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Sudanese *Ziziphus abyssinica* Hochst: Isolation, partial characterization of a flavone and antimicrobial potential

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Abstract
This study was aimed to investigate the major flavonoid of *Ziziphus abyssinica* root and to screen the antimicrobial activity of root ethanol extract. The flavonoids were extracted with aqueous ethanol and the crude extract was purified by paper chromatography where a flavonoid (compound I) was isolated. The structure of this compound has been partially characterized by some spectral tools (UV and ^{1}HNMR). In the antimicrobial assay, the root ethanol extract showed moderate activity against *Staphylococcus aureus* and the yeast *Candida albicans*. The extract also exhibited partial activity against other test organisms(. *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*).

Keywords: *Ziziphus abyssinica*, flavonoid, isolation, antimicrobial activity

Introduction
In developing countries phytotherapy is still playing an important role in primary health care where modern medicines are beyond affordability. For decades, medicinal plants offered potential leads for drug design and drug development. Bioactive plant molecules such as the flavonoids are endowed with a wide range of activities against a panel of bacteria, fungi and several enzymes.
Ziziphus abyssinica Hochst. ex A. Rich is a tree in the family Rhamnaceae that may reach 8m in height [1]. This plant is widely used in African system of medicine. leaves are used against tonsillitis, pneumonia, burns, wounds, tachycardia, pectoral pain and snake bite [7-10]. Some extracts of *Ziziphus abyssinica* were shown to possess antimicrobial, antioxidant, antiulcerative, anti-diarrheal, antiplasmodial and molluscicidal properties [11-17].
In continuation of our interest in the constituents of plants used in Sudanese system of medicine, this study was designed to investigate the flavonoids of the medicinally important *Ziziphus abyssinica*.

Materials and Methods
Plant material
Roots of *Ziziphus abyssinica* were collected from the premises Nyala western Sudan. The plant was identified and authenticated by The Medicinal and Aromatic Plants Research Institute (Sudan). The plant material was shade - dried at room temperature and finally powdered.

Microorganisms
Gram +ve: *Bacillus subtilis and Staphylococcus aureus.*
Gram –ve: *Escherichia coli, Pseudomonas aeruginosa*
Fungal strains: *Candida albicans*
Media for bacterial growth: Mueller Hinton agar
Media for fungal growth: Sabouraud dextrose agar (Oxoid, England) m/Liter
Equipments: A Shimadzu UV spectrophotometer -model UV240) was used for UV measurements; The ^{1}HNMR spectrum was obtained on a Joel- Nuclear Magnetic Resonance (NMR) spectrophotometer, (Brucker AC-250) operating at 500 MHz.

Solvents
Analytical grade solvents were used. Methanol used for spectrophotometric analysis was purchased from Merck, Germany. DMSO-d_6 was used as solvent and TMS as internal standard.

Methods
Extraction and isolation of flavonoids
Powdered plant material (1Kg) was macerated with 95% ethanol for 72h. at room temperature. The extract was filtered and the solvent was removed *in vacuo*. The dried extract of *Ziziphus abyssinica* root was applied on What man 3mm paper (46×57 cm) as narrow strips and run in BAW(6:1:5, v:v:v).After the usual workup, a chromatographically pure flavonoid(compound I) was isolated.

Antimicrobial activity
The antimicrobial activity was evaluated using well diffusion bioassay. An inoculum suspension was swabbed uniformly to solidify and then allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar. Aliquots of test sample (100 mg/ml) were added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37^{o}C for 24 h-for bacteria – and for three days at 25^{o}C for fungi. The resulting inhibition zones were measured in millimeters (mm). The assay was performed in duplicate.

Results and Discussion
Ethanolic extract of *Ziziphus abyssinica* root was purified via paper chromatography to give a flavonoid - compound I.

In the UV most flavonoids exhibit two major absorption bands appearing at: 230-290 nm (called band II) and the other in the range 300-400nm (called band I). The appearance of both bands indicates conjugation between the carbonyl function of the flavonoid and its aromatic (B) ring. The following flavonoids exhibit both bands: flavones, flavonols, chalcones and aurones. However, the appearance of a single absorption band (band II) is a distinctive feature of those flavonoids which are saturated at $C_2 – C_3$ i.e. the flavanones, isoflavones, dihydroflavonols and dihydrochalcones [18-20].

