such as shape, color, odor, and taste were evaluated for each plant sample. The macroscopic characters of leaves were studies as per the produce given in the WHO guidelines [13].

Microscopic studies of the leaves

The microscopic characters of leaves were studies as per the produce given in WHO guidelines [13]. Freehand sections of leaflet, petiole, rachis, and rachilla were taken. Sections were cleared by heating with chloral hydrate solution and examined under microscope. Photomicrographs were taken with Leica USA model 2000ATC (ocular: CPL W10X; objective: ×4, ×10, and ×40). Various identifying characters, such as type of trichomes, type of stomata, and epidermal cells were recorded, and then photomicrography was done [14,15]. Photographs were taken with the help of digital camera (Sony 16 MP).

Study of leaves powder

The shade dried leaves were powdered to very fine powder, which passed through 100 # sieve. Chloral hydrate TS was used for clarification.A small amount of powder was taken onto a microscopic slide and observed under microscope to study the characteristic features. Photomicrographs were taken with Leica USA model 2000ATC (ocular: CPL W10X; objective: ×4, ×10 and ×40). Various identifying characters, such as type of trichomes, type of stomata, and epidermal cells were recorded, and then photomicrography was done [16]. Photographs were taken with the help of digital camera (Sony 16 MP).

Physicochemical parameters
The physicochemical parameters such as moisture content, percentage extractives in different solvents, ash content, acid insoluble ash, water-soluble ash, and moisture content by loss on drying were determined by standard methods as in the WHO guidelines [13].

Fluorescence analysis of powdered drug
Fluorescence study of leaves powder was performed as per reported standard procedures [17]. A small quantity of powder drug was treated with various reagents and observed in visible light, under UV with long wavelength (365 nm). The color observed by application of various reagents in different radiation was recorded [17,18].

RESULTS AND DISCUSSION

Macroscopic studies of leaves
An examination to determine macroscopic and microscopic characteristics is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any further tests are undertaken [13]. The macroscopic study showed that the leaves were bipinnate with 3–11 pairs of pinnae, each containing 7–25 pairs of leaflets. The leaflet was linear with parallel margins and a rounded at the apex, color was dark green, odor was characteristic and taste was astringent (Fig. 1).

Microscopic studies of leaves
Hand sections of leaflets, petiole, rachis, and rachilla were used for micro-characterization. The results were as the following:

Leaflets
The microscopic study of upper and lower surfaces of leaflets showed the presence of polygonal and rectangular epidermal cells in the center of the lamina and rectangular at the edges, paracytic stomata, and large number of cluster of calcium oxalate crystals. Unicellular, non-glandular erect, and curved trichomes were found rarely in the edge of the lamina (Figs. 2 and 3).

Venation pattern
The lamina was examined under a microscope, so a reticulate venation was observed and large vein islets including vein terminations were identified. The vein terminations are branched, sometimes simple and slender (Fig. 4).

Petiole
The microscopic study of petiole showed the presence of square, polygonal and rectangular epidermal cells, also barrel like cells were observed (Fig. 5). Unicellular, non-glandular, and warty trichomes, with thick cell wall, an acute apex, and rounded base found abundantly. Trichomes have different shapes such as elongate, swollen conical-shaped, and wavy. Some trichomes have a pedestal (Fig. 6).

Rachis
The microscopic study of raches showed the presence of polygonal and rectangular cells, non-glandular, worthy and conical trichomes. Trichomes may be with the thick cell wall, an acute apex, and rounded base, some of them have a pedestal. Paracytic stomata were very rare (Figs. 7 and 8).

Fig. 1: Leaves of *Acacia etbaica* subsp. uncinata

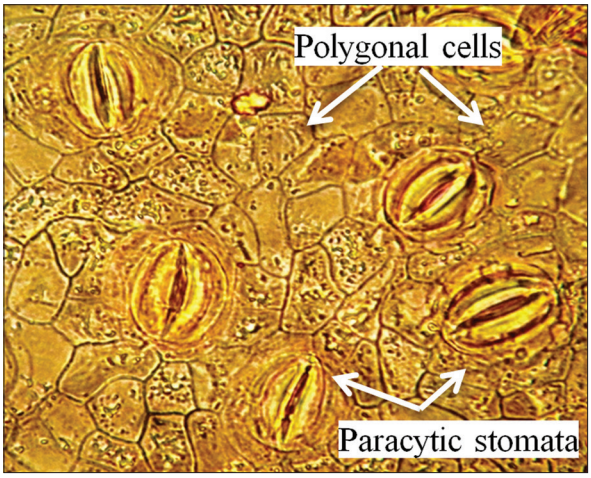

Fig. 2: Surface view of epidermal cells of leaflet (10×40)

