Isolation and Partial Characterization of Flavone from Sudanese *Acacia nilotica* subsp. *adstringens* Pods and Antibacterial activity of Pod Fractions

Mohamed,A.M.[1(*)], Mayada,I.S.[2] and Weam.A.K.[3]

[1]El Sheikh Abdallah Albadry University,Faculty of Pharmacy(Sudan).
[2]Sudan University of Science and Technology, Faculty of Science(Sudan).
[3]Omdurman Islamic University, Faculty of Pharmacy(Sudan).

Abstract

The methanolic extract of *Acacia nilotica* subsp. *adstringens* pods was fractionated over silica gel plates to give a flavonoid-compound I . The structure of compound I was partially characterized via some spectral data: UV, ^{1}HNMR . Different fractions of *acacia nilotica* were assessed for their antimicrobial activity against Gram negative *(Escherichia coli* and *Pseudomonas aeruginos*) , Gram positive (*Bacillus subtilis* and *Staphylococcus aureus)* and the yeast *Candida albicans*. The n-butanol and ethanol fractions showed moderate activity against *Pseudomonas aeruginosa*.The ethyl acetate fraction exhibited moderate activity against *Bacillus subtiles* while the chloroform fraction showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus.*

Keywords*: Acacia nilotica* subsp. *adstringens,* Flavonoid, Isolation, Antimicrobial Activity.

Introduction

Flavonoids are bioactive phytochemicals that occur naturally both in the free state and as glycosides. Their chemical structure is based on a C_{15} skeleton consisting of two benzene rings connected by a generally closed three-carbon chain[1].

Flavonoids are polphenolics which appear as secondary metabolites of plant[2] . They are present inside the cells or on the surface of different plant organs. The structures of these plant phenolics may be modified by alkylation, acetylation, hydroxylation, O-glycosylation of hydroxyl groups as well as C-glycosylation . Sometimes additional rings are condensed to the basic skeleton of the flavonoid core[3].

The health benefits of the flavonoids have long been recognized and the antioxidant, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic properties have been reported[4,5].However,the most important biological activity of the

flavonoids is their ability to act as potential[6-14] antioxidants. The cardiac stimulation and vasoconstrication of some flavones and their conjugates has been reported[15]. A number of flavonoids have anti-protozoal activities[16]. Some flavonoids have an anti HIV-I potency at non-toxic concentration[17]. Some of the minor flavonoids have anti-microbial and cytotoxic properties[18,19] . Some anthocyanins have been reported as potent sex determining hormone[20] .

Acacia nilotica subsp. *adstringens* is a medium size tree commonly occurring in Sudan **,** Algeria, Burkina Faso, Cameroon, Togo and other African countries[21].Different Acacia species are extensively used in Sudanese system of medicine and *Acacia nilotica* subsp. *adstringens* is no exception .Crushed seeds of *Acacia nilotica* subsp. *adstringens* are used traditionally against haemorrhoids and gingivitis. The powdered bark is used as a natural remedy for acute diarrhea and leprosy[22]. Pods are claimed to treat cough, diabetes, dysentery, abdominal pain and worms[21] . Aerial parts are used against malaria and sore throat . The root is traditionally claimed to cure impotency[23] . Extracts of this plant were found to be inhibitory to at least four species of pathogenic bacteria[23] .

Materials and Methods

Plant material

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

Extraction and isolation of flavonoids

Powdered shade-dried pods (900g) of *Acacia nilotica* subsp. *adstringens* were extracted with 80% methanol (4L) at room temperature for 72hr. The solvent was removed *in vacuo* to give a crude product.The crude extract was purified over silica gel TLC plates developed with 5% acetic acid. The chromatograms were visualized and located under UV light .A Chromatographically pure flavonoid-compound I(R_f 0.75) was thus isolated.

Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were a aseptically distributed onto agar slopes and incubated at 37º C for 24 hours.

Aliquots(20ml) of the incubated nutrient agar were distributed into sterile Petri dishes; the agar was left to settle and in each of these plates two cups (10mm in diameter) were cut using sterile cork borer (No.4).