The UV spectrum of compound I (Fig.1) showed λ_{max}(MeOH) 274 nm. Such absorption is given by: the flavanones, isoflavones, dihydroflavonols and dihydrochalcones. Isoflavones are easily distinguished [18, 20] by a shoulder in the range: 300-340nm. Such shoulder was not detected in the UV spectrum of compound I. Also the UV shift reagent – sodium methoxide which is diagnostic [18, 20] of 3-and 4`-OH groups – revealed (Fig.2) only a small bathochromic shift indicating absence of a 3-OH function which is a characteristic feature of dihydroflavonols. The ^{1}HNMR spectrum (Fig.5) showed a multiplet around 2.80ppm and signals in the range δ5.4-5.65ppm accounting [19, 20] for the resonances of C_2 and C_3 protons of flavanones.

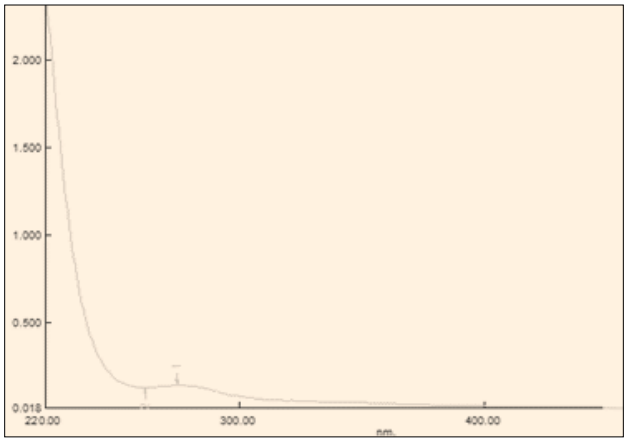


Fig 1: UV spectrum of compound A

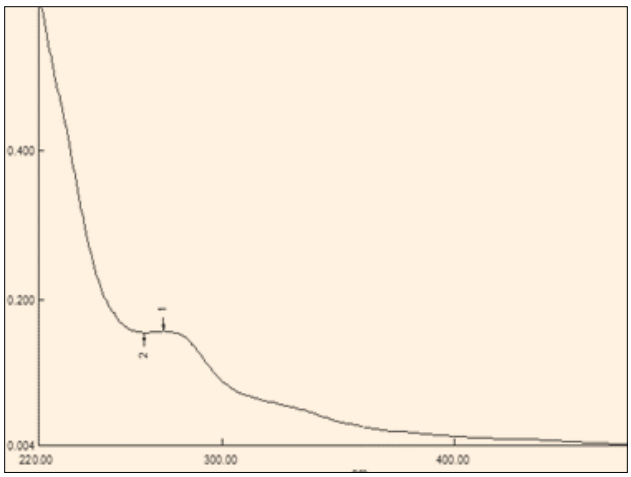


Fig 2: Sodium methoxide spectrum of compound I

Very useful structural features are gained by using different UV shift reagents such as sodium acetate and aluminum chloride. Sodium acetate is diagnostic of a 7-OH where a bathochromic is observed when the sodium acetate spectrum is recorded. No bathochromic shift was observed in the sodium acetate spectrum of compound I (Fig.3) indicating absence of a 7 – OH function.

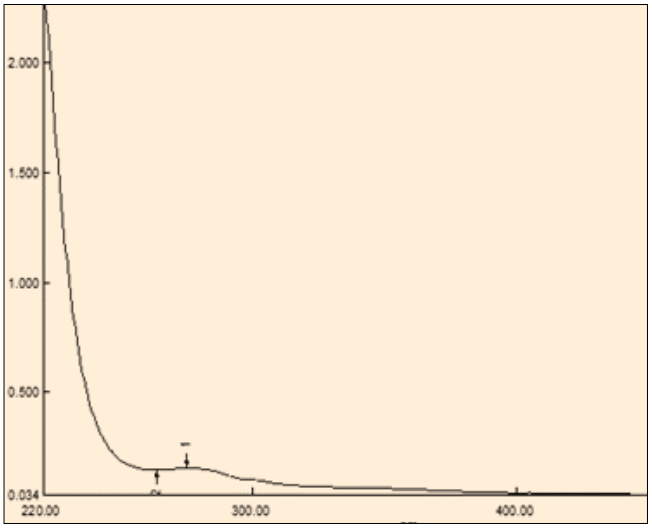


Fig 3: Sodium acetate spectrum of compound I

In the chemistry of flavonoids, aluminum chloride is an extremely useful complexing agent which afford diagnostic bathochromic shifts characteristic of: 3-, 5-OH as well as catechol systems. The aluminum chloride spectrum (Fig.4) failed to reveal a bathochromic shift indicating absence of 3-, 5- OH groups and catechol systems.

