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ISOLATION, PARTIAL CHARACTERIZATION OF A FLAVANONE FROM *ALBIZIA AMARA* (LUGUMINACEAE) STEM BARK AND ANTIBACTERIAL ACTIVITY OF ROOT FRACTIONS

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ABSTRACT

This study was carried out to investigate the major flavonoid of *Albizia amara* stem bark and to evaluate the antibacterial activity of root ethyl acetate and n-butanol fractions. The flavonoids were extracted with ethanol and the crude extract was purified by paper chromatography where a flavanone was isolated. The structure of this compound has been partially characterized by UV and ^{1}HNMR data. In the antimicrobial assay, the ethyl acetate and n-butanol fractions from *Albizia. Amara* roots were assessed for antibacterial activity. These extracts showed responses against the bacterial strains: *Streptococcus mutans* and *Lacto bacillus* . Both of the ethyl acetate and the n-butanol fractions showed significant activity against *Lacto bacillus* within the test concentracions(100,200mg/ml). However, within the test concentrations, the ethyl acetate fraction was more effective against *Streptococcus mutans* than the n-butanol fraction.

KEYWORDS: Albizia amara, Isolation, Flavanone, Antimicrobial activity.

INTRODUCTION

The genus *Albizia* comprises more than hundred species mainly distributed throughout tropical and sub-tropical regions of Africa, Asia and Australia.

These species are valuable source of gum and high quality timber.[1]

Albizia amara (Luguminaceae) is a large tree reaching 10m in height. In Africa it extends from Sudan and Ethiopia southwards towards Zimbabwe and Botswana.[2] *Albizia amara* is a potential medicinal plant and its various extracts are widely used in ethnomedicine.[3] Seed oil is used traditionally against leucoderma and leprosy.[4] while flowers are applied externally for swelling, boils and eruptions.[5]

It has been shown that the ethanol extract of *Albizia amara* contains some saponins which are known for their biological activity.[3] The petroleum ether extract of leaves contains phenolic glycosides, flavonoids and saponins.[6] Seed extract was shown to contain bioactive alkaloids,[7] while seed oil was found to contain high linoleic and palmitic acid content.[8]

Alkaloids with significant bactericidal properties has been reported from seeds.[5] Some of these alkaloids showed cytotoxic activity against some human cell lines.[5]

Significant antiinflammatory and analgesic activity has been associated with the ethanol extract of *Albizia amara*.[9] The antihyperlipidemic activity of bark extracts has been documented.[10] It has been demonstrated that the methanol extract of *Albizia amara* possess significant free radical scavenging capacity.[11-13]

MATERIALS AND METHODS

Plant material

Albizia amara was collected from Nyala, western Sudan. The plant was identified and authenticated by direct comparison with a reference herbarium sample.

Test organisms

The bacterial strains: *Lacto bacillus* and *Streptococcus mutans* were used in this study.
- Positive control: Ampicilin.
-Media for bacteria: Mueller –Hinton agar.

Equipments

1- Ultraviolet-Visible spectrophotometer (Shimadzu

model UV240).
2- Joel- Nuclear Magnetic Resonance (NMR) spectrophotometer operating at 500 MHz.

Solvents
Solvents of analytical purity were used in this study. Methanol (Merck, Germany) was used for spectrophotometric analysis. DMSO-d_6 was used as NMR solvent and TMS as internal standard.

Methods
Extraction of flavonoids
Plant material (1Kg) was extracted with 95% ethanol for 72h at room temperature. The extract was filtered and the solvent was removed *in vacuo*.

Isolation of flavonoids
Plant extract was concentrated and applied on Whatman 3mm paper (46×57 cm) and run in BAW(6:1:4;v:v:v). The dried papers were viewed under UV light. The chromatograms were then located. Similar bands were joined and cut into small pieces and slurred with methanol. After several hours of contact the solvent was removed. Compound I was thus isolated from *Albizia amara* stem bark as yellow amorphous solid.