The agar discs were removed, and cups were filled with 0.1ml of each test sample and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37C^{o} for 24 hours.The test was performed in duplicates and the diameters of inhibition zones were measured (in mm) and averaged as indicator of antibacterial activity.

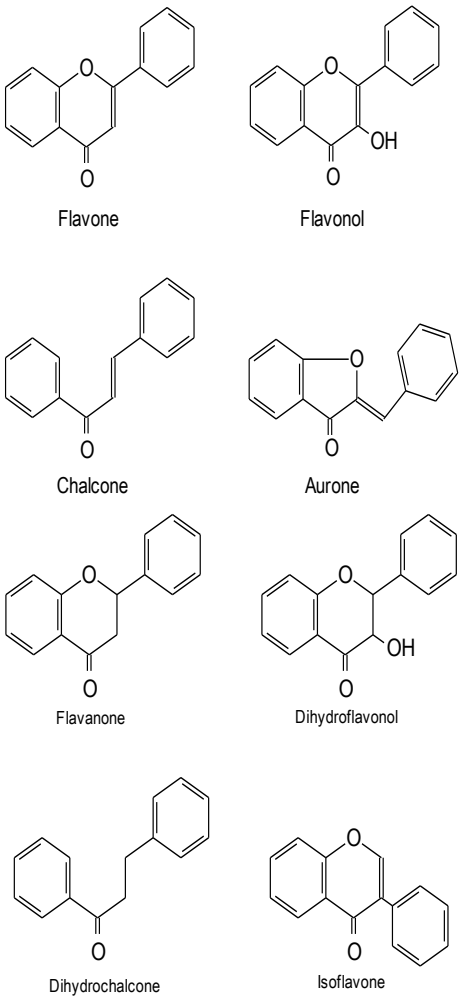
For antifungal activity, the above procedure was repeated but Sabouraud dextrose agar was used instead of Mueller Hinton agar and incubation continued for three days at 25^{o}C.

Results and Discussion

The structure of Compound I was partially characterized by some spectral data(UV and ^{1}HNMR)..

In most cases the UV spectra of flavonoids can afford valuable structural information that could serve in distinguishing between the various classes of flavonoids. Some flavonoids, namely, flavones , flavonols, aurones and chalcones show two absorption bands : one appears in the range 240-285nm (called band II) and the other appears in the range 300-550nm(called band I).These flavonoids are characterized by unsaturation at C_2- C_3 link. Such unsaturation results in conjugation between the carbonyl function at position-4 and the aromatic (B) ring of the flavonoids.Consequently two chromophores (benzoyl and cinnamoyl)appears. The cinnamoyl chromophore gives rise to band I , while the benzoyl chromore gives rise to band II.

Another class of flavonoids (flavanones, isoflavones, dihydrochalcones and dihydroflavonols) is characterized by saturation at C_2-C_3 . Consequently, and due to loss of conjugation between the carbonyl function at C_4 and the B ring , these flavonoids show only one absorption band – band II, which originates from the benzoyl chromophore.



Compound I was isolated from pods of *Acacia nilotica* subsp. *adstringens* as yellow powder. In the UV , it absorbs at λ_{max}(MeOH) 220,273,320nm. Such absorption is characteristic of flavones. The hydroxylation pattern of this flavone was studied by using different UV shift reagents; sodium methoxide(diagnostic of 3- and 4` groups) ; sodium acetate(diagnostic of 7-OH); aluminium chloride(diagnostic of 3-, 5-OH groups and catechols) and boric acid which is diagnostic of catechol systems.

The sodium methoxide spectrum (Fig.2) showed a bathochromic shift diagnostic of a 4`-OH group.The sodium acetate spectrum(Fig.3) did not reveal a bathochromic shift indicating absence of a 7-OH function. Also the aluminium chloride spectrum (Fig.4) did not reveal a bathochromic shift indicating absence of 3- and 5-OH groups as well as catechol systems.No bathochromic shift was detected in the boric acid spectrum(Fig.5) which confirms absence of catechol moieties.

