International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-2, Issue-9 September2016
ISSN: 2395-3470
www.ijseas.com

Isolation , Identification and Antibacterial Activity of a Flavone from Sudanese *Acacia nilotica* var *adstringens*

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Abstract : *Phytochemical investigation of Acacia nilotica var adstringens heartwood led to the isolation of a flavone : 7,3`,4`-trihydroxy-3-methoxyflavone from the methanolic extract. The crude extract was purified by a combination of chromatographic techniques(polyamide and Sephadex columns and paper chromatography) . Structure of isolate was elucidated on the basis of extensive spectroscopic procedures including : IR, UV, ^{1}HNMR and MS. The isolated flavonoid was evaluated for its antibacterial potential against Gram negative (Escherichia coli and Pseudomonasa eruginos) , Gram positive (Bacillus subtilis, Bacillus cereus and Staphylococcus aureus) bacteria. Compound I showed varying antibacterial responses. It showed high potency against Gram positive human pathogens : Staphylococcus aureus and Bacillus subtilis.*

Keywords : *Acacia nilotica var adstringens, Isolation ,Characteization,Flavone,Antibacterial Activity*

I- Introduction

Flavonoids occur 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 three-carbon chain (C_6-C_3-C_6). The three – carbon chain is generally closed to form a heterocyclic ring (C-ring). (Pengely, 2004).

Flavonoids are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The flavonoids may be modified by hydroxylation, methylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton. In addition, alkyl groups (or phenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core (Grotewold., 2006).

A number of flavonoids and chalcones have anti-protozoal activities (Wright and Phllipson., 1998). Some flavonoids like myricetin and kaempferol-3-glucoside have an anti HIV-I potency at non-toxic concentration (Hostettman *et al*., 1995). Some of the minor flavonoids have very interesting activities. They have anti-microbial, anti-fungal and cytotoxic properties (Harbrone., 1988). The

anthocyanin – isorhamnenetin- of the algae *Chlamydomonas* is a highly potent sex determining hormone (Kuhn *et al*., 1994).

The health benefits of flavonoids is mainly attributed to their antioxidant properties (Middleton and Kandaswami., 2000). Isoflavones, the bioactive ingredient in leguminous vegetables, not only cause a small reduction in blood cholesterol but also reduce blood pressure, arterial dimensions, and oxidative stress (Anderson and Anthony., 1999).

Acacia nilotica var *adstringens* is distributed through Sudan, Algeria, Burkina Faso, Cameroon and other African countries(Barnes *et al*., 2002). This subspecies is a source of gums and tannins. The bark and pods are used for tanning leather and as a dye source. The gum is locally used for making ink. The subspecies is commonly planted as a shade tree in the Sahelian regions.The crushed seeds are used by local healers for treatment of hemorrhoids . A decoction of the pods is used for coughs, and swallowing the juice formed by chewing is said to treat dry cough. The flowers are a source of pollen and nectar for bees (Barnes *et al*., 2002).

The plant is used in Sudanese ethno medicine in treatment of malaria, sore throat (aerial part) and toothache (bark). The powdered bark of the plant with little salt is used for treating acute diarrhea (Mann *et al*., 2003). The root is said to cure impotency (James and Duke., 1983). Astringent bark is used for diarrhea, dysentery, and leprosy. Bruised leaves are employed as poultice onto ulcers. The gum or bark is used for cancer of ear, eye, or testicles, indurations of liver, spleen and excess flesh. Bark, leaves, and young pods are strongly astringent due to tannins and are chewed in Senegal as anti-scorbutic. Bark decoction is drunk for intestinal pains and diarrhea. Other preparations are used for coughs, toothache and syphilitic ulcers. In Lebanon, the resin is mixed with orange-flower and used as infusion for typhoid convalescence. Egyptian Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods (Duke., 1983) Extracts of plant were found to be inhibitory to at least four species of human pathogens (Umalkar,. 1977).

2- Materials and Methods

The heartwood of Acacia *nilotica* var *adstringens* was collected in April 2016 from Elobied **(**western Sudan**)**. The plant was authenticated by the Dept. of Botany, University of Khartoum.

2.1 Extraction of flavonoids

Powdered shade-dried heartwood (1kg) of *Acacia nilotica* var *adstringens* was macerated with 80% methanol (5L) at ambient temperature for 48hr. The solvent was removed *in vacuo* to give a crude product(140g).