Antimicrobial assay
For bacteria an inoculum suspension (20 ml Mueller-Hinton agar) was swabbed uniformly to solidify, and then allowed to dry. Holes of 6 mm in diameter were made in the seeded agar. Aliquots from each plant extracts (100 and 200 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 then incubated at 37^{o}C for 24 h. The resulting inhibition zones were measured in millimeters (mm). The assays were repeated in triplicate and the concurrent values were taken. The activity is expressed as less active, if the zone of inhibition is 9-12 mm, moderate 13-18 mm and high if greater than 18 mm.

RESULTS AND DISCUSSION

Compound I was isolated from stem bark of *Albizia amara* by paper chromatograph. In the UV it absorbs (Fig.1) at λ_{max}(MeOH)279nm, hence it belongs to those flavonoids which are characterized by saturation at C_2 – C_3 linkage i,e. it could be: a flavanone, dihydroflavonol, dihydrochlcone or an isoflavone. The latter class is ruled out since it is characterized in the UV by a shoulder in the UV range: 300-340nm and such feature was not detected in the UV spectrum of this compound. On the other hand dihydroflavonols are known to possess a 3-OH function. This group was not detected by the sodium methoxide spectrum – no bathochromic shift (Fig.2).

However, flavanones and dihydrochalcones are distinguishable by their ^{1}HNMR. Flavanones, unlike dihydrochalcones give double multiplets around δ2.80 and δ5.20ppm. One of these multiplets is due to mutual splitting of the magnetically unequivalent protons at C_3. The double doublet arising from such splitting suffers further splitting by the neighboring C_2 proton yielding a multiplet. The other multiplet is due to the splitting of C_2 resonace by the neighboring unequivalent C_3 protons. Flavanone multiplets were detected in the ^{1}HNMR spectrum of compound I(Fig.6). Hence the isolated flavonoid is a flavanone.

The sodium acetate spectrum (Fig.3) did not afford any bathochromic shift confirming absence of a 7-OH function. The aluminium chloride and the boric acid spectra (Fig.4 and 5) were also devoid of bathochromic shifts suggesting absence of catechol systems as well as 3- and 5-OH groups.

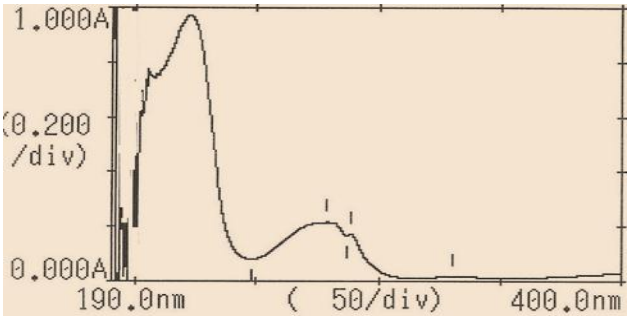


Fig. 1: UV spectrum of compound I.

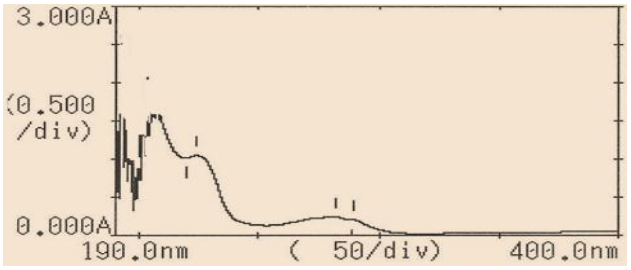


Fig. 2: Sodium methoxide spectrum of compound I.

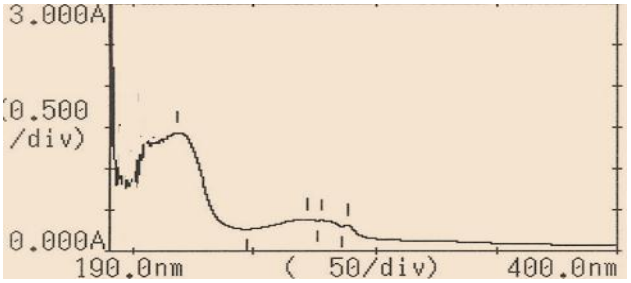


Fig. 3: Sodium acetate spectrum of compound I.

