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Isolation, characterization and biological activity of a flavone from seeds of the traditional Sudanese medicinal tree (*Acacia nilotica*)

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Abstract
Flavonoids are secondary metabolites compounds produced by plants which are found in a glycone or glycoside which attached to a sugar molecule, have basic structure consisting of a C_6-C_3-C_6 skeleton. *Acacia nilotica* is medicinal plant is used to treat some diseases such as cancer tumors, tuberculosis, smallpox, diabetes, cough, diarrhea, dysentery and leprosy. The seeds powder of *Acacia nilotica* were extracted by 95% ethanol flowed by TLC chromatography in developing solvent BAW (5:2:6; v: v: v) to give a product which was flowed by IR, UV, MS, NMR to identify compound 1.

Keywords: *Acacia nilotica*, flavonoids, phytochemical, *Bacillus subtitles*

Introduction
Flavonoids are formed in plants from the aromatic amino acids: phenyl alanine and tyrosine [1]. The basic structure consists of a C_6-C_3-C_6 skeleton contain two benzene rings connected by a linear 3-carbon chin. Flavonoids are secondary metabolites chemical compounds produced by plant and other lower organisms they are found in one of two form a glycone or glycoside form (attached to a sugar molecule) [2, 3]. Flavonoids compounds one of the most characteristic classes of compounds in higher plants. Many flavonoids are responsible of flower pigment in most families plant flowering [4]. However, their occurrence is not restricted to flower only but comprise all parts of the plants sap barks, stems, wood, root, heartwood, leaves, fruits and seeds. Some kinds of flavonoid compounds are more characteristic of specific tissues [5, 6].

Many type flavonoids are endowed with medicinal uses, such as anti-inflammatory, anti-allergic, anti-ischemic, and anti-platelet, immunomodulatory and anti-tumor activity [7, 8]. Flavonoids have protective effects against many contagions bacterial diseases, viral diseases and degenerative diseases [9]. The presence of flavonoids in foods and other compounds is thought to be one of the reasons for the beneficial influence on human health [10]. They have long been recognized in folk medicine [11].

Acacia nilotica is a big tree with height 5-20 m with a dense aspheric crown, have dark to black colored stem and branches, fissured bark, grey-pinkish slash, exuding a reddish low quality gum. The tree has thin, straight, grey spines in axillary pairs, always in 3 to 12 pairs, with long 5 to 7.5 cm in young trees, mature trees usually without thorns. Have bright golden-yellow color Flowers in globules head with dimeter 1.2-1.5 cm, set up either axillary or whory on peduncles 2-3 cm long located at the end of the branches [12]. *Acacia nilotica* produce timber, e.g. rail way sleepers, structural timber, boat building. *Acacia nilotica* makes a good protective hedge because of its thorn. The gum or bark is used to treatment many cancer tumors of eye, testicles, ear and indurations of spleen and liver. The root is used to treat tuberculosis and the smallpox is treatment by wood. The bark is taken for cough. It act as an astringent and it is used to treat dysentery, tuberculosis, leprosy, diarrhea, diabetics and smallpox [13].

Material and methods
Sample collection and preparation
The *acacia nilotica* seeds were collected in May 2018 from Khartoum State (Alsonot forest). The plant material was authenticated by the Department of Botany, University of Khartoum and a voucher sample was kept in the herbarium of this department.

Instruments
The UV Shimadzu 1601 Spectrophotometer was used to obtained spectra and UV lamp was used for determination of fluorescent spots on TLC. The IR spectrum was measured by using a Shimadzu (Perkin-Elmer 1310) Spectrophotometer. Nuclear Magnetic Resonance spectra were run on (EM-360-300 MHZ) NMR Spectrophotometer. Melting points were determined on a Kofler Hot-Stage Apparatus but the Mass spectra were recorded on (Finnigan-MAT SQ-700) Spectrometer.

Preliminary phytochemical screening
The ethanolic extract of the seeds of *acacia nilotica* was screened for flavonoids, tannins, saponin, steroids, alkaloids, glycosides and anthroquinone according to the methods described by Harborne (2001).