2.2 Isolation of flavonoids

The methanolic extract of *Acacia nilotica var adstringens* (2g) was applied on top of a polyamide(800g) column(100x5cm) and stepwise gradient elution(water : methanol) started with 100% % water and ended with 100% methanol. Fractions of 100 ml were collected and investigated by PC (Whatman No.1) using three solvent systems: BAW, 15% AcOH and distilled water. Chromatograms were visualized under UV light before and after exposure to ammonia vapors. Similar fractions were companied and concentrated to dryness under reduced pressure to obtain main fractions. Detected compounds were isolated by subsequent PPC from column fraction using different solvent

systems (BAW, 15% acetic acid).The isolated compounds were further purified by rechromatography on Sephadex LH-20 column using MeOH , (MeOH: H_2O)and H_2O as eluents.

2.3 Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto hutment agar slopes and incubated at 37ºC for 24 hours.The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100ml of normal saline to produce a suspension containing about 108 – 104 colony forming units per ml. The suspension was stored in the refrigerator at 4º C until used. The average number of viable organism per ml of the saline suspension was determined by means of the surface viable counting technique.

Serial dilution of the stock suspension were made in sterile saline in tubes and one drop volumes (0.20ml) of the appropriate dilution were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates.The plates were allowed to stand for two hours at room temperature for drop to dry, and then incubated at 37ºC for 24 hours.

2.4 Testing for antibacterial activity

The cub-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of test sample. (2ml) of the standardized bacterial stock suspension were mixed with 200ml of sterile molten nutrient agar which was maintained at 45º C in water bath. (20ml) Aliquots of the incubated nutrient agar were distributions into sterile Petri dishes; the agar was left to settle in each of these plates which were divided into two halves. Two cubs in each half (10mm in diameter) were cut using sterile cork borer (No.4). Each of the holes was designed for a test sample. Separate Petri dishes were designed for standard antibacterial chemotherapeutic (ampicillin and gentamycin).

The agar discs were removed, a hamate cups were filled with (0.1ml) of test samples using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for two hours.

The above procedure was repeated for different concentrations of the isolated compound and the standard antibacterial chemotherapeutics. After incubation, the diameters of resultant growth inhibition zones were measured in duplicates and averaged.

3-Results and Discussion

3.1-Identification of compound I

The IR spectrum of compound I (Fig.1)showed : ν (KBr) 662(C-H, Ar. bending), 1070(C-O), 1450, 1460(C=C,Ar.),1730(C=O,α,β-unsatd.) ,2863,2929(C-H, aliph.) and 3396cm1 (OH)

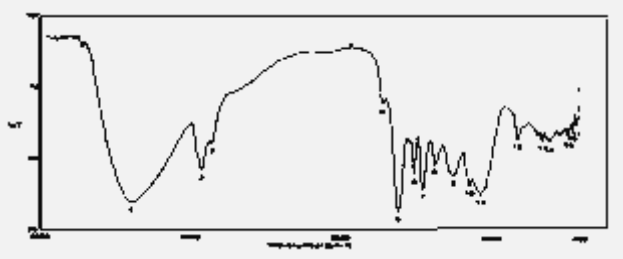


Fig1: IR spectrum of compound I

The UV spectrum gave λ_{max} (MeOH) 248,348nm (Fig.2) which is a characteristic absorption of flavones (Mabry *et al*., 1970).Useful structural features are obtained by using UV shift reagents. Addition of the shift reagent -sodium methoxide- to a methanolic solution of compound I gave a 49nm bathochromic shift(Fig.3) in band I, without decrease in intensity, indicating a free 4`-OH(Harborne,1989). The sodium acetate spectrum(Fig.4) gave a 5 nm bathochromic shift at band II which is indicative of a 7-OH function (Mabry et. al.,1976). The boric acid spectrum(Fig.5) revealed a bathochromic shift indicative of a B-ring catechol moiety. When aluminium chloride was added to a methanolic solution of compound I, band I shifted bathochromically by 29nm(Fig.6). The aluminium chloride spectrum degenerated in HCl indicating a catechol system. Thus supporting the previous findings with boric acid .

