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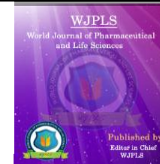

ISOLATION AND PARTIAL CHARACTERIZATION OF A DIHYDROFLAVONOL FROM SUDANESE *ACACIA NILOTICA* SUBSP. *NILOTICA* PODS AND ANTIBACTERIAL ACTIVITY OF POD FRACTIONS

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ABSTRACT

From the methanolic extract of, *Acacia nilotica* subsp. *nilotica* a flavonoid - compound I has been isolated. The extract was purified by thin layer chromatography and the isolated flavonoid was partially characterized via some spectroscopic data (UV, ^{1}HNMR). Some fractions of *Acacia nilotica* were evaluated for antimicrobial potential against Gram negative *(Escherichia coli* and *Pseudomonas aeruginosa*); Gram positive (*Bacillus subtilis* and *Staphylococcus aureus)* and the fungal species *Candida albicans*. The ethyl acetate and ethanol fractions showed moderate activity against *Pseudomonas aeruginosa*, while the n-butanol fraction exhibited a partial activity. The chloroform and ethanol fractions showed partial activity against *Staphylococcus aureus*

KEYWORD: Acacia nilotica subsp. nilotica, Flavonoid, Isolation, Antimcirobial Activity.

INTRODUCTION

Flavonoids are one of the largest group of phytochemicals found in plants.[1,2] This polyphenolics are almost present in nearly every part of the plant.[2,3] In their basic nucleus, the flavonoids have a C_6-C_3-C_6 configuration consisting of two benzene rings (A and B) linked by a three carbon bridge.[4] Flavonoids are endowed with marked physiological potential including: anti-inflammatory, antimicrobial, antiallergic, antimutagenic, antiviral and anticancer activities.[5] However, the most important biological effect of flavonoids is their ability to act as potential antioxidants.[6-16]

Acacia nilotica L. is a common, medium sized tree in the family Mimosaceae. The genus *Acacia* which comprises about 1380 species is the most significant genus in its family.[17,18] *Acacia nilotica* grows to 15-18 m in height.It is widely distributed in subtropical and tropical Africa. In Asia it is of common occurrence in Pakistan and India.[19]

Acacia nilotica is a medicinal plant of many attributes. It is traditionally used as anti-cancer, astringent, diuretic, antiscorbutic, antispasmodic and as nerve stimulant. The plant is also used against intestinal pains, diarrhea, cold, congestion, cough, dysentery and fever.[20] Seeds are claimed to have antimalarial, antidiabetic, antihypertensive and antispasmodic activities. The leaves and pods,which are rich in protein, possess antinflammatory properties. The bark is used in the treatment of hemorrhages, cold, tuberculosis and leprosy. The root is used as an (aphrodisiac) and the flowers for treating syphilis lesions.[21]

In continuation of our interest in bioactive molecules in plants of family Mimosaceae , hereby we report the isolation, partial characteization of a flavonoid isolated from *Acacia nilotica.*We also report the antimicrobial potential of different fractions of this plant.

MATERIALS AND METHODS

Pods of *Acacia nilotica* subsp. *nilotica* were collected from a forest reserve in Khartoum (Sudan). The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Extraction and isolation of flavonoids

Powdered air-dried pods (1kg) of *Acacia nilotica* subsp. *nilotica* were macerated with 80% methanol (4L) at room temperature for 72hr. The solvent was removed under reduced pressure to give a crude product.

The methanol extract was fractionated over silica gel plates developed with 5% acetic acid. The chromatograms were located under UV light. A Chromatographically pure flavonoid- compound I (R_f 0.80)was eluted from silica by methanol.

Antimicrobial activity

The antimicrobial activity was evaluated using agar well diffusion bioassay as described by Adeiyi *et.al.*[22]. Muellar Hinton agar was used as medium for bacterial culture, while fungal culture was maintained on Sabouraud dextrose agar.

