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RESEARCH ARTICLE

Isolation, Characterization and Antimicrobial Activity of a Flavanone from Sudanse *Albizia amara* (Fabaceae) leaves

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Abstract

Phytochemical screening of ethanolic extract of *Albizia amara* leaves revealed the presence of flavonoids, glycosides, alkaloids, saponins and tannins. A flavanone: 5-methoxy-3`, 4`, 5`- trimethylflavanone was isolated from the leaves and its structure was deduced on the basis of its spectral data(IR,UV,^{1}HNMR and MS). The isolate was evaluated for its antimicrobial activity against six standard human pathogens : two Gram positive(*Staphylococus aureus* and *Bacillus subtilis*) , two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria and two fungal species **(***Aspergillus niger*, *Candida albicans*) and promising results suggested that the flavanone is a plausible candidate for further optimization.

Introduction:-

Medicinal plants are widely spread in Sudan and their biological and phytochemical properties are not thoroughly evaluated. *Albizia Amara*(Fabaceae) , known locally as Arrad , is a deciduous tree, often rounded or spreading crown, reaching 10 m in height but often smaller. Bark dark brown and roughly cracked. Leaves compound with numerous small leaflets. Leaves and twigs are covered with distinctive soft golden hair. Seeds of *Albizia amara* are regarded as astringent, and used in the treatment of piles and diarrhea(Karuppannan *et.al.* ,2013**)** . The leaves and flowers have been applied to boils, eruptions, and swellings. They are also regarded as an emetic and as a remedy for cough, ulcer, wounds dandruff and malaria(Yadava and Reddy,2001; Woongchon *et.al.*,1991; Dharani**,**2007).Preliminary phytochemical screening of leave extract revealed the presence of flavonoids.

Flavonoids are a group of polyphenolic compounds that are widely distributed in the plant kingdom. They occur naturally as plant pigments in a broad range of fruits and vegetables as well as beverages(Orwa *et.al.*,2009; Joule and Smith,1972) . Flavonoids are phenolics comprising 15 carbons, with two aromatic rings bound together by three carbon atoms that form an oxygenated heterocyclic ring (C_6-C_3-C_6). They are found throughout the plant kingdom and in particular in leaves and in the skin of fruits. Based on the variation of their heterocyclic ring , flavonoids are divided into different sub-classes: anthocyanidins, flavans, flavanones, flavones, flavonols, isoflavones, dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones and aurones. The basic C_6-C_3-C_6

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flavonoid skeleton can have numerous substituents (e.g. hydroxyl, acetyl, methoxyl and methyl groups) and the majority of the flavonoids exist naturally as glycosides(Crozier *et.al.*,2009; Proestos *et.al*.,2006)
Flavonoids have gained recent interest because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological effects including antimicrobial(Yenjai *et.al*.,2004), cytotoxicity(Furuta *et.al*.,2004) , anti-inflammatory(Xia *et.al*.,2000) as well as antitumor activities(Aura *et.al*.,2005; Graf *et.al*.,2005). Flavanones exhibit anti-oxidant, immunomodulatory, chemopreventive and anticancer properties(Harborne ,2001). Flavanones with a hydroxyl functions at $C_{4'}$ and C_6 have shown significant cytotoxic effects against tumor cells, compared with other structurally related flavanones. The hydroxylation at C_6 plays an important role in antioxidant activity of flavanones(Ko *et.al*.,2004**)** .

Materials and Methods:-

Plant material:-
The leaves of *Albizia amara* were collected in April 2015 from a forest reserve around "Maleet" -western Sudan.The plant was kindly authenticated by the Dept. of Botany , University of Khartoum.

Extraction of plant constituents:-
Powdered shade- dried leaves (1kg) of *Albizia amara* were exhaustively macerated with 95% ethanol at room temperature for 48 hours, The solvent was removed under reduced pressure to afford a solid. The crude extract was suspended in water and partitioned with organic solvents in order of increasing polarity: petroleum ether, chloroform ethyl acetate and n-butanol.

