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ISOLATION OF A FLAVONOL FROM STEM BARK OF SUDANESE *ACACIA TORTILIS (*FORSK.)HAYNE AND ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT

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ABSTRACT

This study was set to investigate the flavonoids of *Acacia tortilis* which is a key species in indigenous medicine. This plant which showed many pharmacological properties is used traditionally against many diseases. A flavonol was isolated from *Acacia tortilis* stem bark via chromatographic techniques and its structure was partially characterized on the basis of its spectral data(UV,^{1}HNMR and IR). The ethanol extract exhibited good activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli.*

KEYWORDS: *Acacia tortilis*, Isolation, Flavonol, Antimicrobial Activity.

INTRODUCTION

Flavonoids are phenolic compounds widely present in plants and foods of plant origin.[1-4] Flavonoids contain fifteen carbon atoms in their basic nucleus- flavan, arranged in a C_6-C_3- C_6 configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third heterocyclic ring (C). There are six major subgroups: chalcones, flavones, flavonols, flavanones anthocyanins and isoflavonoids. Flavonoids encompass a large group of polyphenolic substances with marked physiological potential including: antibacterial, anti-inflammatory, antiallergic, antifungal, antimutagenic, antivirl and vasodilator effects.[5-7] Acacia (Fabaceae) is a large genus comprising around 1350 species. Acacia species are rich source of timber,gum and tannins and are mainly distributed in worm and dry regions-Arabia, Australia and Africa.[8-11] Most of these plant species are rich in bioactive molecules including phenolics.[10] Traditionally, Acacia species are used as antidiabetic, antidiarrhoeic, antiinflammmatory, antimicrobial and as hypotensive.[12-14] *Acacia tortilis*(**Forsk.) Hayne** is a plant of potential medicinal attributes. It is used traditionally against many diseases including inflammation, skin diseases and cough.[11] The pharmacological therapeutic potential of *Acacia tortilis* has been explored. The antifungal activity of the stem bark has been reported.[15] Bark tannins proved to be useful for cattle suffering from diarrhea[16].Stem bark extracts were reported to treat infections.[17] Some polysaccharides isolated from gum exudates exhibited antidiabetic effect.[18] It has been shown that the root bark possesses antimalarial activity.[19] Some *in vivo* studies demonstrated that *Acacia tortilis* aqueous extract decreased serum total cholesterol and LDL levels.[20]

MATERIALS AND METHODS

MATERIALS

Plant material

Stem bark of *Acacia tortilis* was collected from White Nile state(Sudan). The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute(Sudan).

Instruments

UV spectra were run on a Shimadzu 2401PC UV-Visible Spectrophotometer. The IR spectra were run on a Perkin- Elmer 1310 Infrared Spectrophotometer. NMR spectra were performed on a Joel ECA 500MHZ NMR Spectrophotometer.

Test organisms

The antimicrobial activity of the ethanol extract was evaluated using the following standard microorganisms: *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans***.**

METHODS

Extraction and isolation of flavonoids

Powdered stem bark of a *Acacia tortilis* (1.5 kg) were macerated at room temperature with ethanol (95%) for 72h. The solvent was evaporated under reduced pressure

to dryness to give a crude product. The crude ethanol extract was fractionated via paper chromatography using the 30% acetic acid as mobile phase. The chromatograms were viewed and located under UV light and a flavonoid-compound I- was eluted from paper with methanol**.**

Antimicrobial activity

Preparation of bacterial suspensions

A (24) hours broth culture of test organisms were aseptically distributed onto nutrient agar slopes and incubated for 24h at 37º C. Bacterial growth was washed off with 100 ml sterile normal saline giving approximately 10^8- 10^9 C.F.U/ ml.. The average number of viable organisms per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37 °C for 24 h.

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

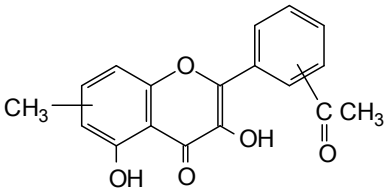
Testing of antibacterial susceptibility

Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) soaked with a solution of each test sample were placed on the surface of the seeded agar. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The test was done in two replicates and the diameters (mm) of the inhibition zones were measured and averaged.

