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PARTIAL CHARACTERIZATION OF AN ISOFLAVONE FROM SUDANESE *COMBRETUM ACULEATUM* (COMBRETACEAE) ROOTS AND ANTIMICROBIAL ACTIVITY OF LEAVES METHANOL EXTRACT

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ABSTRACT

Combretum aculeatum is a climbing shrub growing up to 4 m. In African system of medicine, the plant is used for treating some human diseases. This study was designed to investigate the major flavonoid of *Combretum aculeatum* roots and to screen the antimicrobial activity of leave methanol extract. The flavonoids were extracted with ethanol and the crude extract was purified by paper chromatography where an isoflavone has been isolated. The structure of this compound has been partially characterized by some spectral tools (UV and ^{1}HNMR). In the antimicrobial assay, the methanolic extract showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. It showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. The extract exhibited significant antifungal activity against the fungi: *Candida albicans and Aspergillus niger*.

KEYWORDS: *Combretum aculeatum*, flavonoids, isolation, antimicrobial activity.

INTRODUCTION

Combretum aculeatum (Combretaceae) is sub-Sahelian dry zone species. In Africa it is distributed from Senegal to Mauritania and from Somalia to Tanzania. *Combretum aculeatum* is a climbing shrub growing up to 4 m, even taller if support is available.[1] The plant is an important nutrient for animals, which consume the leaves and flower.[1,2] In African system of medicine, the plant is used for treating some human diseases.[1,3] The leave and root extracts showed antibacterial activity.[4] The plant is purgative and diuretic. It is used to treat blennorrhoea, colic, diarrhoea, intestinal worms, wounds, fever, gastritis and loss of appetite. Aqueous extract of the leaves is used to promote micturition in cases when venereal disease obstructs the urethra. In some African countries *Combretum aculeatum* is used against leprosy. Traditionally the root is used against catarrh and eye troubles. Boiled roots are taken in Kenya for stomach upsets. Macerations of roots are used to enhance wound healing. In Sudanese ethnomedicine, the aqueous extract of roots is used as a purgative and as a poultice for skin tuberculosis. Some members of genus *Combretum* are well known in traditional medicine where they are used against a wide range of diseases including abdominal pain, back-pain, cough, cold, diarrhea, earache, fever, headache, worms, infertility in women, leprosy, scorpion stings and snake bite.[5,6]

Some interesting phytochemicals have also been reported from *Combretum* species including: a substituted bibenzyl from *C. molle*,[7] Some triterpenes and their glycosides were isolated from *C. laxum*.[8] The alkaloids combretine and betonicine were isolated from the leaves of *C. micranthum*. Kaempferol and other flavonoids were reported from *C. erythrophyllum*,[9] *C. apiculatum* and *C rdamonin*. A Chalcone was isolated from *C.apiculatum*,[10] and ellagic acid derivatives from *C. kraussii*.[12] Several *Combretum* species were reported to contain a group of stilbenes known as combretastatins.[12]

MATERIALS AND METHODS

Plant Material

The roots of *Combretum aculatum* were collected from a forest reserve around Hawata western Sudan. The plant was authenticated by direct comparison with a reference herbarium sample.

Equipments

Ultraviolet spectra were recorded in spectroscopic methanol on a Shimadzu UV -Visible Spectrophotometer. ^{1}HNMR spectrum was measured in

spectroscopic grade DMSO-d_6 on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz.

Methods

Isolation of flavonoids

Powdered roots of *Combretum aculeatum* (950g) were macerated with 95% ethanol for 72 hours. The solvent was evaporated under reduced pressure. The resulting extract was applied on Whatman No. 3mm papers as concentrated narrow zones. The bands were developed with BAW(4:1:5;V:V:V). The developed papers were air-dried and examined under UV light (λ_{max} 366nm). The equivalent bands from each paper were combined, cut into small pieces and the product was eluted with methanol. The solvent was evaporated to give a powder - compound I.

Different UV shift reagents (sodium methoxide, sodium acetate and aluminium chloride) were used to elucidate the hydroxylation pattern on the nucleus of the isolated flavonoid and they were used as follows: the UV spectrum of compound I, in methanol, was first recorded; 3 drops of sodium methoxide reagent were added to the sample and the sodium methoxide spectrum was recorded; 6 drops of $AlCl_3$ reagent were added to the fresh sample and the $AlCl_3$ spectrum was recorded; powdered sodium acetate was then added to the fresh sample, the mixture was shaken and the sodium acetate spectrum was recorded.