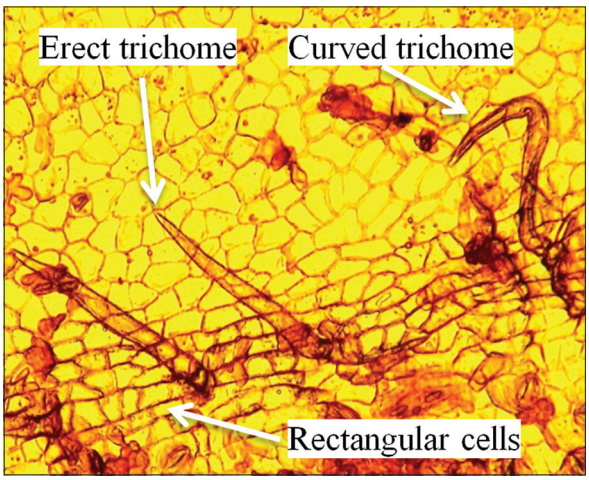

Fig. 3: Surface view of leaflet with non-glandular trichomes (10×10)

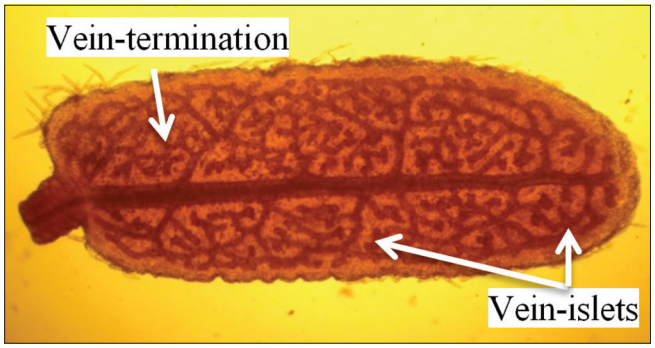

Fig. 4: Surface view of venation pattern (10×10)

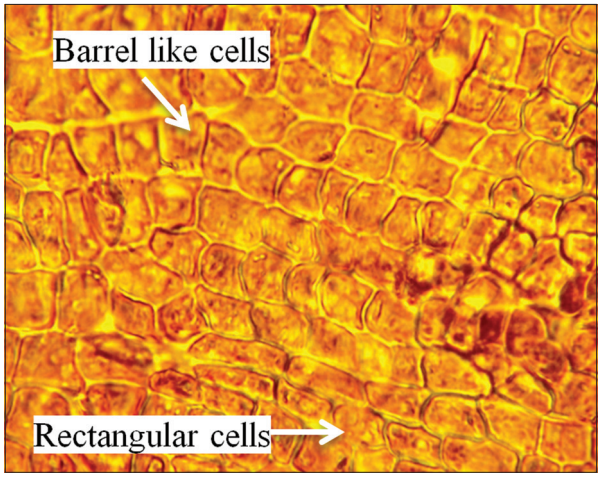

Fig. 5: Surface view of epidermal cells of petiole (10×10)

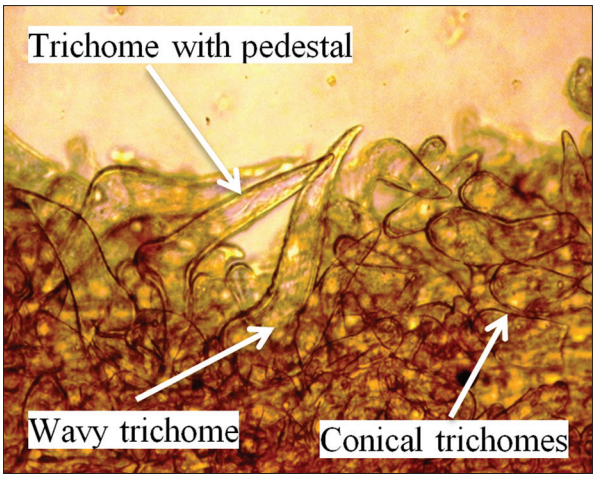

Fig. 6: Surface view of epidermal cells of petiole (10×40)

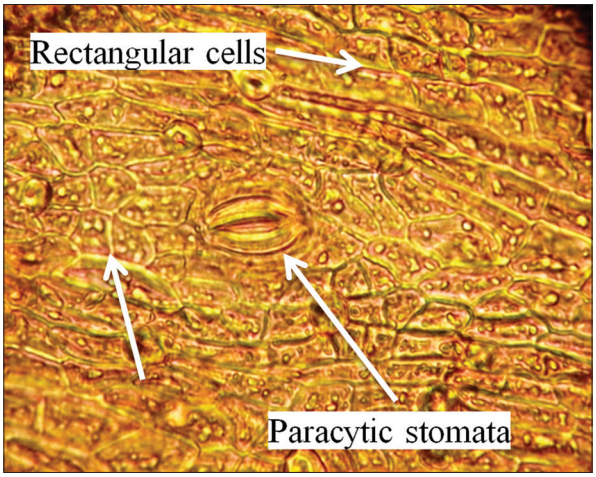

Fig. 7: Surface view of epidermal cells of rachis (10×10)