Isolation of flavonoids
(1 kg) seeds dried powdered of *acacia nilotica* were macerated at room temperature with 95% ethanol for (5 days). The solvent was evaporated under reduced pressure and the crude extract was dissolved in a pure methanol followed that applied on Whatman paper (No 3mm-46 x 57 cm). The bands were irrigated with BAW (n- butanol- acetic acid-water; 5:2:6: v:v:v). The developed chromatograms were air-dried and examined under both visible and UV light (ʎ366, 245 nm). The chromatograms were exposed to ammonia vapor for about 2-3 seconds and immediately re-examined to observe possible changes that may eventually appear in color or fluorescence under a long wavelength UV lamp. The equivalent bands from each paper were then cut out, combined and cut into small strips and slurred with methanol. After several hours of contact with occasional shaking, the liquid was filtered and evaporated under reduced pressure to ensure compound 1.

Anti-microbial Assay
The ethanolic fractions of *acacia nilotica* were tested for their antimicrobial activity against six stander human pathogens *Pseudomonas aeruginosa* (P.s), *Bacillus subtilis* (B.s), *Escherichia coli* (E.c), Staphylococcus *aureus* (S.a), *Condida albacans* (Ca), and *Aspergillus niger* (A.s).
Compound I was also screened for its antimicrobial activity.

Preparation of Bacterial Suspensions
The agar diffusion method was used. Sterile nutrient agar powder was prepared by dissolving 14 g of the agar powder in 500 ml distilled water, boiled to ensure complete dissolution and sterilized at 121 ºC for 15 minutes and dispensed into labelled petri dishes and allowed to gel. Wells were bored into the nutrient agar using a 4 mm sterile cock borer.

Preparation of Fungal Suspensions
Fungal cultures were maintained on saturated dextrose agar incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline, and finally suspended in (100 ml) of sterile normal saline, and the suspensions were stored in the refrigerator until used for tested.

Testing for Antibacterial Activity
0.2 g of crude extract was weighed into sample bottles and dissolve with 1 ml of its solvents, 9 ml sterile distilled water was added to obtain 20 mg/ml of the extract. And pure compounds was isolated using adjustable volume micro titer pipette, and allowed to diffuse at room temperature for 2 hours. The plates were then incubating in the upright position at 370 °C for 24 hours. After incubation, the diameter of the resultant growth inhibition zones were measured, and average.

Testing for Antifungal Activity
Fungal strains grown on nutrient agar at 37 °C for 24 hours were suspended in a saline solution (0.9% NaCl), but instead of nutrient agar saturated dextrose agar was used. 10 µl of the extract solution of Sample was used to inhibition zone around each disc was measured the Antifungal activity.

Result and discussion
Phytochemical screening
The result of the preliminary phytochemical screening showed in table (1) that *acacia nilotica* contains flavonoids, tannins, saponin, alkaloids, and anthraquinone and absent of steroid, triterpens and glucosides.

Table 1: Phytochemical screening of *acacia nilotica* leaf extract

Compound	*Acacia nilotica* results
Anthraquinones	+ve
Tannins	+ve
Saponins	+ve
Triterpenes	-ve
Steroids	-ve
Glycoside	- ve
Alkaloids	+ve
Flavonoids	+ve

Identification of compound I
Compound I was isolated from ethanolic extract of *acacia nilotica* as brown color powder. The IR spectrum of compound I was obtained bands at ν (KBr): 3223cm-1 (OH), 2835.8, (C-H, alkane), 1544.81 (C = O), 15324, 1452 (C = C, aromatic), 1142.50 (C-O, ether), and 1054.29(C – O, phenolic). Since the IR revealed a C = O function, hence compound I cannot be an anthocyanin or catechin. These classes are devoid of such function.
The UV spectrum in fig (1) showed λ_{max} (MeOH) 265,335 nm. The occurrence of both band I (due to cinnamoyl system) but band II (due to benzoyl system) is a characteristic feature one of: flavonols, flavones, aurones and chalcones are shown in fig (2).

