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RESEARCH ARTICLE

Isolation and Characterization of a Flavone From Acacia orfota (Forssk.) Schweinf and Biological Activity of Total Extract

Abdel Karim. M.1(*) , Abdelwahab A.2, Sayed,E.3, Mahmoud,I.N.4

1-Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry

2- University of Zalingei , Sudan

3- National Research Centre, Dept. of Tannins Chemistry , Dokki,Cairo,12622,Egypt

4-Department of Natural Compounds Chemistry, National Research Centre (ID: 60014618), Dokki, 12311, Cairo, Egypt.

Correspondence
Abdel Karim. M.
Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry

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ABSTRACT

Information on the constituents of medicinal plants used in Sudanese ethnomedicine is very scarce. Hence, this study was set to investigate the phenolics of the medicinally important species Acacia orfota which is widely used in ethnomedicine to treat a wide array of human disorders. A Flavone was isolated from the leaves and its structure was partially elucidated on the basis of its spectral data(UV,1HNMR and MS). Antimicrobial activity of the total extract was screened in vitro against a panel of Gram positive and Gram negative bacterial pathogens and fungi, in comparison with control drugs and significant results were obtained.

INTRODUCTION

Acacia genus (Family Fabaceae) involves about 1350 species [1] . These species are considered as a rich source of gallic and ellagic acids [2] . Acacia species are characterized by the presence of flavonoids and phenolics [3]. Some Acacia species find wide applications in ethnomedicine as antidiarrhoeic, antidiabetic, antiamoebic, anti-inflammatory and hypotensive [4]. Many Acacia species were found to exhibit antimicrobial activity[5].

In Sudanese ethnomedicine Acacia nilotica is used as a remedy for malaria, sore throat , cough, intestinal worms and wounds[6-9].The plant is used commercially in Sudan for leather tanning[10]. The gum from Acacia seyal- Gum Arabic- is considered as a safe dietary fiber by the United States Food and Drug Administration since 1970s. Although its effects were extensively studied in animal models , there is paucity of data regarding quantified use in humans[11].Acacia gum has been used in pharmaceuticals as demulcent . It is also used topically for healing wounds and has been shown to inhibit periodontic bacteria and early deposition of plaque[12].

The ethanolic extract of the leaves of Acacia nilotica ,which is rich in phenolics, showed potent antioxidant activity against stable DPPH radical [13]. Also in DPPH bioassay, Duduku et. al.[14] evaluated the antioxidant capacity of the medicinally important species Acacia auricoliformis.

Little information is available about Acacia orfota (Forssk.) Schweinf., growing in Sudan.The present study deals with the isolation and identification of flavonoids of Acacia orfota leaves as well as the antimicrobial activity of the total extract.

Materials and Methods

General

An Agilent Cary Series Spectrophotometer was used to obtain UV spectra. NMR spectroscopy was carried out using a Bruker 400 MHz in MeOH-d4

Plant material

The leaves of Acacia orfota were collected from Kordofan, west Sudan in March 2016 and identified by direct comparison with a herbarium sample.

Extraction and isolation of flavonoids

Powdered shade-dried leaves of Acacia orfota (1.5Kg) were exhaustively extracted by 70% methanol. The dry extract was suspended in water and fractionated successively with n-hexane, chloroform,

ethyl acetate and n-butanol. The n- butanol fractions were evaporated to dryness, yielding 34.5g. Two dimentional paper chromatography of n-butanol fractions revealed the presence of several purple and blue bands for phenolic compounds. Further fractionation on a polyamide 6S column eluted by water/methanol in decreasing polarity gave 30 fractions which were finally collected to seven major fractions after examination by PC. Purification on a Sephadex LH-20 column gave compound I in chromatographically pure form.

In-vitro antimicrobial activity

The methanol extract of Acacia orfota was screened in vitro against a panel of Gram positive and Gram negative bacterial pathogens, and fungi, in comparison with control drugs: Thiophenicol (Thiamphenicol, Sanofiaventis, France) as an antibacterial agent, and Treflucan (Fluconazole, Egyptian International Pharmaceutical Industries Company- EIPICO) as an antifungal agent, by the agar diffusion technique[15,16].