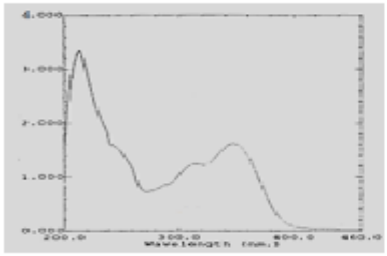


Fig.2: UV spectrum of compound I

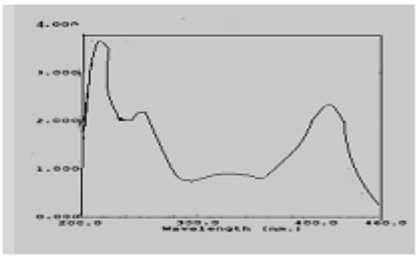


Fig.3: Sodium methoxide spectrum of compoundI

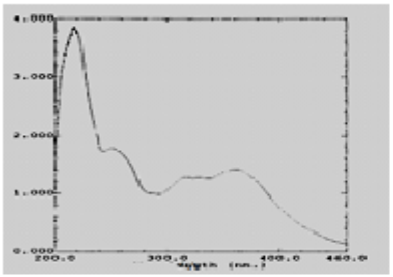


Fig4: Sodium acetate spectrum of compound I

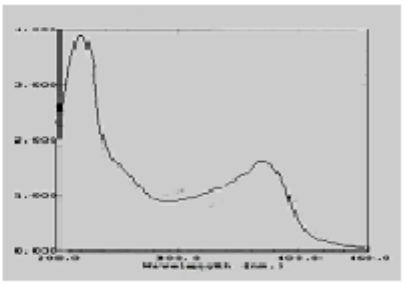


Fig.5: Boric acid spectrum of compound I

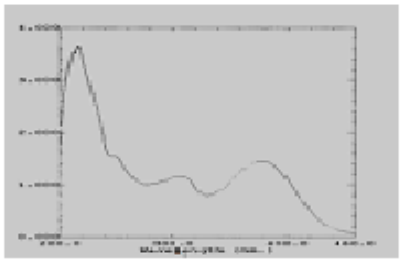
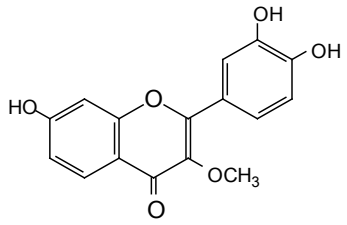


Fig 6: Aluminium chloride spectrum of compound I

^{1}HNMR spectrum (Fig.8) showed a doublet at δ 7.8ppm (1H), characteristic of C_5-proton. The doublet at δ. 6.8ppm (2H) is characteristic of C_6-and C_8- protons, while the doublet at δ. 7.5ppm (2H) accounts

for $C_{2`}$- and $C_{6`}$- protons of (B) ring. The resonance at δ.7.3ppm (1H) is characteristic of $C_{5`}$ -proton(ring B). The singlet at δ. 3.7ppm (3H) was assigned for a methoxy function .The Mass spectrum gave m/z 300 for the molecular ion. Comparison with available literature data showed that compound I is a :7, 3`, 4`-trihydroxy-3- methoxyflavone:



Compound I

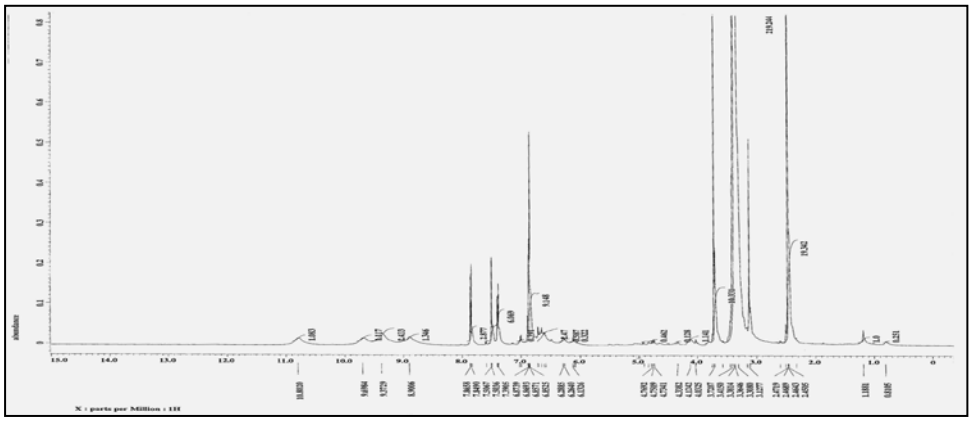


Fig.8: ^{1}HNMR spectrum of compound I

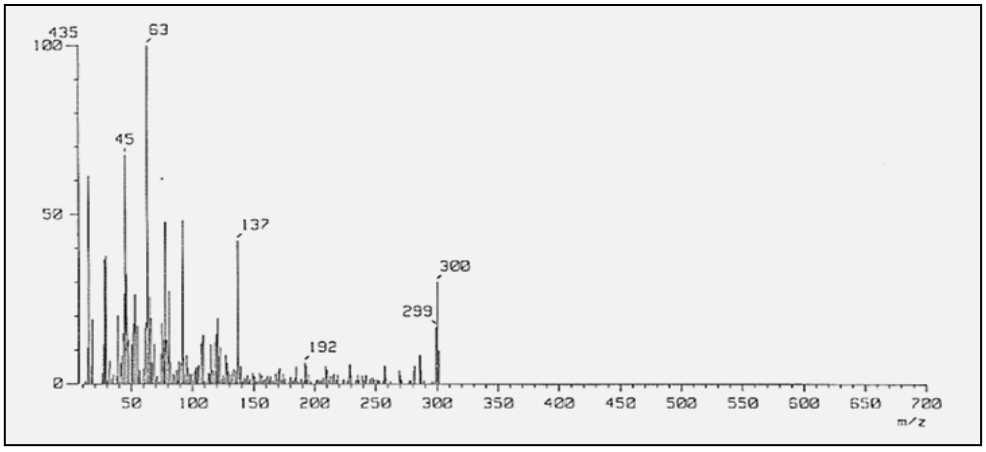
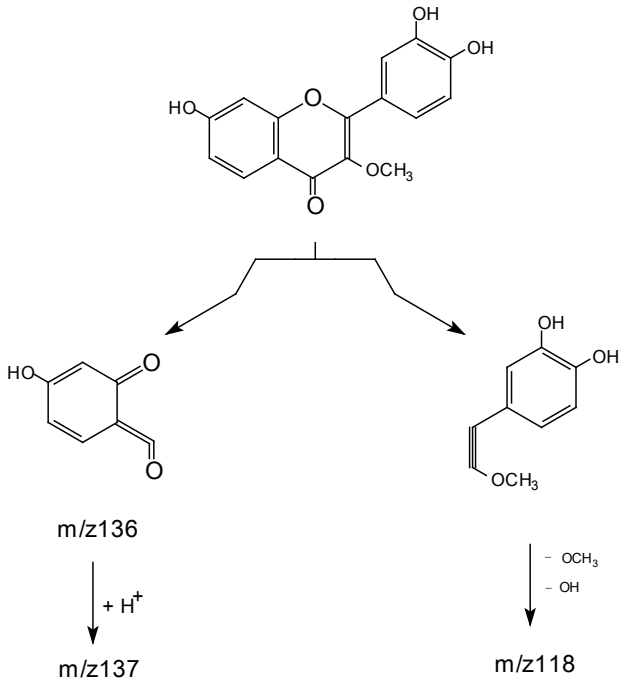


Fig. 9: MSS spectrum of compound I

Additional evidence in favor of the proposed structure comes from the retro Diels-Alder fission (Scheme I) where the ions m/z137 and m/z118 which correspond to intact A and B rings were detected in the electron beam.



Scheme I : Retro Diels - Alder fission

3.2-Antimicrobial activity

Compound I was examined for antimicrobial activity against Gram positive bacteria(*Bacillus subtilis*, *Bacillus cereus* and *staphylococcus aureus*) *and* Gram negative bacteria (*Escherichia coli*, *pseudomonas aeruginose*).The obtained results (Table 1) revealed that the test compound showed different antimicrobial responses. It revealed high potency against Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus.*

Table 1: Antibacterial activity of compound I

Bacteria	Gram stain reaction	Control (Methanol)	Compound I
Bacillus cereus	Positive	0.0	13
Bacillus subtilis	Positive	0.0	21
Staphylococcus aureus	Positive	0.0	20
Escherichia coli	Negative	0.0	10
Pseudomonas aeruginosa	Negative	0.0	13

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