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ISOLATION OF AN ISOFLAVONE FROM SUDANESE *ACACIA NUBICA* STEM BARK AND ANTIMICROBIAL ACTIVITY OF THE ETHANOL EXTRACT

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ABSTRACT

A flavonoid - compound (I)- was isolated from the ethanol extract of *Acacia nubica* stem bark by thin layer chromatography and its structure was partially characterized on the basis of its spectral data (UV, NMR). The ethanol extract of *Acacia nubica* was screened for antimicrobial activity against five standard human pathogens : *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and the fungal species *Candida albicans*. The ethanolic extract exhibited significant antibacterial and antifungal activity against test organisms.

KEYWORDS: *Acacia nubica*, Isolation, Isoflavone, Biological Activity.

INTRODUCTION

Flavonoids are plant phenolics widely present in plants and foods of plant origin.[1-4] The basic structure of flavonoids consists of fifteen carbon atoms, arranged in a C_6-C_3- C_6 configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third heterocyclic ring(C). Major subgroups of flavonoids include: chalcones, flavones, flavonols, flavanones anthocyanins and isoflavonoids. Flavonoids are endowed with marked physiological potential including: antibacterial, anti-inflammatory, antiallergic, antifungal, antimutagenic, antiviral and vasodilator effects.[5-7]

Acacia (Fabaceae) is a large genus comprising around 1350 species. Acacia species are rich source of timber, gum and tannins and are mainly distributed in worm and dry regions of the world.[8-11] Most Acacia species are rich in bioactive molecules including phenolics.[10] Acacia species are reputed in traditional medicine as antidiabetic, antidiarrhoeic, antiinflammmatory, antimicrobial and as hypotensive[12-14].*Acacia nubica* **Benth.** is a herb reaching a height of 1-5m. It is distributed in Egypt, Sudan, Saudi Arabia and Iran. However, information on *Acacia nubica* is very scarce.This study was designed to isolate the major flavonoid of *Acacia nubica* and to assess the biological activity of the ethanol extract.

MATERIALS AND METHODS

Materials

Plant material

Stem bark of *Acacia nubica* were collected from White Nile state (Sudan). The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum – Sudan.

Instruments

UV spectra were run on a Shimadzu 2401PC UV-Visible Spectrophotometer.NMR spectra were performed on a Joel ECA 500MHZ NMR Spectrophotometer.

Test organisms

The antimicrobial activity of *Acacia nubica* was evaluated using the following standard microorganisms: *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans.*

Methods

Extraction and isolation of flavonoids

Powdered stem bark of a *Acacia nubica* (1.5 kg) were macerated at room temperature with ethanol (95%) for 72h. The solvent was evaporated under reduced pressure to dryness to give a crude product. The crude ethanol extract was fractionated via paper chromatography using 30% acetic acid as mobile phase. The chromatograms

were viewed and located under UV light and a flavonoid - compound I- was eluted from paper with methanol.

Antimicrobial activity

A (24) hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated for 24h at 37º C . Bacterial growth was washed off with 100 ml sterile normal saline giving approximately 10^8- 10^9 C.F.U/ ml. The average number of viable organisms per ml of the stock suspension was determined.

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 and then incubated at 37°C for 24 hours.

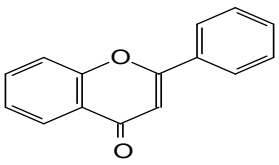
The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) soaked with a solution of each test sample were placed on the surface of the seeded agar.the inoculated plates were incubated at 37 °C for 24 h.The test was done in duplicates and the diameters (mm) of the inhibition zones were measured and averaged.

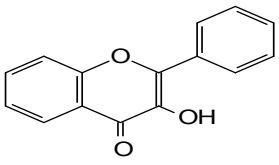
The above mentioned method was adopted for antifungal activity, but instead of nutrient agar Sabouraud dextrose agar was used and incubation continued or four days at 25^OC. Samples were used here by the same concentrations used above.

RESULTS AND DISCUSSION

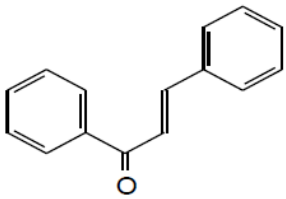
The flavonoids are plant phenolics characterized by a flavan basic skeleton which consists of two aromatic rings (designated A and B) joined by a 3 carbon chain which may or may not form a third heterocyclic ring (designated C). Flavonoids are classified into the following categories: i) anthocyanins ii) flavans iii) flavones iv) flavonols v) chalcones vi) aurones vii) flavanones viii) isoflavones ix) dihydroflavonols x) dihydrochalcones.



