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# Isolation and Characterization of a Dihydrochalcone from Roots of Sudanese *Acacia nilotica* subspecies *tomentoza* (Linn.) Willd. ex Del. (Fabacea)

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**Abstract**
Information on the constituents of medicinal plants used in Sudanese system of medicine is very scarce. Hence, this study was set to investigate the flavonoids of Sudanese *Acacia nilotica* subspecies *tomentoza* which is a key species in indigenous medicine. A dihydrochalcone was isolated from roots using a combination of chromatographic techniques and its structure was partially elucidated on the basis of its spectral data (UV, $^1$HNMR and MS). The antimicrobial activity of compound I was evaluated and significant activity was observed.

**Keywords:** *acacia nilotica*, isolation, flavone, partial structure

## Introduction

Over centuries and medicineral plants are employed in primary health care and many modern medicines are of plant origin. In developing countries, where modern medicines are beyond affordability, different communities extensively use herbs to fight diseases. Recently extensive studies have been carried out to document the constituents of many key species in herbal medicine and to study their pharmacological effects, such research could result in leads necessary for drug design and drug discovery and could lend a rationale for the ethnomedical uses of these herbs.

*Acacia* is a large genus of trees and herbs in the subfamily Mimosoideae of the family Fabacea. Many species of this genus are used in traditional medicine and many *Acacia* species are globally planted for their economic importance including *Acacia mangium, Acacia mearnsii, Acacia saligna* which are considered as sources of wood products, tannins and firewoods [1].

*Acacia nilotica* (Linn.) Willd. ex Del. Is a legume tree widely distributed in Sudan. It can tolerate air dryness and temperatures exceeding 50ºC [2]. The antimicrobial activity of *Acacia nilotica* has thoroughly been investigated [3-9]. *Acacia nilotica* which is rich in polphenolics showed significant radical scavenging capacity in the DPPH assay [10]. The free radical scavenging, cytotoxic and hemolytic activities of ethyl gallate isolated from leaves were evaluated [11]. Also it was testified that the root extract was active, *in vivo*, against *Plasmodium bergei* and *Plasmodium falciparum*[12].The antiplasmodial properties of this species has also been reported by Tahir *et al*. [13]. It has been reported that powdered bark is extremely useful for diarrhea [14]. Also in caster oil-induced model, the acetone fraction showed significant antidiarrhoeal activity [15]. In some studies, the molluscicidal activity of *Acacia nilotica* has been documented [16-18]. In animal model studies, seed aqueous extract exhibited spasmogenic properties [19].

## Materials and Methods

### Materials

#### Plant material

The roots of *Acacia nilotica* subspecies *tomentoza* were collected from Khartoum, Sudan. The plant was identified and authenticated by the Institute of Aromatic and Medicinal Plants, Khartoum, Sudan.

#### Solvents

All solvents used are of analytical grade. Methanol HPLC grade was used for spectroscopic purposes (BDH, England).

#### Instruments

In thin layer chromatography, the ultraviolet lamp used in visualizing plates was a multiband UV $\lambda_{max}$ (254 / 365 nm) portable ultraviolet lamp, a product of Hanover lamps (6 watt S/Y and L/W). Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV -Visible Spectrophotometer (Shimadzu).

The electron impact ionization (EIMS) mass spectra were obtained on a solid probe using Shimadzu QP-class-500. $^1$HNMR spectra were run on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-$d_6$. The chemical shifts values are expressed in δ (ppm) units using (TMS) as an internal standard and the coupling constants (J) are expressed in Hertz (Hz).

### Methods

#### Isolation of Flavonoids

Powdered shade- dried roots of *Acacia nilotica* were macerated with 95% ethanol at room temperature for 48 hours. The ethanol extract was rich in phenolics. The extract (3g) was mounted on top of a silica gel column(85x4cm) and the column was eluted successively with chloroform: methanol (4:1; 3:2 and 1:4, v:v). The fraction: chloroform:

methanol (1:4) was rich in phenolics. It was fractionated on silica gel TLC developed with chloroform: metahano (1:4, v:v). The plates were air-dried and examined under both visible and UV light ($\lambda_{max}$ 366,245nm). The equivalent bands from each plate were then combined and slurred with methanol. The solvent was evaporated *in vacuo* to afford a flavonoid- compound I- in a chromatographically pure form.

**Antimicrobial Assay**

The isolated flavonoid was screened for antimicrobial activity against four human pathogenic bacterial strains: Gram-positive (*Staphylococus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and fungal species *Candida albicans*.

