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RESEARCH ARTICLE

PHYSICOCHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF A DIHYDROFLAVONOL FROM SUDANESE *ALBIZIA AMARA*(ROXB.)B.BIOVIN LEAVES.

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Abstract

A dihydroflavonol was isolated from the leaves of Sudanese *Albizia amara*. The isolate was purified by different chromatographic techniques and identified via a combination of spectral tools (IR, UV, ^{1}HNMR and mass spectroscopy). The isolated compound was screened for its antimicrobial activity against six standard human pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillas niger*, *candida albicans)*, and promising results were obtained.

Introduction:-

Since, ancient times nature has been an important source of medicinal agents and a large number of natural products have been identified and developed from natural sources based on their use in traditional medicine(Bhaskar and Balakrishnan,2009). Numerous medicinal plants are of global interest today because of their therapeutic and economic significance(Kokila *et.al*.,2013).

The genus *Albizia* comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa(King and Young,1999). The current literature revealed that some plants belonging to genus *Albizia* have great medicinal values(Hong *et.al*.,2005). Recently, an antitumour saponin- *julibroside* -was isolated from the stem bark of *A. julibrissin*(Ravishankar and Shukla,2007). It is also reported that *A. lebbeck* has antiseptic, antidysentric and anti-tubercular activities(Khan *et.al*.,2010).

Albizia amara (Leguminaceae)is a deciduous tree, often rounded or spreading crown, reaching 10 m in height but often smaller. Leaf and flowers of this plant show antiinflammatory activity and are usually used for boils and ulcers(Kumar *et.al*.,2008). Seeds showed astringent, antidiarrhoeal and antibacterial effects , whereas different plant extracts showed DNA binding properties and antibacterial activity.They also inhibited platelet aggregation and human lymphocyte transformation beside antiinflammatory and cytotoxic activity(Gasper and Nshimo,1988). The oil from the seeds is said to cure leprosy and leucoderma(Pal *et al*.,2010). In traditional medicine the roots are chewed and applied to an eye infection of cattle.Fruits are used traditionally as an emetic and for treating coughs and malaria. Saponins were extracted from the roots and leaves. Tannins and gums were extracted from the bark.Gum is used against ulcers; fruits are said to cure malaria and coughs. Bark stem decoction taken three times per day serves as an emetic to induce vomiting and to treat malaria(Dharani,2002). Leaves are said to be used in the treatment of wounds(Mukhtar and Ghori,2012).

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In continuation of our interest in bio-constituents of plant species used by local healers in Sudan, we planned to investigate the flavonoids of *Albizia amara* which is used traditionally to treat an array of human disorders.

Materials and Methods:-

Materials:-

The UV spectra were recorded on a Shimadzu 1601 Spectrophotometer and UV lamp was used for localization of fluorescent spots on TLC and PC. The IR spectrum was recorded as KBr disc, using Perkin-Elmer, FTIR 1600-Jasco. Nuclear Magnetic Resonance spectrum was run on a Bruker AM 500 MHZ NMR Spectrophotometer. The mass spectrum was recorded by direct probe EIMS using a Shimadzu QP-class-500 spectrometer.

Plant material:-

The leaves of *Albizia amara* were collected in April 2015 from "Maleet" (western Sudan) The plant was identified and kindly authenticated by the Dept. of Botany, University of Khartoum.

Methods:-

Isolation of flavonoids:-

Powdered shade- dried leaves (1kg) of *Albizia amara* were macerated with 95% ethanol at room temperature for 48 hours. 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. The n-butanol fraction was rich in phenolics. It was dissolved in methanol and applied to Whatman paper (No. 3 mm – 46x 57cm). The bands 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 vapour 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 evaporated *in vacuo* to dryness. In this way a flavonoid-compound I was isolated in chromatographically pure form as yellow amorphous powder.

Antimicrobial assay:-

Compound I was screened for antimicrobial activity against six standard human pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillas niger* and C*andida albicans).*

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(Wikler,2007). 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 suspensions were stored in the refrigerator until used.

Testing of antibacterial activity:-

The cup plate agar diffusion assay was adopted to screen the antibacterial activity. 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 10cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliter 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 test solution. The plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured in triplicates and averaged.

Testing of antifungal activity:-

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

Results and Discussion:-

Identification of compound I:-

Compound I was isolated as yellow amorphous powder from n-butanol fraction of *Albizza amara* leaves. The structure of this flavonoid was deduced on the basis of its spectral data (IR, UV, ^{1}HNMR and MS).

The IR (KBr) spectrum of compound I (Fig 1) showed : v (KBr)615,744 (C-H, bending , Ar.), 1024 (C-O), 1456-1558(C=C, Ar.), 1650(C=O), 2923(C-H, aliph.) and 3382 cm^{-1}(OH).