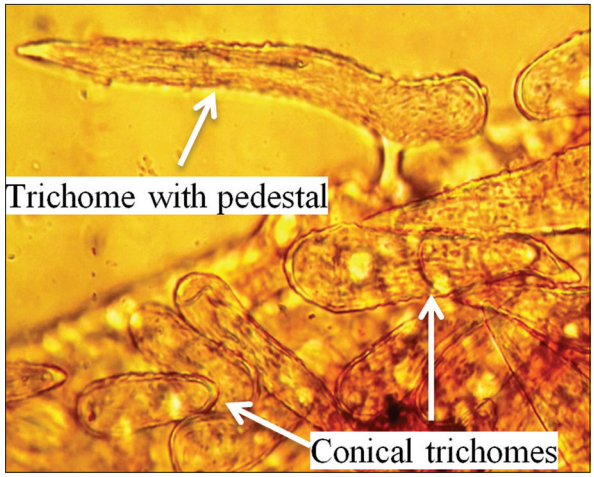

Fig. 8: Surface view of epidermal cells of rachis with rtichomes (10×40)

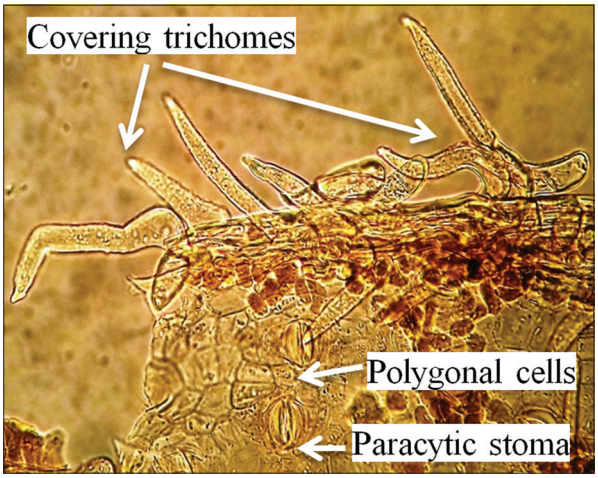

Fig. 9: Surface view of epidermal cells of rachilla (10×40)

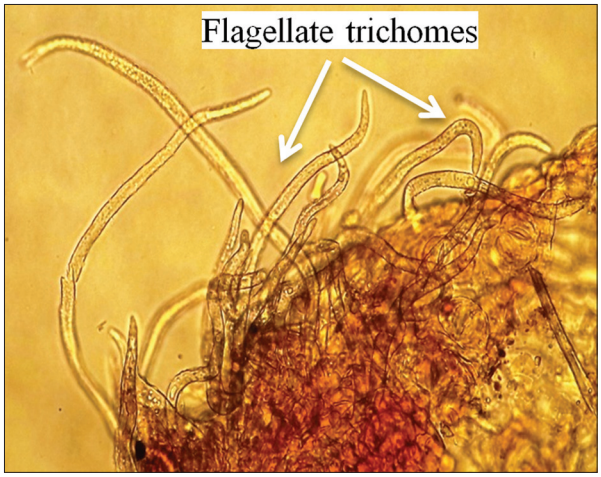

Fig. 10: Surface view of epidermal cells of rachilla (10×40)

Rachilla

The characteristic microscopy of the rachilla showed the presence of polygonal cells, paracytic stomata, and non-glandular trichomes. Trichomes with an acute apex and rounded at the base were found abundantly, they are found in various shapes as flagelliform, curved, erect, and wavy (Figs. 9 and 10).

Powder microscopy

Microscopical study of powders showed the following characters: fragment epidermis with paracytic stomata (Fig. 11a), fragment of the epidermis with trichomes (Fig. 11b), non-glandular worthy trichome with an acute apex, rounded base, and pedestal (Fig. 11c), Non-