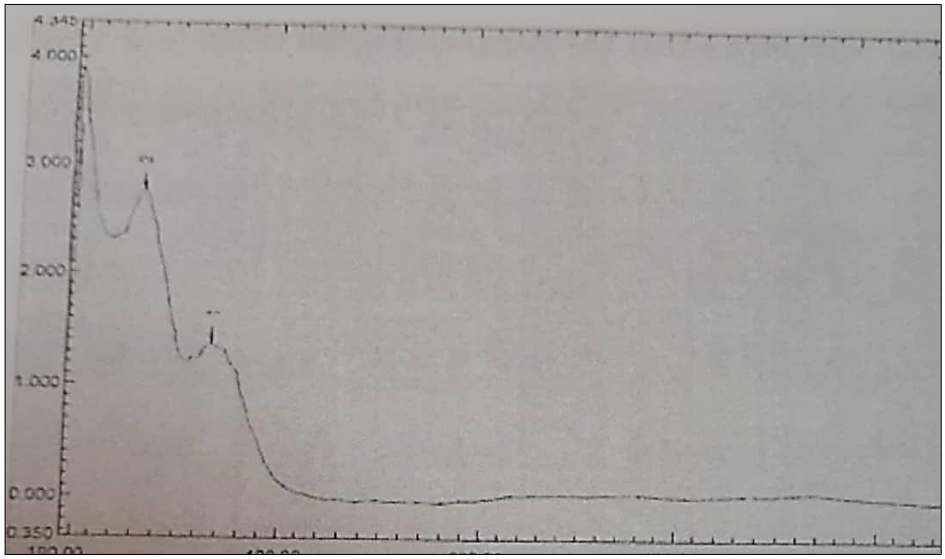
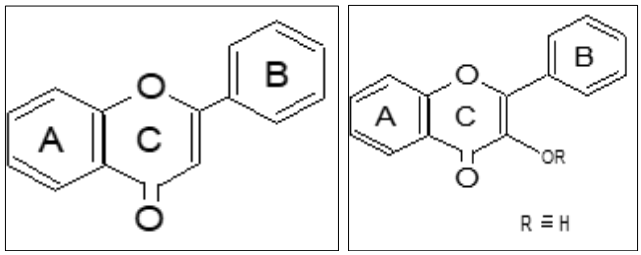
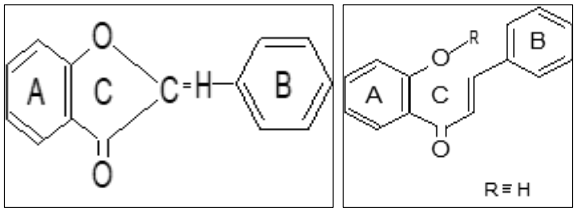


Fig 1: UV spectrum of compound 1



Flavone Flavonol



Aurone Chalcone

Fig 2: Some class of flavonoids

Chalcones and aurones exhibit band I in the ranges: 365-390 and 390-430 nm respectively, while flavones and flavonols absorb in the ranges: 320-350 and 350-390 respectively. Thus compound I is probably a flavone.

Very useful structural features are acquired by employing the some reagents called UV-shift reagents: sodium methoxide, sodium acetate, boric acid and aluminum chloride. Addition of NaOMe to a methanolic solution of compound I did not appear any bathochromic shift in band II and this is diagnostic absence of free 4`-OH show fig(3). No showable bathochromic shifts were observed in the boric acid and sodium acetate. This is indicates absence of a 7- OH subsequently absence of catechol systems.