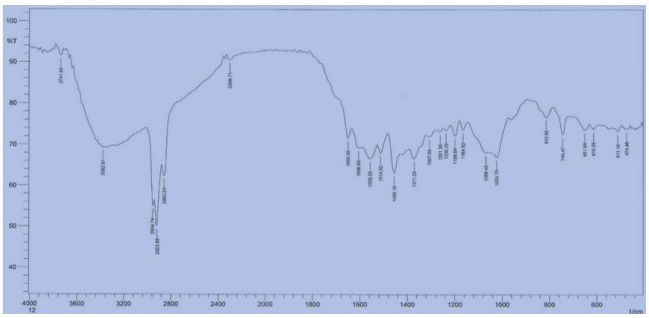


Fig. 1:- IR spectrum of compound I

In the UV, compound I absorbs at λ_{max} (MeOH) 267nm (Fig.2). No shoulder characteristic of isoflavones (Mabry and Markham ,1975) appeared in the range: 300-340nm.

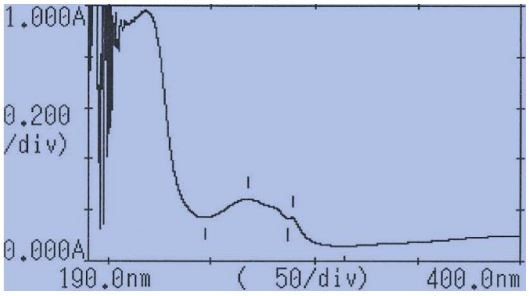


Fig.2:- UV spectrum of compound I

The sodium methoxide spectrum (Fig.3) revealed a bathochromic shift with decrease in intensity diagnostic of a 3-OH function(Mabry and Markham,1975). Thus compound I is a dihydroflavonol.

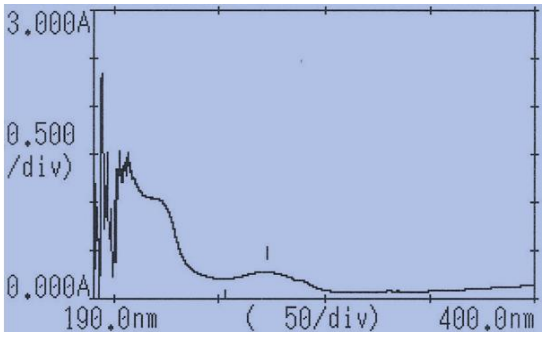


Fig.3:- Sodium methoxide spectrum of compound I

No detectable bathochromic shifts were observed in the sodium acetate and boric acid spectra (Figures: 4 and 5). This clearly indicates absence of a 7-OH as well as catechol systems. However, the aluminium chloride spectrum (Fig. 6) gave a bathochromic shift diagnostic of a 3-OH function(Mabry and Markham ,1975).