Preliminary phytochemical screening:-
The ethanolic extract of *Albizia amara* leaves was screened for steroids, alkaloids, flavonoids, glycosides, saponins and tannins according to the method described by Harborne(2001).

Isolation and characterization of flavonoids:-
The n-butanol fraction *Albizia amara* leaves was rich in phenolics. It was dissolved in methanol and applied on Whatman papers (No. 3 mm ; 46x 57cm). The papers were irrigated with: BAW (n-butanol:acetic acid:water-4:1:5;v:v:v). The developed chromatograms were air-dried and examined under both visible and UV light (ʎ 366,245nm). 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 colour 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 afford compound I. The infrared spectrum of compound I was obtained in potassium bromide (KBr) discs using a Perkin-Elmer FTIR 1600-Jasco spectrophotometer. Ultraviolet absorption was measured in spectroscopic methanol on a Perkin-Elmer UV –Visible spectrophotometer. The UV shifting reagents: sodium methoxide, sodium acetate, aluminium chloride and boric acid were separately added to the methanolic solution of the investigated material and UV measurements were then recorded to determine the hydroxylation pattern on the nucleus of the flavonoid. The electron impact ionization (EIMS) mass spectrum was obtained on a solid probe using a Shimadzu QP-class-500. ^{1}HNMR experiment (1D experiment) was obtained on a Bruker AM 500 spectrophotometer operating at 500 MHZ. DMSO-d_6 was used as solvent and chemical shifts values were expressed in δ (ppm) units using (TMS) as an internal standard.

Antimicrobial assay:-
The isolated flavonoid was screened for its antimicrobial activity against four bacterial strains: Gram-positive (*Staphylococus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and two fungal species **(***Aspergillus niger*, *Candida albicans*). The cup plate agar diffusion method was used.

Preparation of bacterial suspensions:-
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, 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. Suspension was stored in the refrigerator until used.

Testing of antibacterial susceptibility:-
The cup plate diffusion method was used to screen the antibacterial activity of compound I and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines(Wikler,2007). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ 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 20 µl of a solution of compound I. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured in duplicates and averaged.

Testing of antifungal susceptibility:-
The above mentioned method was adopted for antifungal activity, but dextrose agar was used. Samples were used here by the same concentrations used above.

Results and Discussion:-
Preliminary phytochemical screening of the ethanolic extract of *Albizia amara* leaves revealed the presence of alkaloids, saponins ,flavonoids and tannins.

Compound I(R_f 0.89;BAW) was isolated as yellow powder from n-butanol fraction of *Albizza amara* leaves.The IR spectrum of compound I revealed a pattern characteristic of flavonoids(Table 1).

Table 1:- IR data of compound I

υ cm^{-1}	
671,745,827	C-H, bending Ar.
1033	C-O, ether
1458,1514	C=C, Ar.
1649	C=O
2925	C-H, aliph.
3334	OH

In the UV ,compound I absorbs at λ_{max} (MeOH) 205,278nm(Fig.1). Such absorption indicates absence of conjugation between the 4 keto function and the (B) aromatic ring of the flavonoid nucleus. Compounds which reveal such absorption belong to one of the following classes: isoflavones, flavanones and dihydroflavonols(Mabry *et.al.*,1970).
Other classes of flavonoids, namely, flavones, flavonols , chalcones and aurones afford two peaks originating from two chromophores; the benzoyl system and the cinnamoyl system. These two peaks are commonly referred to as band I (usually 300 – 400nm) and band II (usually 240 – 290 nm) (Mabry *et.al.*,1970). The isoflavones, flavanones and dihydroflavonols all give only band II due to lack of conjugation between the carbonyl function and the aromatic (B) ring.

