

PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHY OF *ACACIA ETBAICA SSP. UNCINATA* LEAVES

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ABSTRACT

This study were conducted to establishing the phytochemical screening and thin layer chromatography (TLC) of *Acacia etabica* leaves belonging to Fabaceae family. Phytochemical screening of total ethanolic and aqueous extracts showed the presence of alkaloids, flavonoids, tannins, saponin, Anthraquinone, Triterpenes glycosides. TLC studies constituted different colored phytochemical compounds with different R_f values. All spots are colorless in day light but they are colored under UV light. Ethanolic extract showed six spots and dichloromethane (DCM) extract showed six spots, while water extract resulted in only one spot.

KEYWORDS: *Acacia Etbaica Ssp. Uncinata*, Phytochemical Screening, TLC.

INTRODUCTION

Plants produce primary and secondary metabolites with divergent function.^[1] The primary metabolites, as amino acids, simple sugars, proteins and lipids are involved in cellular processes. The secondary metabolites are chemically active compounds (flavonoids, alkaloids, terpenoids, steroids, saponins, etc.). These a secondary metabolites also known as natural products as they elicit effects on other organisms.^[2]

The active principles of many drugs found in plants are secondary metabolites, are widely used in traditional medicine to treat various ailments. It's have demonstrated antimicrobial, anti-inflammatory, anticancer, anti-viral, anti-malarial.^[3,4,5]

The crude extracts of Acacia medicinal plants were screened for antimicrobial activity against many microorganisms. *A. tortilis* and *A. leiocarpus* extracts have showed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.^[6] The phytochemical screening studies showed active compounds in acacia species, as *A. senegal*, *A. nilotica*, *A. Arabica*, *A. raddiana*.^[7,8,9,10,11,12]

Acacia etbaica is a shrub. *A. etbaica* has a number of subspecies namely: subsp. *etbaica*, mainly found in Sudan and Somalia, subsp. *uncinata* Brenan found in Somalia, Uganda and Kenya and subsp. *platycarpa* Brenan found in Kenya and Tanzania.

Acacia etbaica ssp. uncinata, is one of the most widespread plants in Yemen, locally known as 'Qarad'.^[13] Very few works have been carried out on the leaves of this plant toward documenting its ethnomedicinal uses and establishing its chemical constituents. Traditionally in Yemen the leaves of *A. etbaica ssp. uncinata* are crushed and mixed with water and taken orally to reduce stomach pain.^[14] In East Africa the bark is chewed as a stimulant and is also used in the treatment of gonorrhoea.^[15] In this present study we established the *A. etbaica ssp. Uncinata* leaves phytochemical constituents using phytochemical screening and TLC analysis.

MATERIAL AND METHODS

Plant materials: The leaves of *A. etbaica ssp. uncinata* were collected in September 2013 from Khober, Aldhala, Yemen, dried in shaded area and then manually grinded and stored at room temperature.

Extraction: The powdered material was subjected to hot maceration extraction method, successively with different known solvents in increasing order of polarity; dichloromethane (DCM), ethanol and water. Each time before extracting with next solvent, the powdered material was dried. Each extract was then concentrated by evaporation the solvent on water bath.^[16,9] All the extracts were stored in refrigerator for qualitative analysis..

Phytochemical Screening: The extracts obtained were subjected to following chemical tests for identification of various phytoconstituents as per the methods given by Harborne^[16], as phytochemical test (table 1).

Thin layer Chromatography: TLC is a chromatographic technique which is used for the separation of mixture of compounds. TLC is performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F254 (Merck). Samples prepared with different solvents were spotted onto the TLC plate as a single spot with capillary tubes.^[11] TLC plates were first viewed in UV chamber and *R_f* values were calculated. The used mobile phase system (solvent system) was ethyl acetate: toluene: formic acid (4:4:1).

RESULT AND DISCUSSION

The crude drug (dried leaves of *Acacia etbaica ssp. uncinata*) undergo hot maceration extraction by dichloromethane(DCM) as an on-polar solvent to remove lipids and fats (defatting), then the marc treated by ethanol and water respectively. These three extracts were subjected to phytochemical screening and TLC analysis.

Phytochemical screening: phytochemical analysis of the DCM, ethanol and aqueous extracts of *A. etbaica ssp. uncinata* leaves were carried out to determine the presence of phytochemicals like alkaloids, tannins, carbohydrates, anthraquinone, glycosides, flavonoids, saponins and triterpenes/sterols (Table 1). Similarly to the previous studies of El-Mousallamy *et al*^[7] and of El Yahyaoui *et al*^[8] on *Acacia raddiana*, the results found show that the leaves extracts contain phenolic compounds such as flavonoids, tannins, coumarins and anthraquinones. They also contain carotenoids, alkaloids and saponins. Also similar findings were reported by^[10] in *A. senegal*, but they did not find flavonoids, anthraquinone. Lawrence *et al*^[11] showed the presence of tannins, carbohydrates, terpenoids, phenols, anthraquinone, cardiac glycosides, flavonoids and alkaloids in *Acacia nilotica*.

TLC Analysis: The extracts of leaf of each solvent were subjected to TLC. All spots are colorless in day light but they are colored under UV light. Ethanolic extract showed six spots of red, yellow, and light blue with *R_f* values 0.90, 0.85, 0.80, 0.79, 0.74 and 0.7 respectively whereas DCM extract showed six spots of red and pink with *R_f* values 0.8, 0.69, 0.6, 0.51, 0.47 and 0.43. TLC of water extract resulted in one spot of light pink with *R_f* values 0.69 (Table 2). The conducted Thin layer Chromatography are used to separate the constituents of

secondary metabolites of plant extracts.^[17] Rf values showed significant diversity of compounds separated from the different researched secondary metabolites. Chromatograms have validated the presence of several types of tannins, flavonoids, coumarins, quinones, carotenoids, saponins, alkaloids and terpenes relative to phytochemicals coloring reactions in *Acacia raddiana* leaves^[8] and in *A. nilotica* bark.^[18] Renuka et al^[19] reported dichloromethane extract of *Acacia senegal* root heartwood to exhibit antibacterial activity against *E. coli* and *S. aureus*. Acetone extract of *Acacia nilotica* has been found to be effective against *E. coli*, *S. pyogenes*, *V. cholera*, *S. aureus*, *P. aeruginosa* etc.^[11]

Table. 1: Results of phytochemical screening of DCM, ethanol and water extracts.

Phytochemicals	Test	Solvent		
		DCM	Ethanol (96%)	Water
Alkaloids	Mayer's test	-	-	-
	Wagner test	-	-	-
	Dragondroff test	-	-	-
Tannins	FeCl ₃ 3%	-	+++	++
Carbohydrates	Fehling's test	-	+++	+++
Anthraquinone Glycosides	Modified Borntragers test	-	-	-
Saponins	Foam test	-	+++	++
Triterpenes/sterols	Salkowskis test	++	+++	++
	Liberman-Burchards test	++	+++	++
Flavonoids	Cyaniding test (Shinoda)	-	+++	++
	Lead acetate test	-	+++	++

Table. 2: Results of Thin Layer chromatography.

Mobile Phase	Extract	Rf Value	Color of spot
Ethyl acetate: Toluene: Formic acid (4:4:1)	Ethanollic	0.9	Red
		0.85	Red
		0.8	Red
		0.79	Yellow
		0.74	Light blue
		0.7	Light blue
	DCM	0.8	Red
		0.69	Red
		0.6	Red
		0.51	Red
		0.47	Red
	Water	0.43	Pink
		0.69	Light pink

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