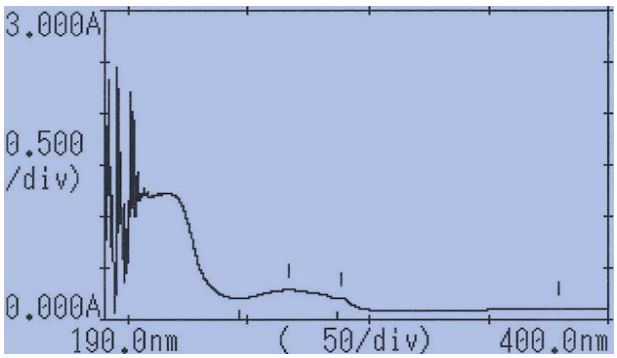


Fig. 4:- Sodium acetate spectrum of compound I

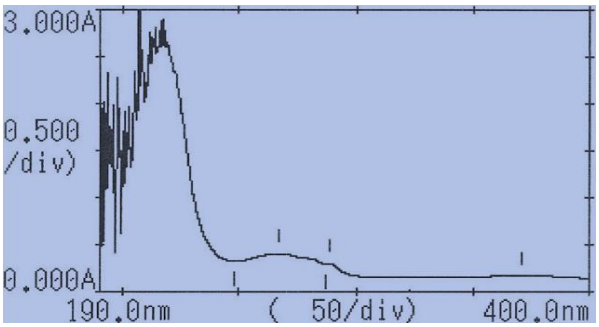


Fig.5:- Boric acid spectrum of compound I

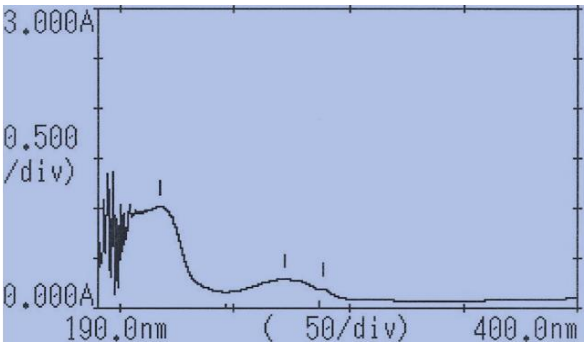


Fig.6:- Aluminium chloride spectrum of compound I

The [1]HNMR spectrum (Fig.7) showed two singlets at δ0.85 and δ1.25 each integrating for 3 protons assigned for two methyl groups. The C_2 proton resonated at δ3.00ppm due to the deshielding influence of the neighbouring oxygen atom. The signal at δ 1.90(3H) was attributed to an acetyl function, while the resonance at δ3.80 (3H) accounts for a methoxyl function. The multiplet at δ3.30-3.70ppm accounts for sugar protons. The signals at δ6.109(1H) and δ6.30(1H) were assigned for C_6 and C_8 protons respectively. The latter usually resonates at lower field relative to the former due to the deshielding effect of the neighbouring oxygen atom .The aromatic protons appeared as multiplet at δ 6.95- 7.80ppm. The mass spectrum gave m/z340 for M+ (aglycone).Glycosidic flavonoids rarely afford discernible molecular ions for the glycoside ,but they do afford a distinguished peak for aglycone-usually base peak(Mabry and Markham ,1975; Cuyckens and Claeys,2004).

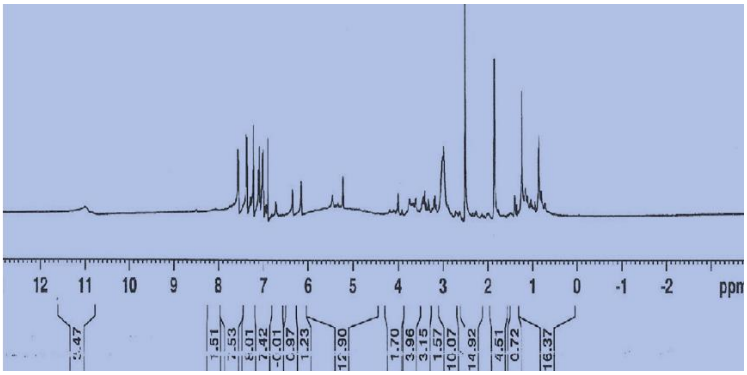
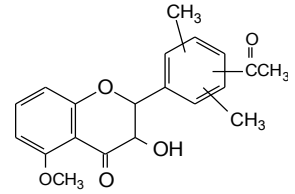
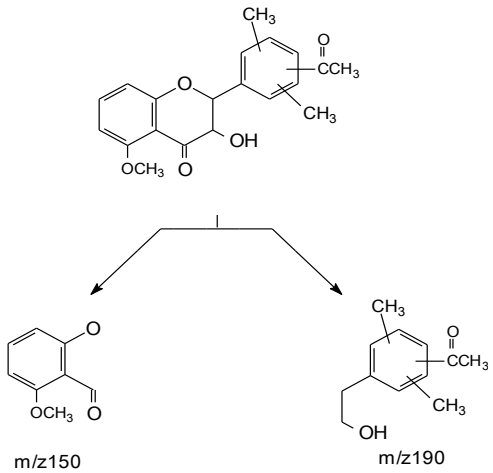


Fig. 7:- 1HNMR spectrum of compound I

The substitution pattern of A ring was deduced from (i) no downfield signal (around 8.00 ppm) was observed for C_5 proton and (ii) the NMR spectrum revealed a 3 proton signal for A ring protons and (iii) the retro Diels-Alder fission (Scheme I) gave m/z 150 for intact A ring. The assignment of two methyl and an acetyl function for B ring was based on the retro Diels-Alder cleavage which revealed a signal at m/z190 for intact B ring. Comparison of the above cumulative data with data published in literature resulted in the following partial structure for compound I:



Compound I



Scheme I:- Retro Diels –Alder fission of compound I

Antimicrobial activity:-

The mean diameters of inhibition zones (MDIZ) and the minimum inhibitory concentration (MIC) produced by compound I on standard microorganisms are presented in Table (1). The results were interpreted in commonly used terms : (<) 9 mm : inactive ; 9-12 mm : partially active ; 13-18 mm : active; (>) 18 mm : very active.

Table 1:- The antibacterial activity of compound I

Sample	Inhibition zone diameter (mm / mg sample)					
	Antibacterial activity				Antifungal activity	
	Bs. (G+)	Sa. (G+)	Ec. (G-)	Pa. (G-)	Ca.	An.
Control(Methanol)	00	00	00	00	00	00
100mg/ml	15	15	13	13	15	15
50mg/ml	14	14	12	12	14	15

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

At a concentration of 100mg/ml ,compound I showed antibacterial and antifungal responses against all test organisms.However at 50mg/ml it was active against the bacterial strains *Bacillus subtilis* and *Staphylococcus aureus* and the fungal species : *Candida albicans* and *Aspergillas niger* and partially active against other test organisms.

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