Five different concentrations of the extract were prepared and individually tested against Gram positive bacteria (Bacillus subtilis ATCC6633 and Staphylococcus aureus ATCC29213), Gram negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC27953), and fungi (Candida albicans ATCC 10321, Aspergillus niger NRRL-363, Fusarium oxysporium NRC23, Alternaria alternata NRC43 and Alternaria tenuissima KM651985). All microorganisms used were obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Centre, Cairo, Egypt.

Preparation of the discs

Different concentrations of the extract were mounted on paper discs prepared from blotting paper (5 mm diameter) on a concentration of (2.0, 1.0, 0.5, 0.25 and 0.125 mg/5µL DMSO/disc). Thiophenicol and Treflucan were used as positive controls for antibacterial and antifungal activity, respectively, in a concentration of 100 µg/disc. DMSO showed no inhibition zones and was used as a negative control.

Preparation of agar plates and inoculation procedure

Agar plates were prepared by using 100 mL of suspension containing 1 x108 CFU/mL of pathological test bacteria and 1 x106 CFU/mL of fungi spread on nutrient agar (NA) and potato dextrose agar (PDA) respectively. After the media had cooled and solidified, the discs were applied on the inoculated agar plates and incubated for 24

h at 30 ºC for bacteria and 72 h at 28 ºC for fungi. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition around the disc in millimeters (mm) and compared with that of the controls.

Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentration (MIC) of the extract was evaluated at the final concentrations; 1000, 500, 250, 125 and 62.5 µg/disc. The lowest concentration showing inhibition zone around the disc was taken as the minimum inhibitory concentration.

Results and Discussion

Sequential solvent extraction followed by polyamide and Sephadex columns allowed isolation of a chromatographically pure flavonoid- compound I from *Acacia orfota* leaves. The structure of the isolate was partially elucidated via a combination of spectral techniques(UV, ^{1}HNMR and MS). The total extract was evaluated for *in vitro* antimicrobial activity against a panel of human pathogens.

Compound I

In UV , compound I absorbs(Fig.1) at λ_{max} (MeOH) 268,331nm.Such absorption indicates conjugation between the 4 keto function and the B aromatic ring of the flavonoid nucleus. It is characteristic of flavones [17].

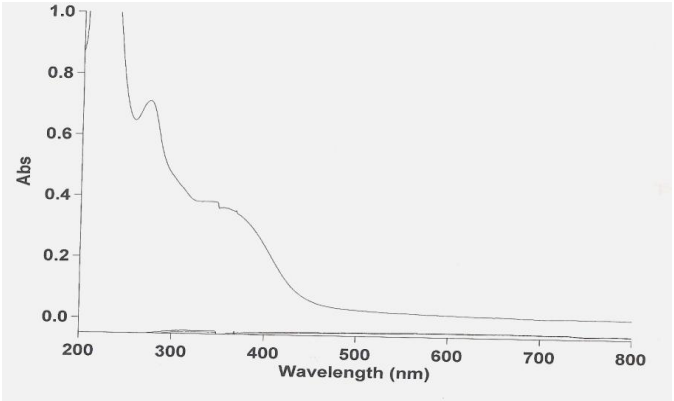
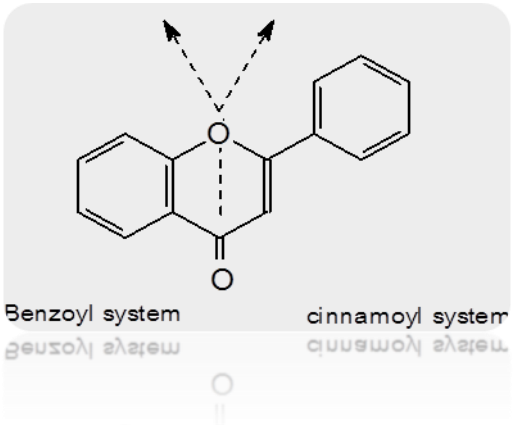
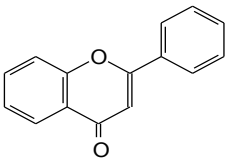