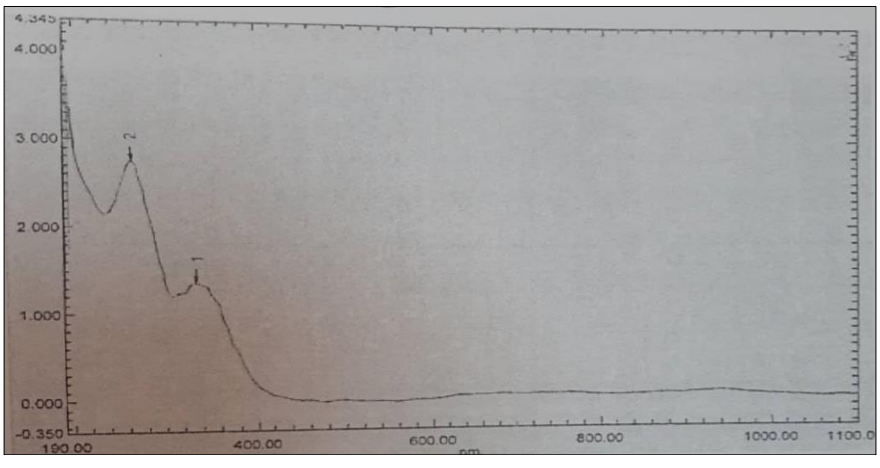
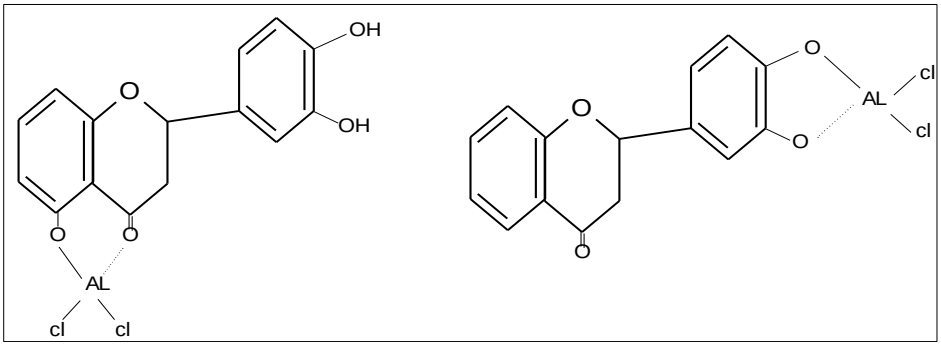


Fig 3: UV of compound 1 and sodium methoxide

Aluminum chloride ($AlCl_3$) is a useful complexing agent for the appeared detection of 3-, 5-OH or dihydroxyl systems. The complexes formed by ($ALCl_3$) and flavonoid with 3- OH (or 5-OH) group or catechol systems are shown below:



5.4 complex 3-, 4-complex

Fig 4: Aluminum chloride conforming complex with catechol

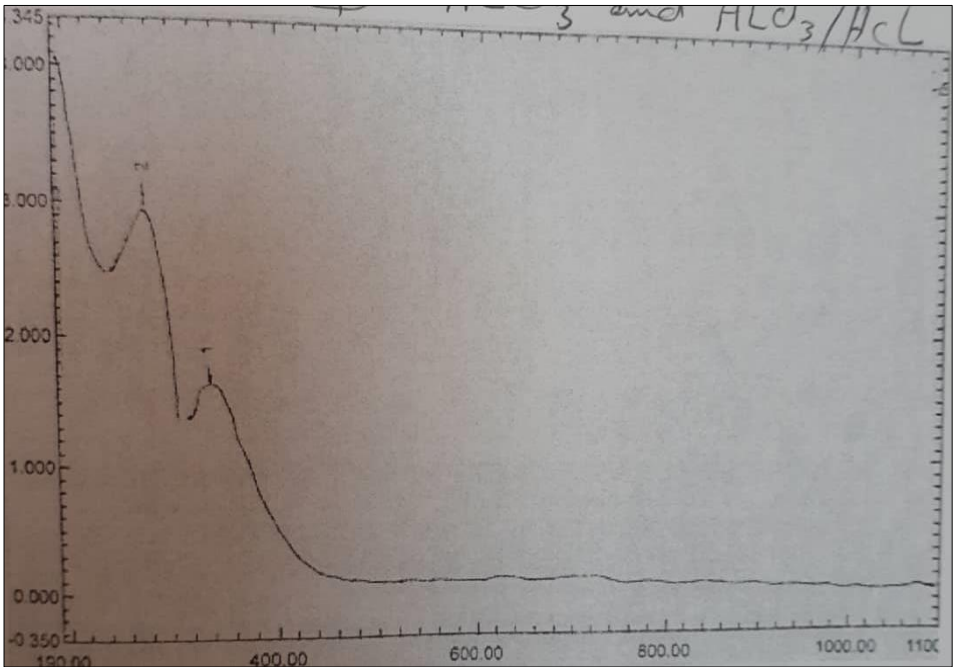


Fig 5: UV spectrum of compound 1 and Aluminum chloride

Distinction between these two types of complexes could be made by the added the solution of (HCl), the presence of (HCl) where the catechol complexes are unstable.