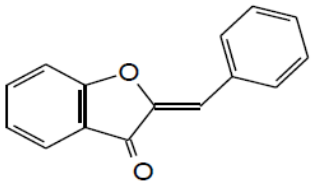
Flavone



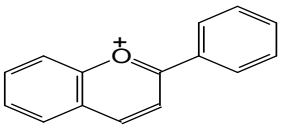
Flavonol



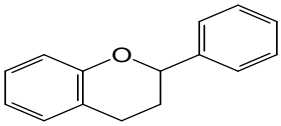
Chalcone



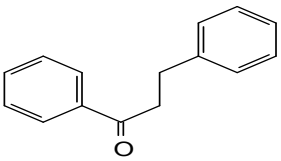
Aurone



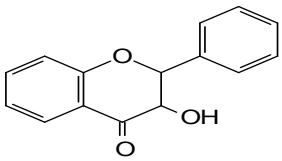
Anthocyanin



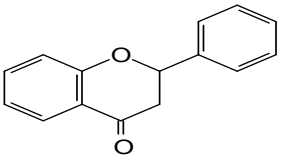
Flavan



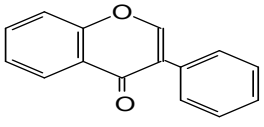
Dihydrochlcone



Dihydroflavonol



Flavanone



Isoflavone

The antocyanins and flavans are easily distinguished since they are devoid of a carbonyl function. Flavones, flavonols, chalcones and aurones exhibit two UV absorption band designated as band I(in the range :300-400nm) and band II(in the range 230-290nm).Other classes(flavanones, isoflavones, dihydrochalcones and dihydroflavonols give only one UV absorption band (band II). These classes loses conjugation between the two aromatic rings(A and B) due to the absence of C_2 – C_3 unsaturation.

Compound I was isolated as pale yellow amorphous powder from the stem bark of *Acacia nubica*. In the UV it absorbs at λ_{max} 264nm (Fig. 1).The appearance of only one band – band II- suggests that this compound is either a flavanone, isoflavone, dihydrochalcone or dihydroflavonols. However the UV spectrum showed a shoulder in the range 300-340nm which is a characteristic feature of isoflavones.

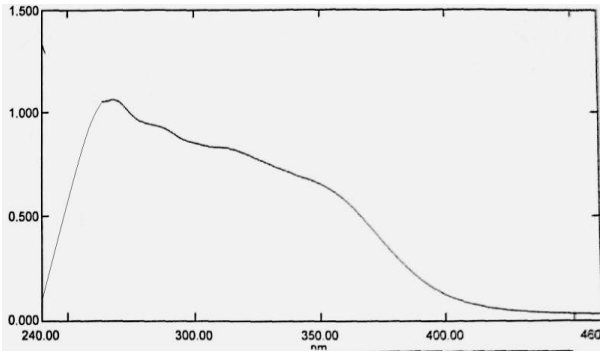


Fig. 1: UV spectrum of compound.

Next the hydroxylation pattern of the isoflavone has been investigated by using some UV shift reagents. These reageants exhibit bathochromic shifts diagnostic of specific hydroxylation pattern.The shift reagent –sodium methoxide- shows a bathochromic shift in presence of 3- or a 4`-OH function. In case of 3-OH group , the shift is accompanied with decrease in intensity.Sodium acetate is another useful shift reagent which exhibits a bathochromic shift diagnostic of a 7-OH substituent.The shift reagen-aluminium chloride is diagnostic of 3- and 5-OH groups as well as catechol systems, while boric acid affords a bathochromic shift in presence of catechol moieties.The sodium methoxide spectrum(Fig.2) of compound I did not reveal any bathochromic shift suggesting absence of 3- and 4`-hydroxylation. The aluminium chloride spectrum (Fig.3) showed a bathochromic shift characteristic of a 5-OH function.The boric acid spectrum suggested the absence of catechol systems since it failed to show any bathochromic shift (Fig.4). However, the sodium acetate spectrum (Fig.5) revealed a 6nm bathochromic shift indicating a 7-OH substituent.

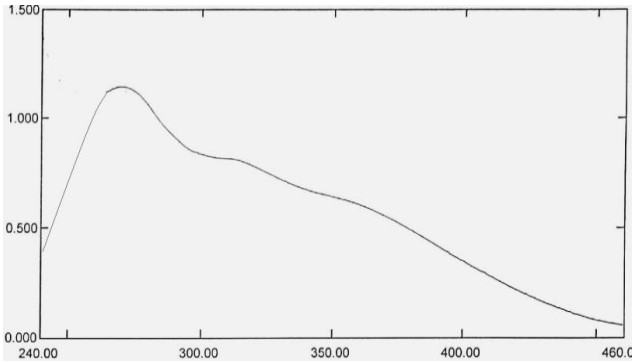


Fig. 2: Sodium methoxide spectrum of compound I.

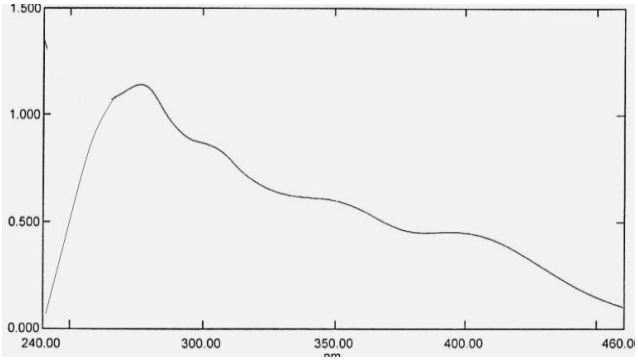


Fig. 3: Aluminium chloride spectrum of compound I.