RESULTS AND DISCUSSION

Characterization of compound I

Compound I was isolated by paper chromatography as yellow powder from *Acacia tortilis* stem bark and its structure was elucidated via a combination of spectral techniques (UV, IR and ^{1}HNMR). The IR spectrum of compound I **(**Fig.1) showed ν(KBr): 619,781,856(C-H, Ar.bending), 1274 (C-O, ether),1496,1514 (C = C, Ar.), 1605 (C = O),2923(C-H) and 3390 cm^{-1}(OH). The appearance of a carbonyl stretching in the IR spectrum suggests absence of two classes of flavonoids characterized by the absence of a carbonyl function-the flavans and the anthocyanins.[21,22] The UV spectrum of compound I showed (Fig.2) λ_{max} 242,366nm. Such absorption is characteristic of flavonols.[21,22] When the UV shift reagent – sodium methoxide - was added to a methanolic solution of compound I, a 44nm bathochromic shift in band I with decrease in intensity (Fig.3) was observed indicating.[21,22] the presence of a 3-OH function which is a characteristic feature of flavonols. When a methanolic solution of compound I was treated with excess powdered sodium acetate, no bathochromic shift in band II (Fig.4) was observed indicating the absence of 7 –OH group. The aluminium chloride spectrum of compound I(Fig.5) showed a 62 bathochromic shift in bamd I indicating.[21,22] a 5 –OH group(the spectrum was stable in HCl). The ^{1}HNMR spectrum of compound I(Fig.6) showed δ(ppm): 1.22 (assigned for a methyl group); 1.84(accounts for an acetyl group); multiplet(4.80-5.40) assigned for a sugar moiety(not identified in this study); multiplet(6.00-7.85) assigned for the aromatic protons. The signals at δ2.5 and δ3.30ppm are due to solvent(DMSO) residual protons and residual water respectively. On the basis of the above spectral data, the following partial structure was proposed for the aglycone of compound I.



Aglycone of compound I

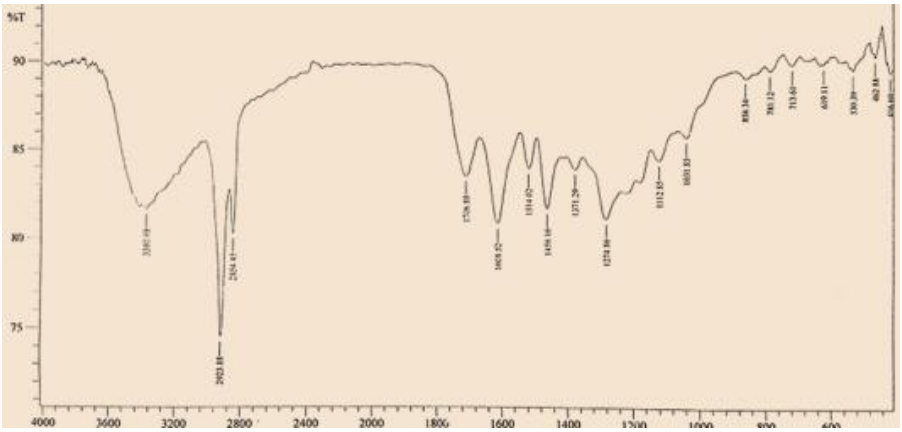


Fig 1: IR spectrum of compound I.

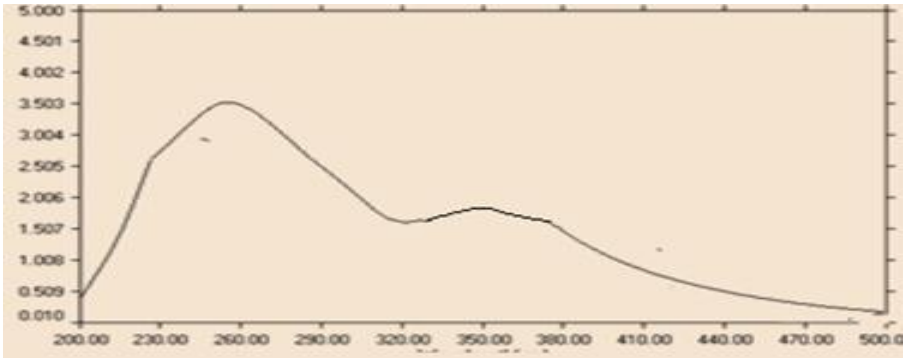


Fig 2: UV spectrum of compound I.

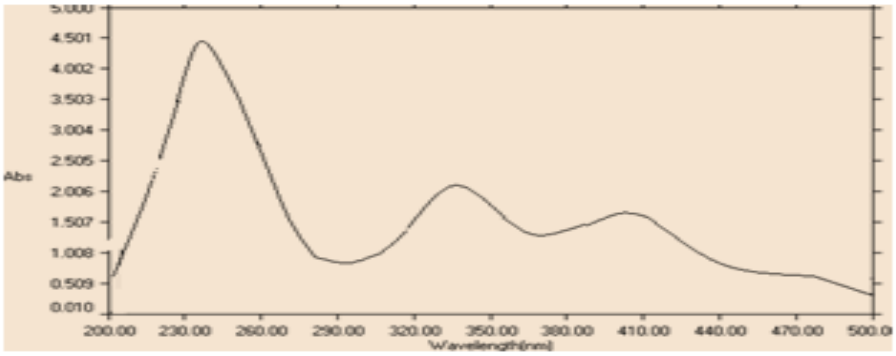


Fig. 3: Sodium methoxide spectrum of compound I.