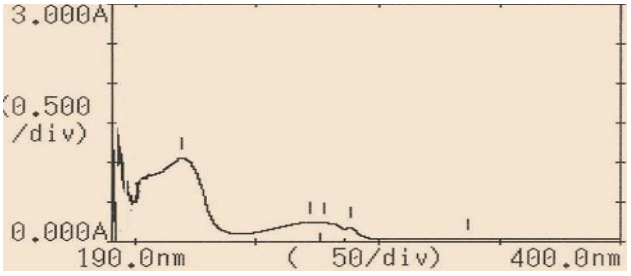


Fig. 4: Alminium chloride spectrum of compound I.

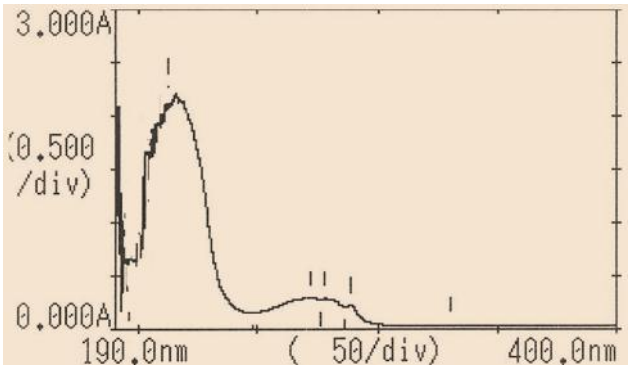
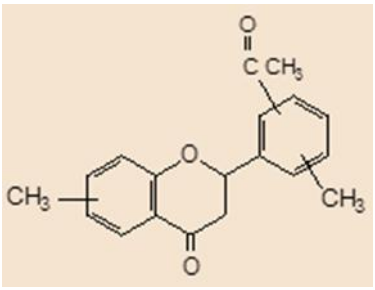


Fig. 5: Boric acid spectrum of compound I.

The ^{1}HNMR spectrum of compound I (Fig.6) gave δ(ppm): 1.35(assigned for two methyl groups); 1.82(acetyl group): 2.70-3.00-multiplet(C_2-protons); 3.30-3.82-multiplet (a sugar moiety- not identified in this study); 5.10-5.40-multiplet (C_3 protons); 6.69-7.60-multiplet (Aromatic protons).

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



Compound I

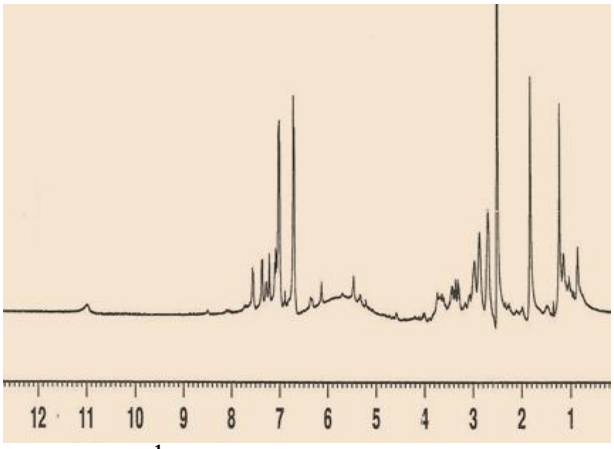


Fig. 6: ^{1}HNMR spectrum of compound I.

Antibacterial activity of root fractions

The ethyl acetate and n-butanol fractions from *Albizia. Amara* roots were assessed for antibacterial activity. These extracts showed responses against the bacterial strains: *Streptococcus mutans* and *Lacto bacillus*. Both of the ethyl acetate and the n-butanol fractions showed significant activity against *Lacto bacillus* within the test concentracions (100, 200mg/ml). However, within the test concentrations, the ethyl acetate fraction was more effective against *Streptococcus mutans* than the n-butanol fraction. Ampicilin was used as positive control and DMSO as negative control (Table 1).

Table 1: Antibacterial activity of root fractions.

Microrganism	Fraction	100 mg/ml	200 mg/ml
Lacto bacillus	Butanol	20	22
	Ethyl acetate	18	19
	AMP	25	25
	DMSO	-	-
Streptococcus mutans	Butanol	14	15
	Ethyl acetate	20	20
	AMP	25	25
	DMSO	-	-

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