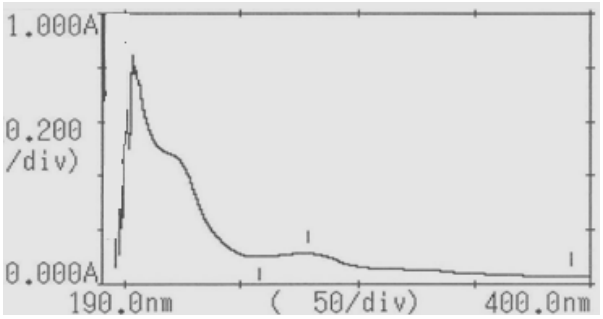


Fig.1:- UV spectrum of compound I

The UV spectrum did not reveal any shoulder characteristic of isoflavones(Mabry *et.al.*,1970) in the range : 300-340nm. Also the sodium methoxide spectrum(Fig.2) did not show any bathochromic shift characteristic of 3- and 4`-OH functions. Such UV data suggest a flavanone(Mabry *et.al.*,1970). The sodium acetate spectrum(Fig.3) did not reveal any bathochromic shift indicating absence of 7-hydroxylation(Mabry *et.al.*,1970).. Also no bathochromic shift diagnostic of 3-,5-OH and catechol systems was observed in the aluminium chloride spectrum(Fig.4). The same trend was observed in the boric acid spectrum(Fig.5) which is diagnostic of catechol moieties. Thus the isolated flavanone lacks: 3-, 5- ,7-, 4`-hydroxylation and catechol systems as indicated by various shift reagents(Mabry *et.al*.,1970).

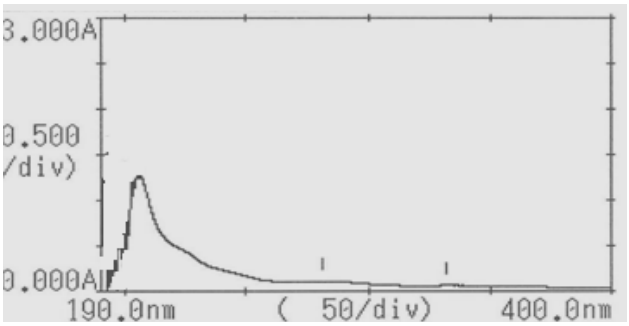


Fig.2:- Sodium methoxide spectrum of compound I

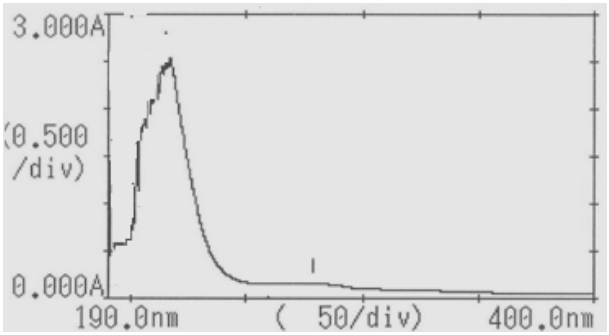


Fig.3:- Sodium acetate spectrum of compound I

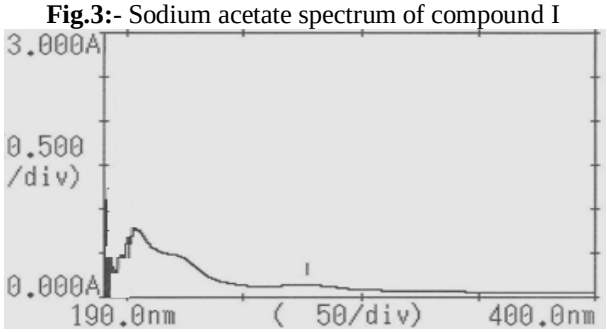


Fig.4:- Aluminium chloride spectrum of compound I

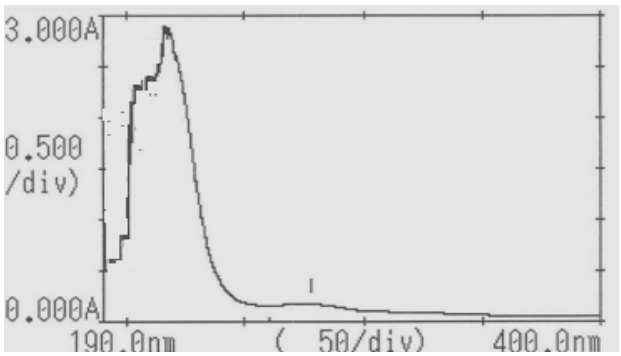
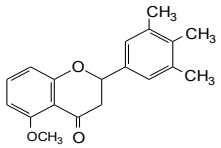