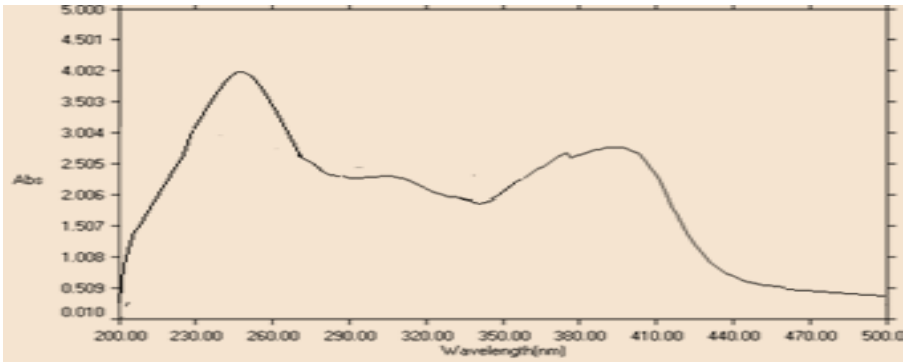


Fig 4: Sodium acetate spectrum of compound I.

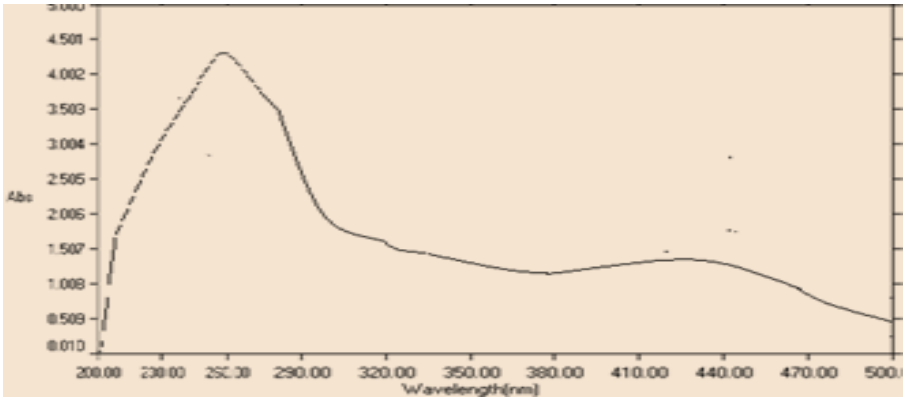


Fig .5: Aluminium chloride spectrum of compound I.

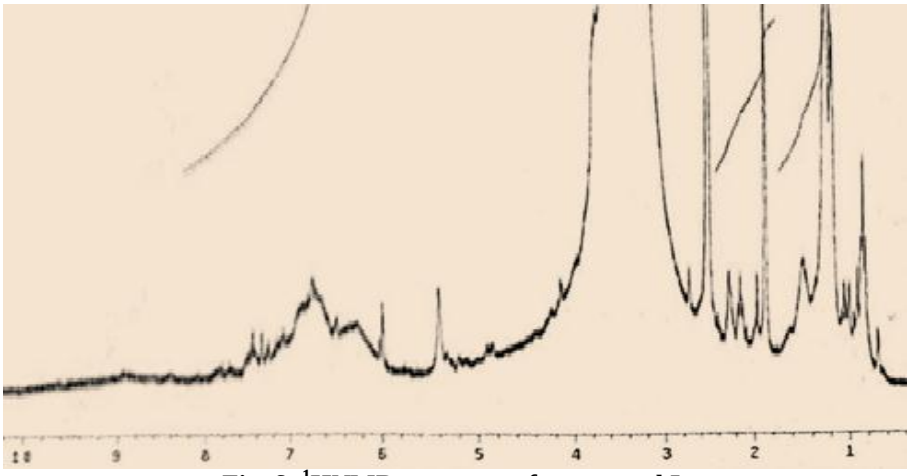


Fig. 6: ^{1}HNMR spectrum of compound I.

Antimicrobial activity

The crude ethanol extract of *Acacia tortilis* was screened for its antimicrobial activity against five standard microorganisms (Table 1). The results are depicted in Table (2). Results were interpreted in the following conventional terms: (< 9 : inactive; 9-12mm: partially active; 13-18mm: active; >18 : very active).

Table 1: Test organisms.

No.	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale. England
*ATCC. American type culture collection, Maryland, USA

Table 2: Inhibition zones (mm/mg sample).

Sample	Ec	Ps	Sa	Bs	Ca
Ethanol extract(100mg/ml)	14	12	15	15	11
Ampicilin(40mg/ml)	-	-	30	15	-
(20mg/ml)	-	-	25	14	-
(10mg/ml)	-	-	15	11	-
Gentamycin(40mg/ml)	22	21	19	25	-
(20mg/ml)	18	15	18	22	-
(10mg/ml)	15	12	14	17	-
Clotrimazole(30mg/ml)					38
(15mg/ml)					31
(7.5mg/ml)					29

Sa. *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Bs: *Bacillus subtilis*
Ca: *Candida albicans*

The ethanol extract exhibited good activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

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