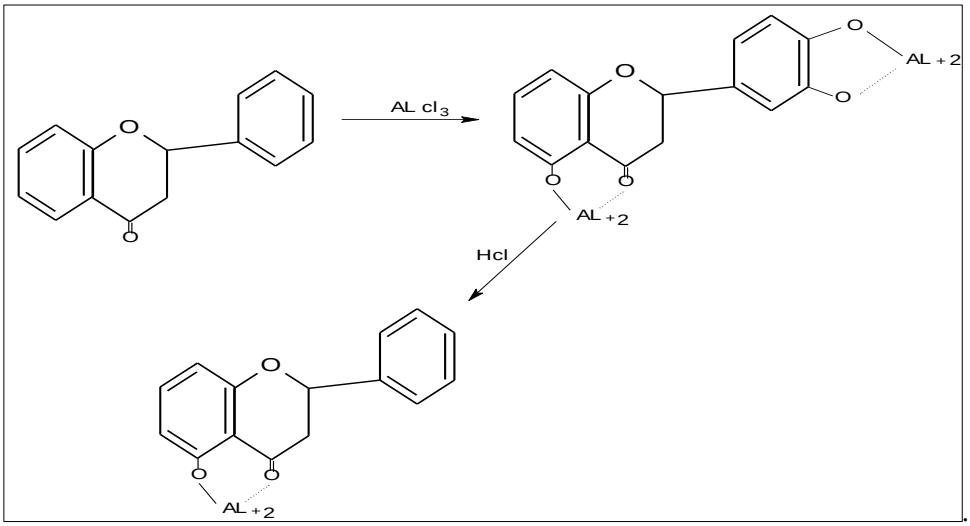


Fig 6: Reaction Aluminum chloride with HCl without catechol

The aluminum chloride spectrum did not showed any detectable bathochromic shift indicating absence of 5- OH and catechol moieties [14].

The ^{1}HNMR spectrum appeared signals at: δ0.82 assigned for methyl group, δ3.52 (3H), δ 3.4(3H) and δ3.76 ppm were assigned to three methoxyl group, Aromatic protons appeared at δ 7.4 and δ8.2 ppm.

The mass spectrum recorded m\z 330 (M^{+} +2) for the aglycone. Other important fragments corresponding to undamaged aromatic rings were shown at m\z 180(ring A) and m\z148 (ring B).