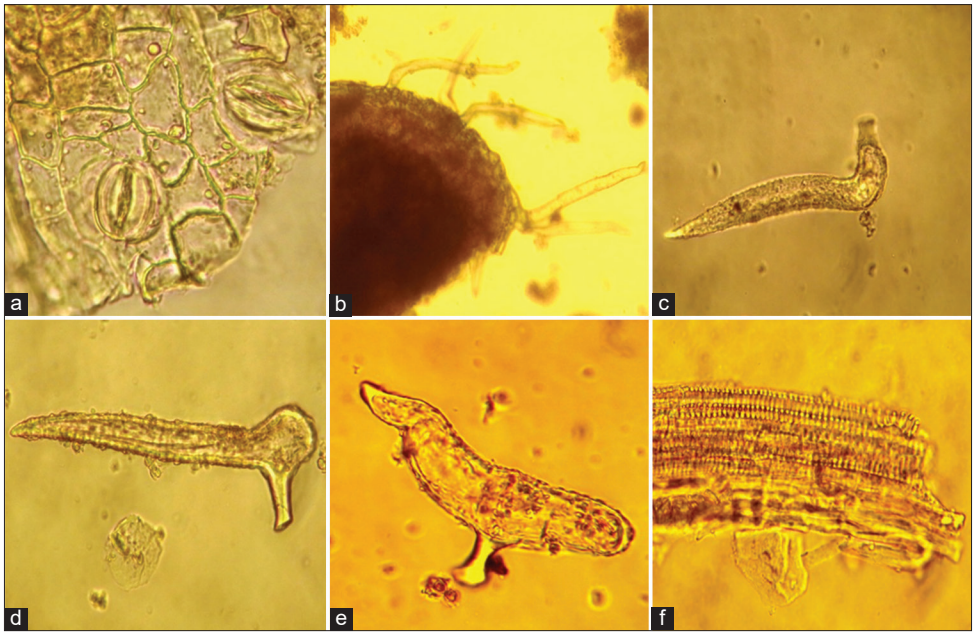

Fig. 11: (a-f) Powder microscopy of leaves of *Acacia etbaica* subsp. uncinata, with different anatomical characters

Table 1: Physicochemical parameter of leaves of Acacia etbaica subspecies uncinata

Parameters	Result%
Ash values	
Total ash	6.11 0.08
Acid insoluble ash	2.50±0.05
Water soluble ash	4.57±0.06
Extractive value	
Water soluble	32.50±0.08
Ethanol soluble	14.10±0.06
Moisture content	
Loss on drying at 110°C	7.26±0.07

Table 2: Fluorescence analysis of leaf powder of *Acacia etbaica* subspecies uncinata

S. No.	Treatments	Observations	
		Day light	Long UV
1.	Powder + 1N NaOH (aqueous)	Brown	Dark brown
2.	Powder + 1N H_2SO_4	Yellow	Deep yellow
3.	Powder + 50% N HNO_3	Brownishred	Brownishyellow
4.	Powder + conc.HNO_3	Brown	Reddishbrown
5.	Powder + dil HNO_3 10%	Light brown	Brown
6.	Powder + Ammonia	yellow	Greenishyellow
7.	Powder + Acetic acid	Brown	Brown
8.	Powder + 50% Iodine	Brownishyellow	Greenishyellow
9.	Powder + 5% $FeCl_3$	Black	Black
10.	Powder + Methanol	Green	Reddish brown
11.	Powder + Water	Brownishyellow	Brown

glandular worthy trichomes with curved base and a pedestal near the end (Fig. 11d) Non-glandular swollen worthy trichome, rounded base with a pedestal in the medium (Fig. 11e) and annular vessels (Fig. 11f).

The pharmacognostic investigations of some physical parameters are helpful in setting standards for a crude drug as these parameters are mostly constant for a plant. Macroscopic and microscopic description of medicinal plants is the first step toward the establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken [16]. Various macroscopic, microscopic, fluorescence, and physicochemical analysis were established. Paracytic stomata and non-glandular, unicellular, straight, or curve trichomes have been found in Fabaceae members [19], including *Acacia auriculiformis* and *A. etbaica* [20,21], and as seen in the studied species. In addition, the presence of flagelliform trichomes, trichomes whith pedestal and barrel-shaped cells are botanical characteristics fore A. *etbaica* subspecies uncinata.

Physicochemical analysis

The determination of physicochemical parameter is important in determination of adulterants and improper handling of powder. The results obtained from the physicochemical parameters are described in Table 1.

Fluorescence analysis of powdered drug

Fluorescence is the most important parameter of pharmacognostic evaluation [22,23] and is used for the identification and standardization of crude drugs. It also indicates the presence of certain phytoconstituents that show fluorescence either in the visible range or under ultraviolet light [24]. The powder was treated with various reagents and the mixture was observed under daylight and under UV light to see the type of fluorescence and results were given in Table 2.

CONCLUSION

In this study, various pharmacognostic parameters such as macroscopic, microscopic, physicochemical, and fluorescence parameters of leaves of *A. etbaica* subspecies uncinata were established. These results can be employed as suitable quality control measures to ensure the quality, safety, and efficacy of this herbal drug. This study is a substantial step and it further requires a long-term phytochemical and pharmacological studies.

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AUTHORS CONTRIBUTION

Dr. Saleh Kassem Algfri has done the research work, data collection, and preparation of the manuscript.

CONFLICTS OF INTEREST

None.

AUTHORS' FUNDING

None.

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