Fig.5:- Boric acid spectrum of compound I

The ^{1}HNMR spectrum(Fig.6) and as anticipated revealed a double multiplets centered at δ2.80ppm and 5.20 ppm characteristic of flavanones. These multipletes are due to mutual spin-spin splitting of the magnetically unequivalent C_3 – protons , which undergo further splitting with the C_2 proton. The signals at δ0.80 , δ1.23 and δ1.45ppm were assigned to three methyl groups , while the resonance at δ 3.39 ppm accounts for a methoxyl function. The multiplet at δ3.30-3.70 accounts for sugar protons. The aromatic protons appeared at δ 6.90 and δ7.39ppm. The mass spectrum gave m/z312 for M^+ (aglycone). Glycosidic flavonoids rarely afford discernible molecular ion for the glycoside, instead, a signal for the aglycone is usually observed (sometime: the base peak) (Mabry *et.al.*,1970)..

The substitution pattern of A ring was deduced from (i) no downfield signal -around δ 8.00 ppm - was observed for C_5 proton and (ii) the NMR spectrum revealed a 3 proton signal for A ring protons at δ6.90ppm.The signal at δ7.39ppm was assigned for meta-coupled $C_{2'}$- and $C_{6'}$ protons of B ring . Comparison with available literature data(Mabry *et.al.*,1970) led to the following structure for compound I:



Compound I

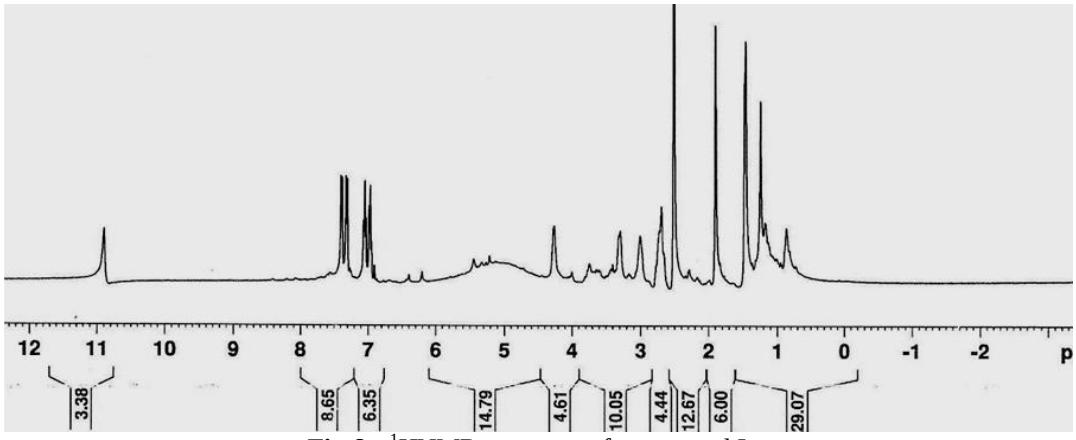
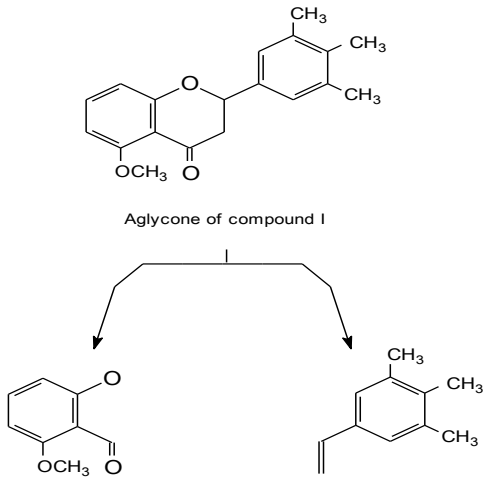


Fig.6:- ^{1}HNMR spectrum of compound I

Further evidence in favor of the proposed structure comes from the retro Diels-Alder cleavage[17] (Scheme I) where the fragments m/z150 and m/z146-resulting from intact A and B rings respectively- were detected in the electron beam.