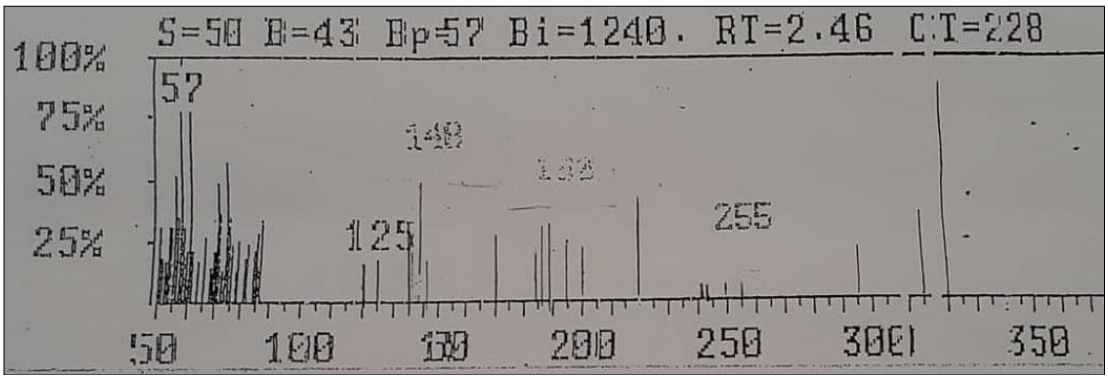
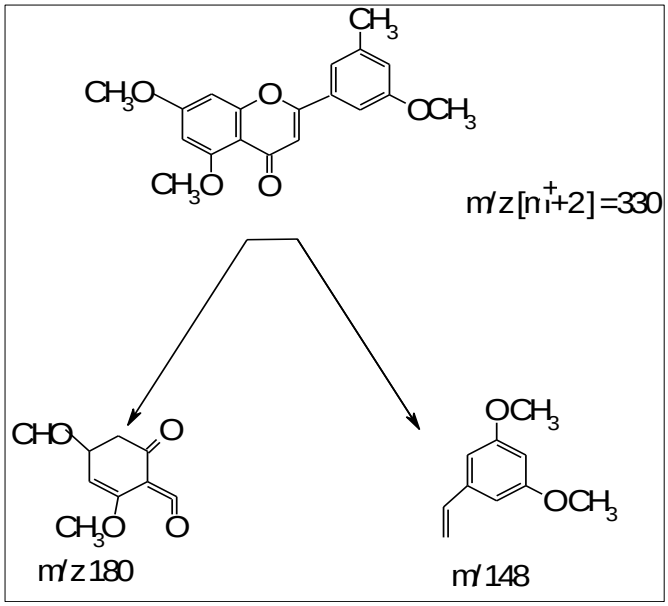


Fig 7: Mass spectroscopy of compound 1

The assignment of the substituent in rings A and B was based on the retro- Diels- Alder fission of scheme I, where the Diels-Alder fragments m/z 148 and m/z 180 were measured in the electron beam.



Scheme I: Retro Diels-Alder fission of compound I

The citation of two methoxyl groups at position 5 and 7 of ring (A) was based on 1H-1H COSY NMR experimental which indicated long range coupling between a methoxyl function and C_6 - H, and another long range coupling between a methoxyl group and the protons at $C_6$and C_8. The third methoxyl function was assigned position 5′ due to long range coupling between a methoxyl group and C_4′-H. A long range coupling between a methyl group and the C_2′ proton suggests methylation at position 3′.

Antimicrobial Activity

To locate the antimicrobial activity of the ethanol extract of *acacia nilotica* and the pure flavonoid isolated from this species against six standard organisms, the cup- plate agar diffusion method was adopted with some modifications. The test organisms were*: Bacillus subtilis* (B.s), *Pseudomonas aeruginosa* (P.s), *Escherichia coli* (E.c), *Klebsiella pneumoniae* (K), *Sallomenatyphic* (S.t), *Aspergillus niger* (A.s) *and Condida albacans* (C.a). The ethanolic extract of the plants to appeared high activity against all organism and also pure compound (I) showed high activity against all test organisms except *Pseudomonas aeruginosa* to give medium activity all these were included in (table 2):

Table 2: The antimicrobial activity crude extractives of *Acacia nilotica* and pure compound I

Organisms	Inhibition growth zone diameter (MIZD) 100 mg\100 ml			
	Crude extract	Compound 1	Ciprofloxacin	NY statin
Bacillus subtitles	24	20	50	-
Pseudomonas aeruginosa	20	18	25	-
Pseudomonas aeruginosa	20	18	25	-
Staphylococcus aureus	22	20	50	-
Aspergillus niger	22	19	-	100
Candida albicans	21	19	-	100

Activity: 10-14 mm→activity is weak, 14-18 mm→activity is medium, 18 - up→activity is higher.

Conclusions

The present study of the ethanolic fraction of *acacia nilotica* seeds indicated that plant contains compounds have biological activity. The feature of these compounds probably contribute to the pharmacological properties and traditional medicinal uses of *Acacia nilotica*. Further isolation and characterization of compound present in it may gain new compounds have biological activity, which can be used to procure compounds in future.

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Conflict of interest: There is no conflict of interest.

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