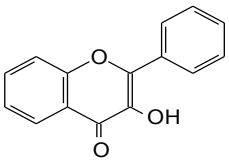
Fig.1 : UV spectrum of compound I

In their UV spectra flavones give both band I(due to cinnamoyl chromophore) and band II(due to benzoyl chromophore) , a feature which is shared by flavonols , chalcones and aurones . Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols afford only one peak originating from the benzoyl system. Band I , usually 300 – 400nm and band II ,usually 240 – 280 nm[126] .

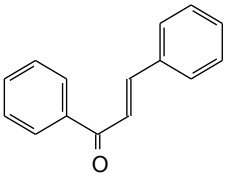




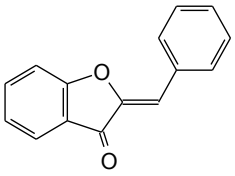
Flavone



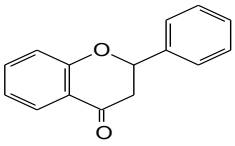
Flavonol



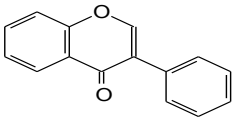
Chalcone



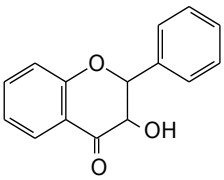
Aurone



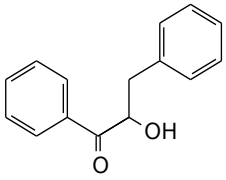
Flavanone



Isoflavone



Dihydroflavonol



Dihydrochalcone

Very significant structural features have been obtained by utilizing the so- called UV shift reagents which produce shifts in the UV absorption maxima in accordance with the location of the various hydroxyl functions in flavonoid nucleus ; these reagents are : sodium methoxide (which is diagnostic of 3- and 4`-OH functions);sodium acetate (diagnostic of 7-OH function); aluminium chloride (diagnostic of 3- , 5-OH and catechol systems) and boric acid (diagnostic of catechol systems)[17].

When sodium acetate was added to a methanolic solution of compound I, a bathochromic shift diagnostic of a 7-OH function was observed. Other shift reagents failed to give any detectable bathochromic shift.

The ^{1}HNMR spectrum(Fig.2) showed : δ 1.35ppm assigned for a methyl group ; δ 3.36 characteristic of a methoxyl function ; δ3.50-4.64 assigned for sugar moiety; δ6.22 assigned for C_6- proton. Other aromatic protons appeared at δ6.47ppm, δ6.94 and 7.40(B ring) , while C_5 proton resonated as anticipated at lower field (δ7.88) due to the deshielding influence of the neighboring 4 keto function. The EI mass spectrum gave m/z283 for M^+ (aglycone).

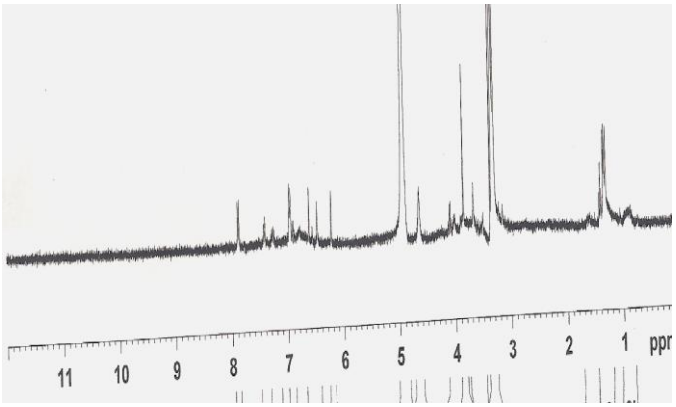
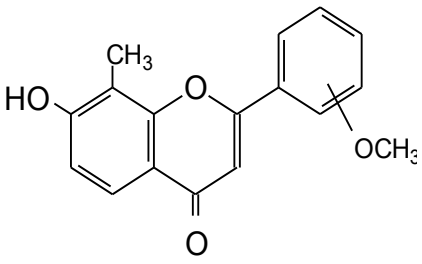