m/z150 m/z146

Scheme I : Retro Diels-Alder fission of aglycone

Antimicrobial activity:-

The mean diameters of inhibition zone (MDIZ) and the minimum inhibitory concentration (MIC) produced by compound I on standard microorganisms are presented in Table (2). The results were interpreted in commonly used terms : < 9 mm considered inactive ; 9-12 mm : partially active ; 13-18 mm : active and more than 18 mm very active. Results displayed in table 2 demonstrate activity of compound I against all test organisms at a dose of 100mg/ml . On the basis of its promising antimicrobial activity, it seems that this flavonoid could serve as a lead molecule.

Table 2:- Antimicrobial activity of compound I

Sample	Inhibition zone diameter (mm / mg sample)					
	Antibacterial activity				Antifungal activity	
	B.subtilis(G^+)	*St.auerus*(G^+)	*E. coli* (G^-)	*P.Aeruginosa*(G^-)	*C.albicans*	*A. niger*
Control Methanol	00	00	00	00	00	00
Comp.I (100mg/ml)	15	15	13	13	15	15
Comp.I (50mg/ml)	14	14	12	12	14	15

Conclusion:-

A detailed phytochemical study of *Albizia amara* leaves was carried out. From the ethanolic extract of *Albizia amara*. a flavanone was isolated by chromatographic techniques. The isolate was identified via spectroscopic tools: IR, UV, ^{1}H NMR, and MS spectroscopy. The isolated compound exhibited promising activity against six standard human pathogens: *Staphylococus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albicans.*

References:-

Aura, A.M., Martin-Lopez, P., O'Leary, K.A., Williamson, G., Oksman-Caldentey, K.M., Poutanen, K., Santos-Buelga, C.(2005), *European journal of nutrition*, **3**,133.

Crozier, A., Jaganath, I.B., Clifford, M.N.(2009) , *Nat Prod Rep*, **26**, 1001 .

Dharani, N.,. Field Guide to Common Trees and Shrubs of East Africa, Struik Publishers, Cape Town, South Africa, ISBN 978 1 86872 640 0 (2007).

Furuta, T., Kimura, T., Kondo, S., Wakimoto, T., Nukaya, H., Tsuji, K., And Tanaka, K..(2004), *Tetrahedron*, **11**, 9375.

Graf, B.A., Milbury, P.E., Blumberg, J.B.(2005), *J. Med. Food*, **8**, 281 .

Harborne, J. B. "Phytochernical Methods", Chapman and Hall Ltd, London, pp111 (2001).

Joule, J.A. and Smith, G.F. "Heterocyclic Chemistry", Van Nostrand Reinhold Company, London (1972).

Ko, C.H., Shen, S., Chen, Y.(2004) , *Free Radic. Biol. Med.*, **36**, 897 .
Karuppannan, K.A., Subramanian D., Venygopal, S.(2013) , *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**, l3.
Mabry, T., Markham, K. R., & Thomas, M. B.,"The Systematic Identification of Flavonoids" Springer-Verlag , New York (1970).
Orwa, C., Mutua, A. Kindt, R., Jamnadass, R. & Anthony, S, (2009) ,Agroforestry Database p 2.
Proestos, C., Boziaris, I.S., Nychas, G.J, Komaitis, M.(2006) ,, *Food Chemistry*, **30**,664.
Wikler, M. A., Ed. "Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement" , Clinical and Laboratory Standards Institute(2007).
Woongchon M, Ghee T.T, Geoffrey A.C, John M.P. (1991) , *J. Nat. Prod*.; **54**, 1531.
Xia, Y., Yang, Z.Y., Xia, P., Bastow, K.F., Nakanishi, Y., and Lee, K.H.(2000), *Bioorganic and Medicinal Chemistry Letters*, **30**,699
Yenjai, C., Prasanphen, K., Daodee, S., Wongpanich, V., Kittakoop, P. (2004), *Fitoterapia*, **31**,89.
Yadava, R.N., Reddy, V.M.(2001) , *J Instit Chemists*, **73**,195.