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^oC for 24 h-for bacteria – and for four days at 25^oC for fungi. The assay was repeated in duplicates and the diameters of inhibition zones were measured and averaged as indicator of activity.

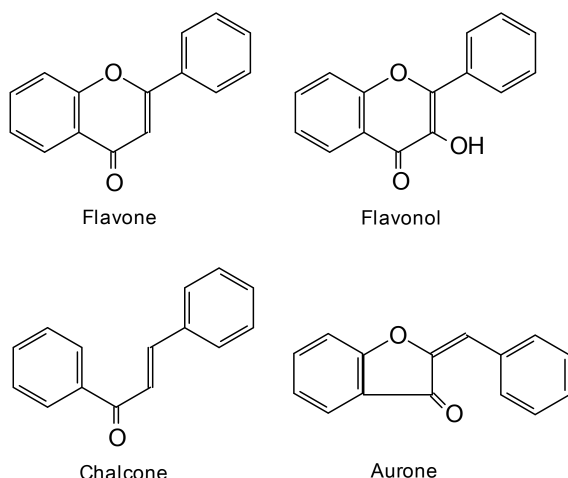
RESULTS AND DISCUSSION

Phytochemical screening of *Acacia nilotica* subsp. *Nilotica* pods revealed the presence of alkaloids, steroids, flavonoids, tannins, carbohydrates and glycosides,

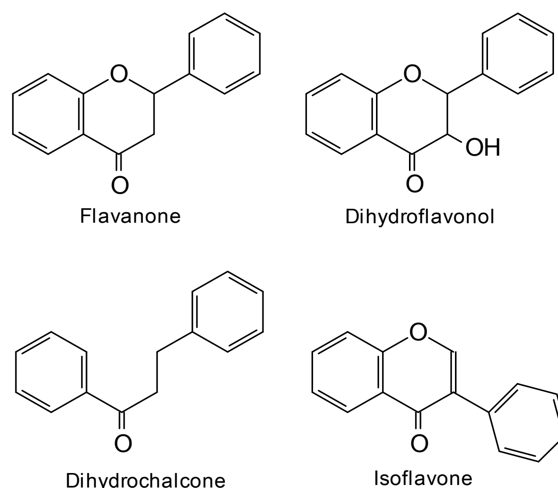
Identification of compound I

Compound I was isolated from pods of *Acacia nilotica* subsp. *nilotica* via TLC experiments. The partial characterization of this compound was mainly based on its UV and NMR data.

Some flavonoids show two UV absorption bands; band I and II. Band I is associated with the absorption of the cinnamoyl system, while band II is considered to originate from the benzoyl system. Flavones, flavonols, chalcones and aurones give both bands due to conjugation between benzoyl and cinnamoyl chromophores .



However, another class of flvonoids joining the flavanones, isoflavones, dihydroflavonols and dihydrochalcones give only one UV band – band II which is associated with the benzoyl chrompophore. These flavonoids are saturated at the $C_2 – C_3$ bond and are thus devoid of a cinnamoyl chromophore.



The UV spectrum of compound I showed(Fig.1) λ_{max}(MeOH) 212,278 nm . Such absorption suggests that this flavonoid is probably an isoflavone, flavanone, dihydrochalcone or a dihydroflavonol. However , the UV shift reagent-sodium methoxide showed(Fig. 2) a bathochromic shift with decrease in intensity . Such findings indicate a 3-OH function which is a characteristic feature of dihydroflavonols.

Considerable structural features have also been obtained by using UV shift reagents: sodium acetate, aluminum chloride and boric acid/ sodium acetate. These reagents produce shifts in the UV absorption maxima in accordance with the location of the various hydroxyl functions in the nucleus of flavonoids.

The sodium acetate spectrum(Fig.3) did not reveal any bathochromic shift indicating absence of 7-hydroxylation. The same trend was observed in the aluminium chloride spectrum(Fig.4) which indicated absence of 3- , 5- OH groups as well as catechol systems. The boric acid spectrum(Fig.5) did not show a blue shift suggesting absence of catechols.