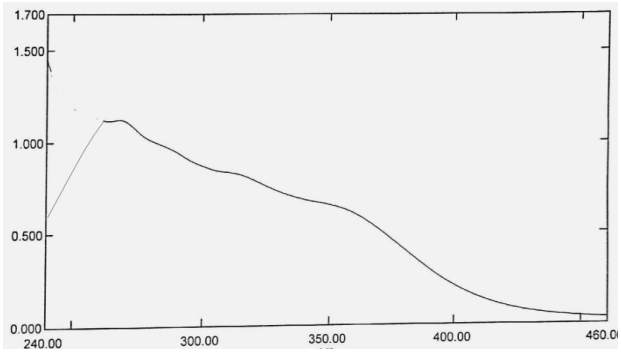


Fig. 4: Boric acid spectrum of compound I.

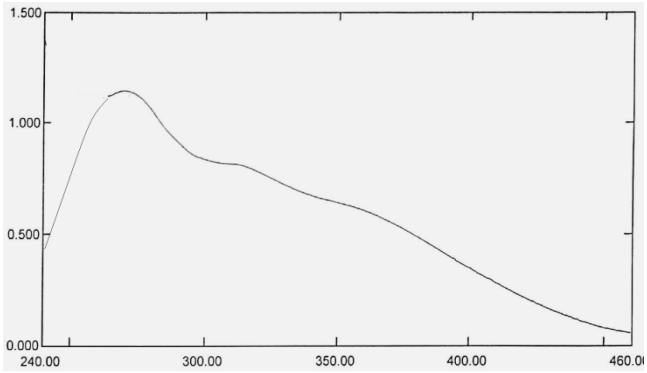


Fig. 5: Sodium acetate spectrum of compound I

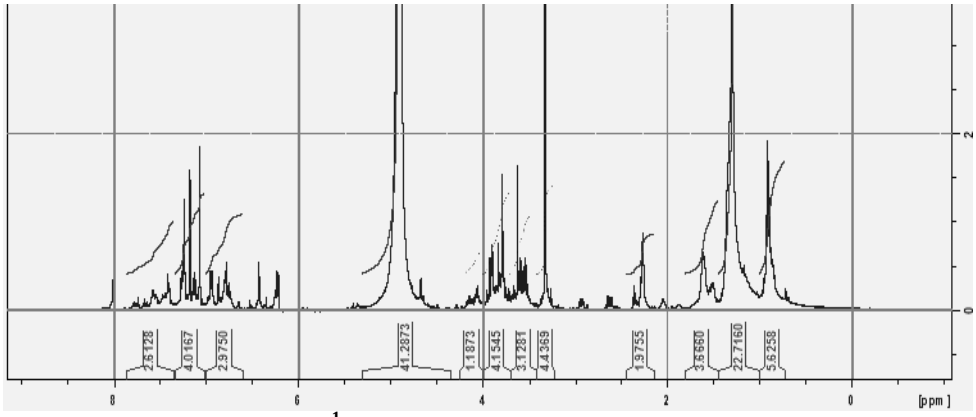
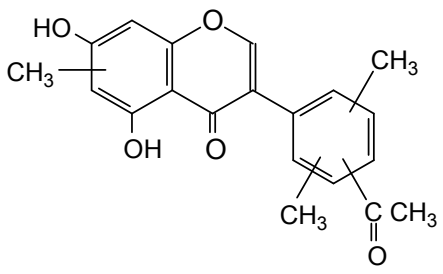


Fig. 6: ^{1}H NMR spectrum of compound I.

The ^{1}HNMR spectrum(Fig.6) showed δ(ppm) : 0.92 (assigned for two methyl groups); 1.63(accounting for one methyl group); 2.25(assigned for an acetyl group); m(3.50-4.20)- assigned for a sugar moiety . The aromatic protons appeared at 6.20, 6.32 and as multiplet(6.60-7.60).The sugar which appeared in this spectrum was not identified in this study.On the basis of this argument, the following partial structure was proposed for aglycone of the isolated isoflavone:



Aglycone of compound I

Antimicrobial activity

Acacia nubica ethanol extract was assessed for antimicrobial activity via the disc diffusion bioassay using five standard human pathogens.. The average of the diameters of the growth of inhibition zones are shown in Table 1 The results were interpreted in terms of

the commonly used terms (<9mm:inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (2) and (3) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 1: Inhibition diameters (mm) of the ethanol extract.

Sample	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
Acacia nubica ethanol extract	100	17	20	21	19	16

Table 2: Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm).

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 3: Antifungal activity of standard chemotherapeutic agent.

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

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

Acacia nubica ethanol extract exhibited significant antibacterial and antifungal activity against test organisms.

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