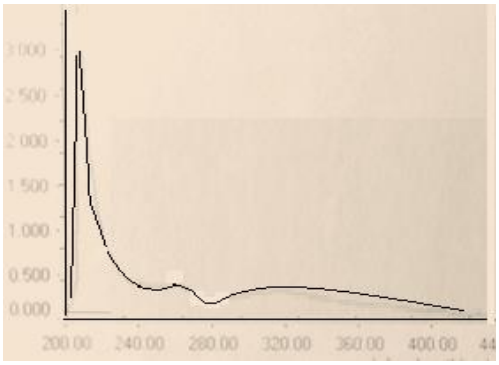


Fig.1: UV spectrum of compound I

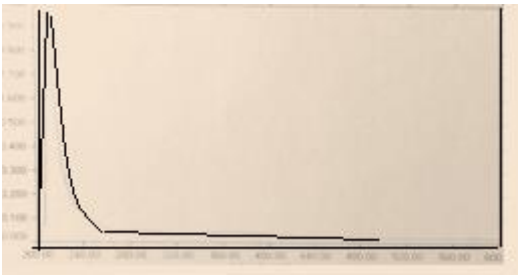


Fig.2: Sodium methoxide spectrum of compound I

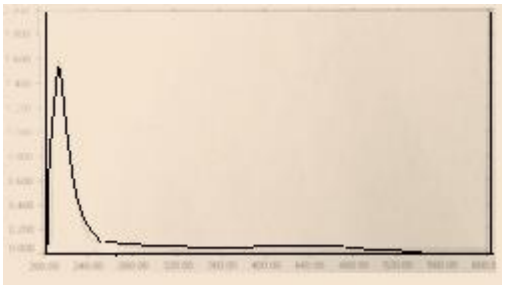


Fig.3: Sodium acetate spectrum of compound I

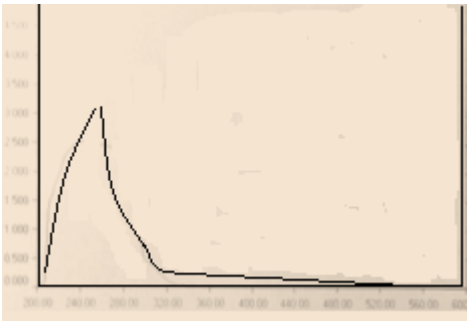


Fig.4: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum(Fig.5) showed δ(ppm) :1.20,1.70 (assigned for two methyl groups); m(2.90-3.80);5.5-(sugar protons-not identified in this study); 4.00(methoxyl); m(6.60-6.70)-

Ar. protons. Signals at δ2.50 and δ3.32ppm are due to solvent (DMSO) residual protons and residual water respectively.

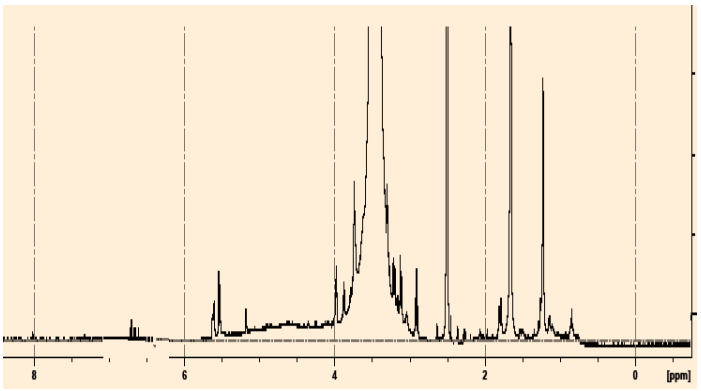
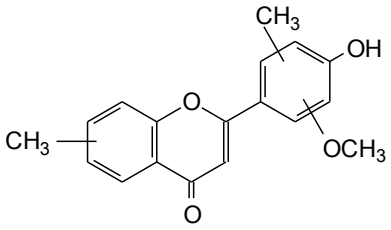


Fig.5 : ^{1}HNMR spectrum of compound I

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



Compound I

Antimicrobial activity

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

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

Table 1 : Antimicrobial activity of different fractions

Extract	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Chloroform	100	15	16	-	-	-
n-Butanol	100	-	-	-	15	-
Ethyl acetate	100	-	15	-	10	-
Ethanol	100	10	-	-	15	-

Table 2 : Antibacterial activity of standard chemotherapeutic 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 : 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*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

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