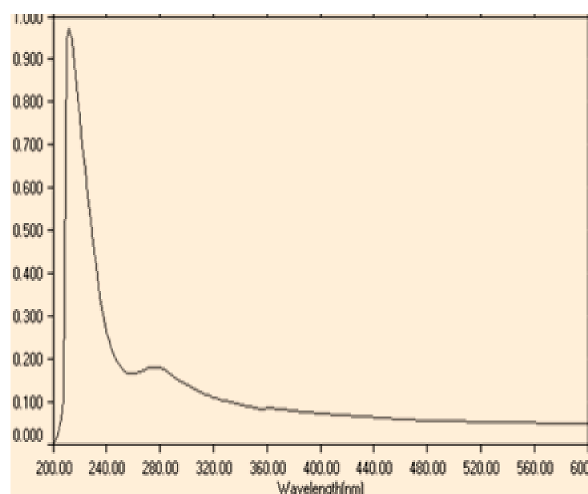


Fig. 1: UV spectrum of compound I.

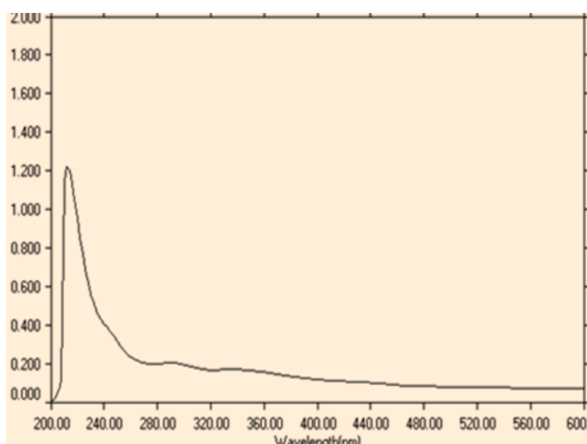


Fig. 2: Sodium methoxide spectrum of compound I.

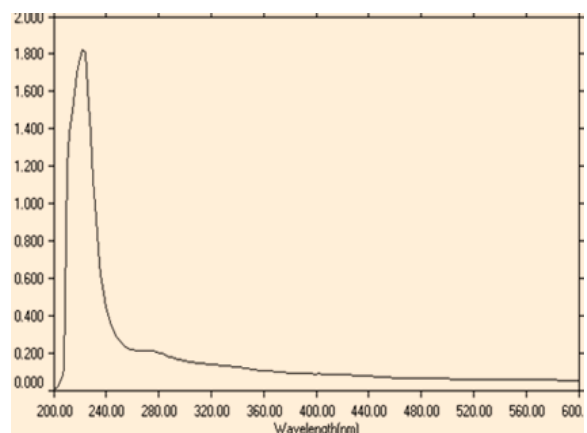


Fig. 3: Sodium acetate spectrum of compound I.

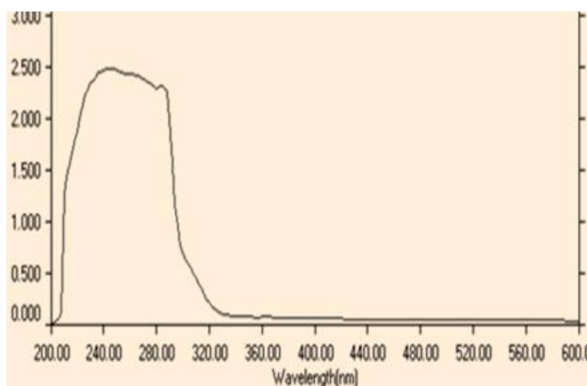


Fig. 4: Aluminium chloride spectrum of compound I.