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline solution to produce a suspension containing about $10^8$- $10^9$ C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.

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 for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

**Preparation of fungal suspension**

The fungal cultures were maintained on 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 was stored in the refrigerator until used.

**Testing of antibacterial susceptibility**

The antibacterial activity of compound I was performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to $10^8$cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with test solution. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were then measured.

**Testing of antifungal susceptibility**

The above mentioned method was adopted for antifungal activity, but instead of agar, dextrose agar was used.

**Results and Discussion**

From the roots of *Acacia nilotica* a flavonoid-compound I- was isolated via a combination of chromatographic techniques. The structure of this isolate was elucidated using spectral tools. The potential antimicrobial activity of this flavonoid was evaluated.

The UV spectrum of flavonoids is an important feature regarding the classification of flavonoids. In flavones, flavonols, chacones and aurones there is conjugation between the C=O function and ring B, hence these compounds give two UV peaks, band II due to benzoyl system and band I due to cinnamoyl. The other groups of flavonoids (flavanones, dihydroflavonols, isoflavones, and dihydrochalcones) give only band II due to loss of conjugation between the carbonyl function and ring B. In UV, compound I absorps at $\lambda_{max}$ 250 nm (Fig1).

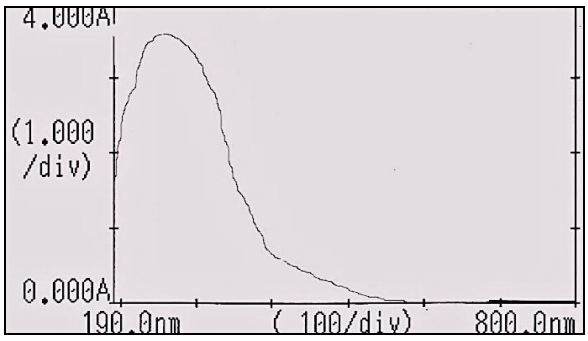



**Fig 1:** UV spectrum of compound I

Such UV absorption is usually given by: isoflavones, flavanones dihydroflavonols and dihydrochalcones. However, no shoulder characteristic of isoflavones (in the 300-340 nm region) was observed in the UV spectrum (Fig1).

Addition of the UV shift reagent- sodium methoxide- to a methanolic solution of compound I gave no bathochromic shift (Fig.2). And this indicates absence of 3- and 4`- OH groups and indicates absence of dihydroflavonols which are characterized by a 3-OH function.

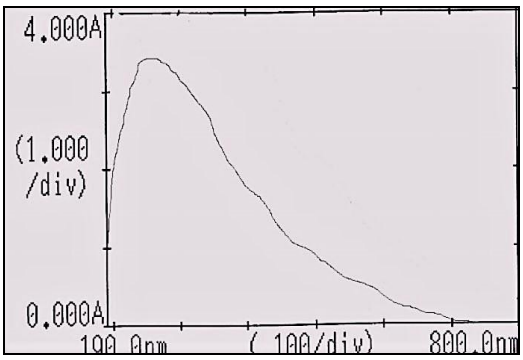



**Fig 2:** Sodium methoxide spectrum of compound I

Flavanones and dihydrochalcones are distinguished by [1]HNMR. Flavanones give a double quartet for the magnetically unequivalent protouns of $C_3$ which split each other into a double doublet. These doublets are further split by $C_2$-H into a pair of quartetes (usually overlapping into multiples) near δ2.80 and δ5.20 ppm. However, no multipltets at δ2.8 and δ5.2 were observed in the [1] HNMR spectrum (Fig.). Thus this isolate is a dihydrochalcone.

Next the hydroxylation pattern of the isolated dihydrochalcone was investigated via various UV shift reagents: sodium acetate, aluminium chloride and boric acid. The sodium acetate spectrum (Fig.3) showed a bathochromic shift diagnostic of a 7-OH function. However, the auminium chloride(Fig.4) and boric acid(Fig.5) spectra did not reveal any detectable bathochromic shifts indicating absence of 3-, 5-OH as well as catechol systems.