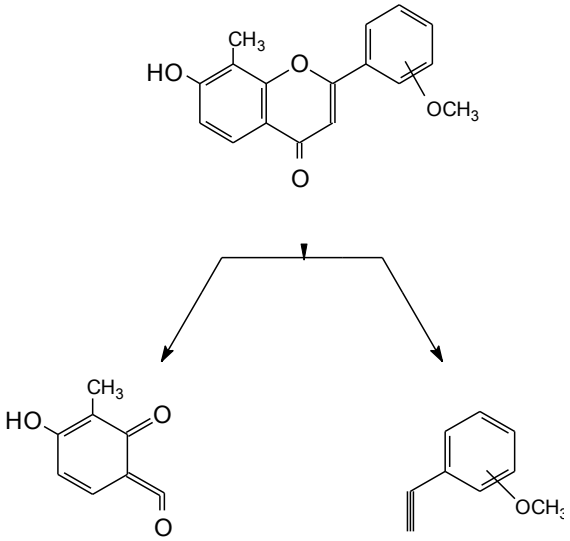
Fig.2 : ^{1}HNMR spectrum of compound I

Two important fragments resulting from retro Diels – Alder fission(Scheme I) and corresponding to intact A and B rings appeared at m/z151 and m/z131 respectively. This lends

evidence for the following partial structure for aglycone of compound I:



compound I(aglycone)



m/z151 m/z131

Scheme I : Retro Diels-Alder fission of compound I(aglycone)

Bioassay

Results of antibacterial test of different concentrations of the total extract are displayed in Table (1) . Different concentrations exhibited different antimicrobial responses against Gram positive and Gram negative bacteria, with zones of inhibition ranging from 6 to 13mm. The extract showed good activity against *B. subtilis*, *P. aeruginosa* and *E. coli* with zones of inhibition of 13, 13 and 12 mm, respectively with minimum inhibitory concentrations (MIC) 62.5, 125 and 125 µg, respectively (Table 3). However it showed moderate inhibitory activity against the Gram positive bacterial pathogen *S. aureus* with zone of inhibition 9 mm.

Different concentrations of the extract were tested for their antifungal activity against *C. albicans* and other four pathogenic fungi. The results are displayed in Table (2) . The extract exhibited a moderate inhibitory activity against the yeast *C. albicans* in comparison with Treflucan.

Conc. (mg/5µL (DMSO/disc)	Gram positive bacteria		Gram negative bacteria	
	B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli* ATCC 25922	*P.aeruginosa* ATCC27953
2.0	13	9	12	13
1.0	12	8	11	11
0.5	11	6	10	8
0.25	10	6	9	6
0.125	8	N.A.	7	6
Thiophenicol	22	11	15	14
Treflucan	N.A.	N.A.	N.A.	N.A.

Table (1): Antibacterial activity of extract

Table (2): Antifungal activity of extract

Conc. (mg/5µL (DMSO/ disc)	*C.albicans*	*A.niger*	*F.oxysporium*	*A.alternata*	*A.tenuissima*
2.00	8	N.A.	N.A.	N.A.	N.A.
1.00	7	N.A.	N.A.	N.A.	N.A.
0.50	7	N.A.	N.A.	N.A.	N.A.
0.250	6	N.A.	N.A.	N.A.	N.A.
0.125	N.A.	N.A.	N.A.	N.A.	N.A.
Thiophenicol	N.A.	N.A.	N.A.	N.A.	N.A.
Treflucan	8	13	15	20	22

Table (3): Minimal inhibitory concentration (µg/disc)

Entry	Gram positive bacteria		Gram negative bacteria		Fungi
	B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli* ATCC25922	*P.aeruginosa* ATCC27953	*C.albicans* ATCC 10321
Crude extract	62.5	250	125	125	250
Thiophenicol	3.13	3.13	25	25	-
Trefluca n	-	-	-	-	25

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