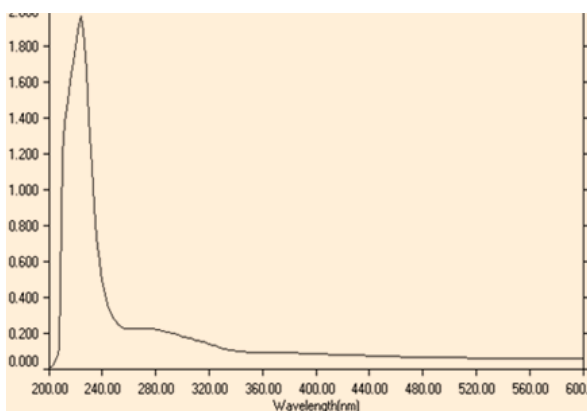
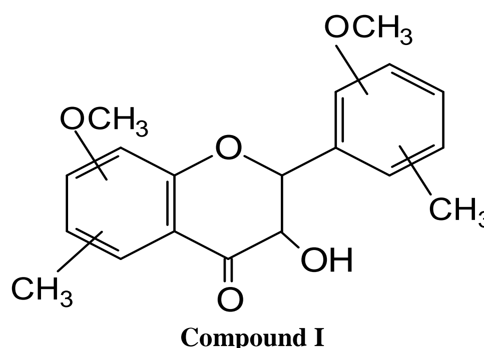


Fig. 5: Boric acid spectrum of compound I.

^{1}HNMR spectrum (Fig.6) showed δ(ppm) : 1.23, 1.66ppm (assigned for two methyl groups);m(3.03-3.82) and m(4.63-5.51) – assigned for a sugar residue(not identified in this study); 3.78, 3.88ppm (two methoxyl groups) ; 6.20,7.10, 7.25, 7.50 ,7.90 and 8.25ppm (Ar.protons). The resonances at δ2.50 and 3.32ppm are due to solvent(DMSO) residual protons and residual water respectively.

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



Compound I

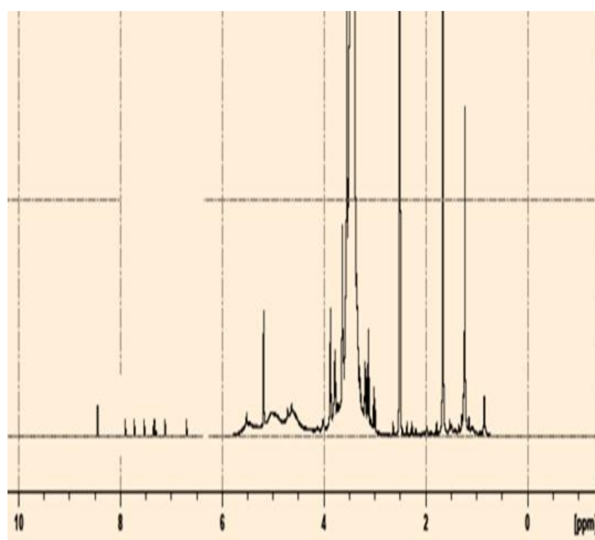


Fig. 6: ^{1}HNMR spectrum of compound I.

Antimicrobial activity

Different fractions of *Acacia nilotica* subsp. *nilotica* were assessed for antimicrobial potential against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (1) .Ampicilin, gentamycin and clotrimazole were used as positive contols (Tables 2 and 3).

The ethyl acetate and ethanol fractions showed moderate activity against *Pseudomonas aeruginosa*, while the n-butanol fraction exhibited partial activity.The chloroform and ethanol fractions showed partial activity against *Staphylococcus aureus*.

Table 1: Diameters of inhibition zones of different fractions.

Extract	Conc. (mg/ml)	Sa	Bs	Ec	Ps	Ca
Chloroform	100	10		-	-	-
n-Butanol	100	-	-	-	10	-
Ethyl acetate	100	-	-	-	15	-
Ethanol	100	10	-	-	14	-

Table 2: Diameters of inhibition zones of standard antibacterial agents.

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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: Diameters of inhibition zones of standard antifungal 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*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

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