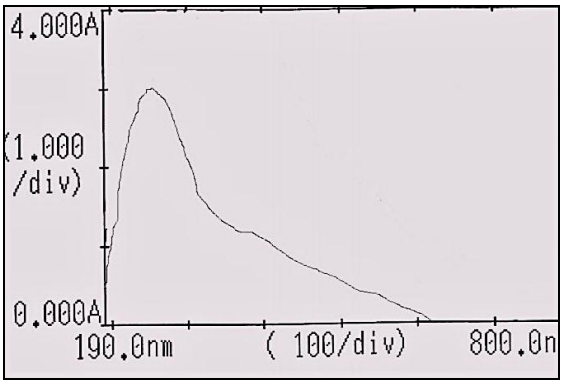



**Fig 3:** Sodium acetate spectrum of compound I

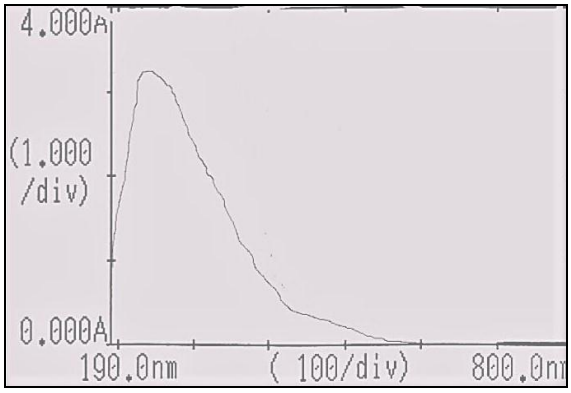



**Fig 4:** Aluminium chloride spectrum of compound I

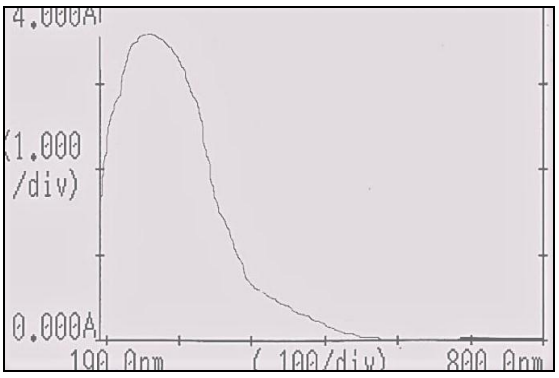
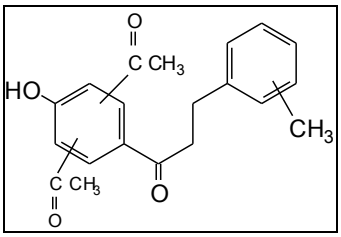



**Fig 5:** Boric acid spectrum of compound I

The [1]HNMR spectrum (Fig. 6) showed: δ1.21(3H) ppm assigned for a methyl group, δ1.66(6H) ppm accounting for two acetyl functions, δ3.00-4.20 ppm assigned for a sugar moiety which was not identified in this study. The sugar anomeric proton resonated well downfield relative to the bulk of other sugar protons at δ5.40ppm.The resonances at δ6.62 (1H) and δ6.95 (1H) account for $C_6$ –H and $C_8$-proton. Due to the deshielding influence of the oxygen bridge at position 1, the $C_8$ - proton usually resonates at lower field relative to $C_6$ – proton. Other aromatic protons appeared at δ7.68 and δ8.41ppm.The mass spectrum (Fig. 7) gave m/z322 for ($M^+$-2H).

On the basis of the above spectral data, the following partial structure was proposed for the aglycone of compound I:



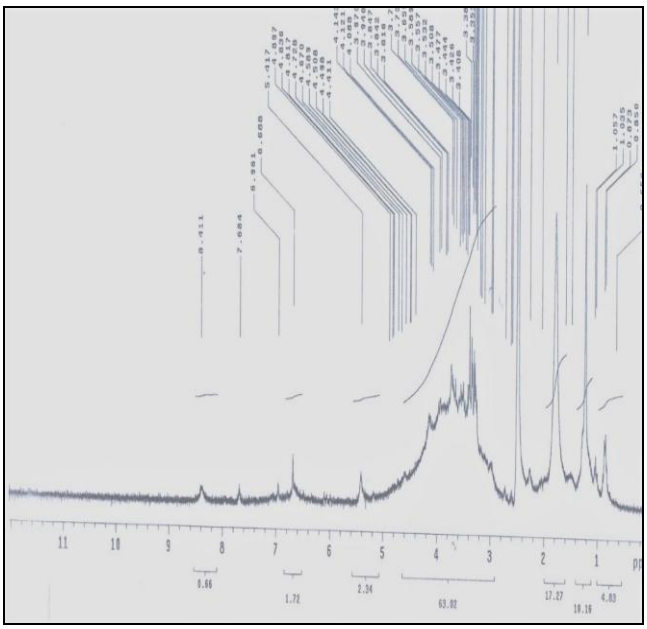



**Fig 6:** H[1]NMR spectrum of compound III

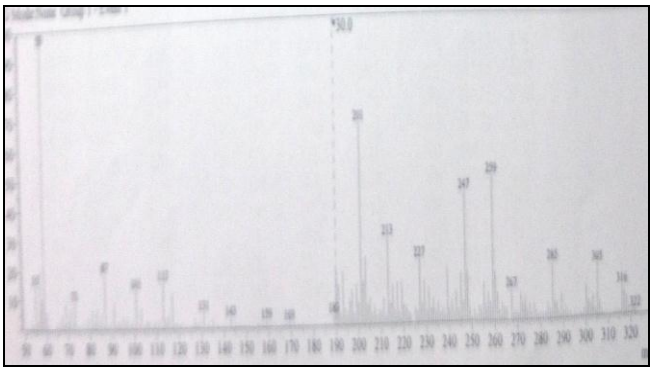
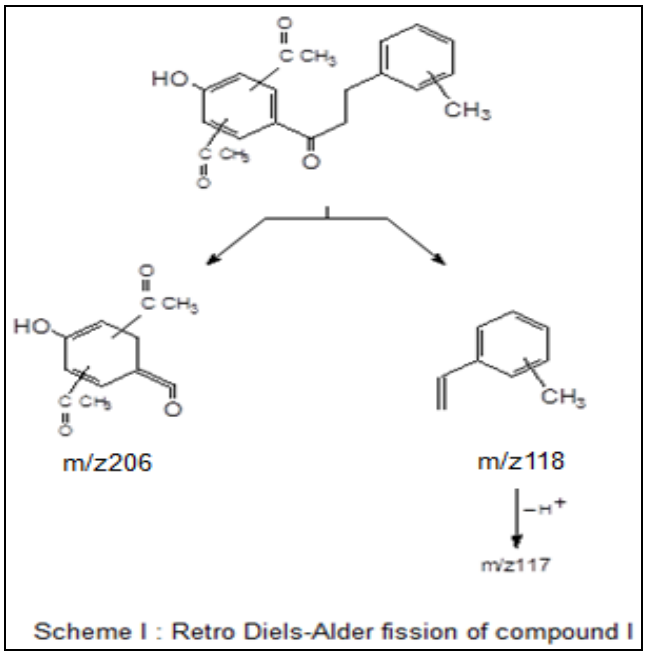


**Fig 7:** Mass spectrum of compound I

The retro Diels-Alder fission of compound I gave m/z220 and m/z118 for intact A and B aromatic rings, thus lending some evidence for the proposed substitution pattern.

**Antimicrobial Activity**

Compound was screened for antimicrobial activity via the cup plate agar diffusion bioassay. The diameters of inhibition zones are displayed in Table (1).




Scheme I : Retro Diels-Alder fission of compound I

Results were interpreted in conventional terms: (<9mm: inative; 9-12mm: partially active;13-18mm: active; >18mm: very active). Tables (2) and (3) represents the antibacterial and antifungal activities of standard drugs respectively.

**Table 1:** Antimicrobial activity of compound I

| Type | Conc.(mg/ml) | Sa | Bs | Ec | Pa | Ca |
|---|---|---|---|---|---|---|
| Comp. I | 100 | 20 | 17 | 19 | 18 | 20 |

Compound I showed significant antimicrobial activity against all test microorganisms at 100mg/ml.

**Table 2:** Antibacterial activity of standard drugs

| Drug | Conc(mg/ml) | Bs | Sa | Ec | Ps |
|---|---|---|---|---|---|
| Ampicilin | 40 | 15 | 30 | | |
| | 20 | 14 | 25 | | |
| | 10 | 11 | 15 | | |
| Gentamycine | 40 | 25 | 19 | 22 | 21 |
| | 20 | 22 | 18 | 18 | 15 |
| | 10 | 17 | 15 | 15 | 12 |

**Table 3:** Antifungal activity of standard drug

| Drug | Conc.(mg/ml) | An | Ca |
|---|---|---|---|
| Clotramizole | 30 | 22 | 38 |
| | 15 | 17 | 31 |
| | 7.5 | 16 | 29 |

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

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