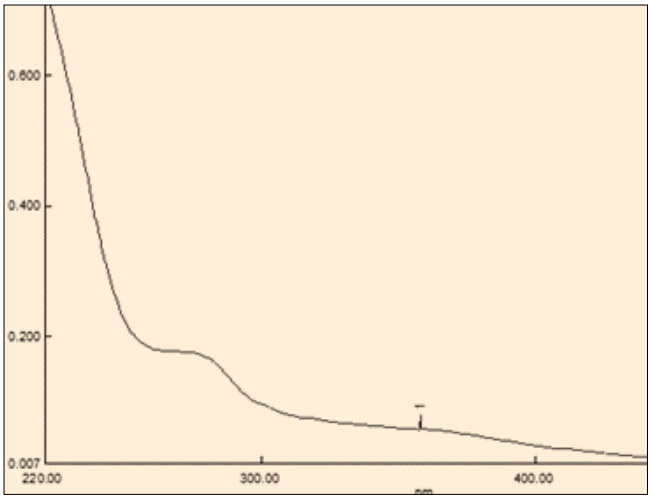


Fig 4: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum of compound I (Fig.5) gave δ(ppm): 1.22(assigned for two methyl groups); 4.11(methoxyl); 5.85(Ar. proton). Other aromatic protons resonated as multiplet in the range δ (6.62-6.90). Signals at δ2.50 and 3.30 are due to solvent (DMSO) residual protons and residual water respectively.

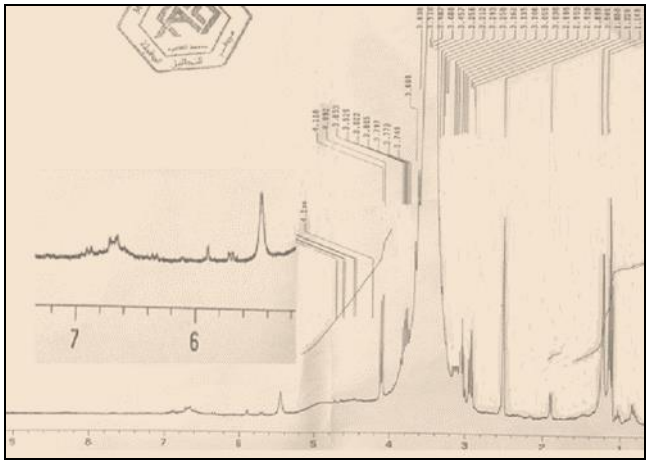
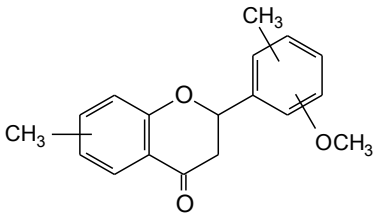


Fig 5: [1]HNMR spectrum of compound I

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



Compound I

Antimicrobial assay

The ethanol extract of *Ziziphus abyssinica* was evaluated for antimicrobial activity against five standard pathogenic bacteria. The results of Table (1) showed moderate activity against *Staphylococcus aureus* and the yeast *Candida albicans*. The extract exhibited partial activity against other test organisms. (*Pseudomonas aeruginosa*, *Bacillus subtiles* and, *Escherichia coli*). Ampicilin, gentamycin and clotrimazole were used as positive controls, while DMSO has been used as negative control.

Table 1: Antimicrobial activity of *Ziziphus abyssinica* ethanol exract

Organism	Inhibition growth zone diameter (MIZD)
	Root extract (100mg/ml)
Bacillus subtilis	12
Staphylococcus aureus	14
Escherichia coli	10
Pseudomonas aeruginosa	12
Candida albicans	13
Organism	Ampicilin (40mg/ml)
Bacillus subtiles	15
Staphylococcus aureus	30
Organism	Gentamycin(40mg/ml)
Escherichia coli	22
Pseudomonas aeruginosa	21
Organism	Clotrimazole(30mg/ml)
Aspergillus niger	22
Candida albicans	38

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