RESULTS AND DISCUSSION

Compound I, which was isolated from *Combretum aculatum* roots via paper chromatography, was partially characterized by its spectral data. The UV spectrum of compound I showed (Fig.1) λ_{max}(MeOH) 281,340sh. nm. The appearance of only one band- band II – together with a shoulder at 340nm suggests an isoflavone.[13] The hydroxylation pattern of this compound was studied using the UV shift reagents; sodium methoxide (diagnostic of 3- and 4`-OH); sodium actate (diagnostic of 7-OH); aluminium chloride (diagnostic of 3- and 5-OH and catechol systems) and boric acid (diagnostic of catechol moieties). The sodium methoxide spectrum (Fig.2) did not show a bathochromic shift indicative of a 3- or 4`-OH functions. The sodium acetate spectrum (Fig.3) did not reveal any bathochromic shift indicating absence of a 7-OH. Also no bathochromic shifts were observed in the aluminium chloride (Fig.4) and boric acid(Fig.5) spectra. This data suggest absence of 3-, 5-OH groups as well as catechol systems.[13]

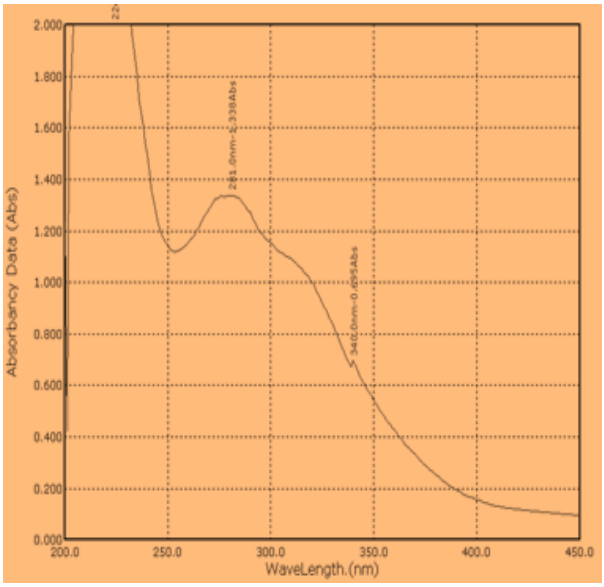


Fig. 1: UV spectrum of compound I.

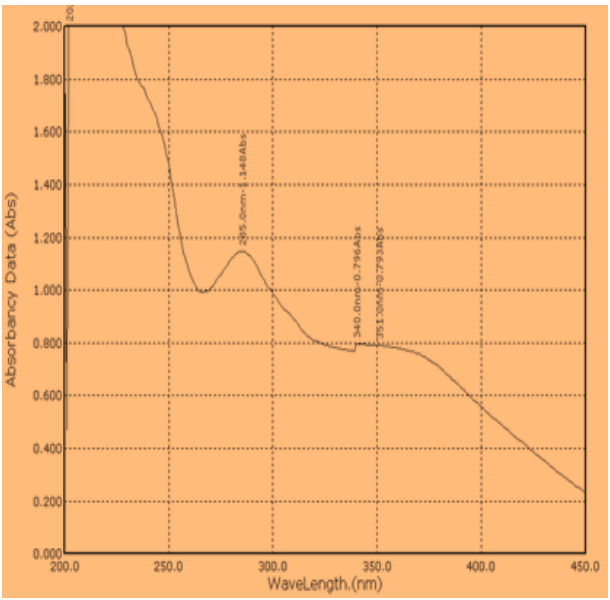


Fig. 2: Sodium methoxide spectrum of compound I.

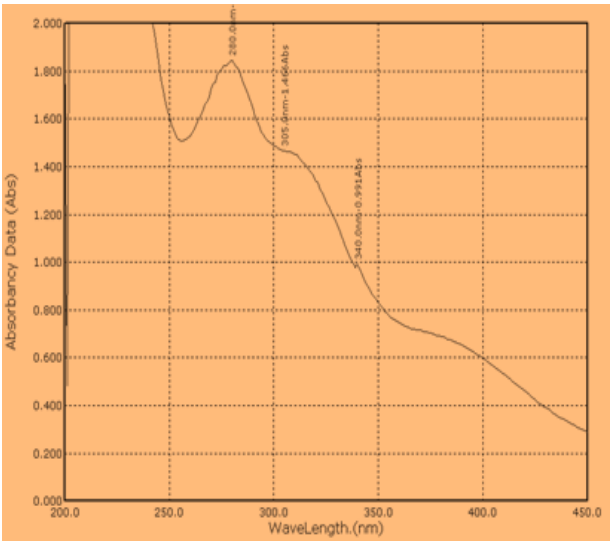


Fig. 3: Sodium acetate spectrum of compound I.

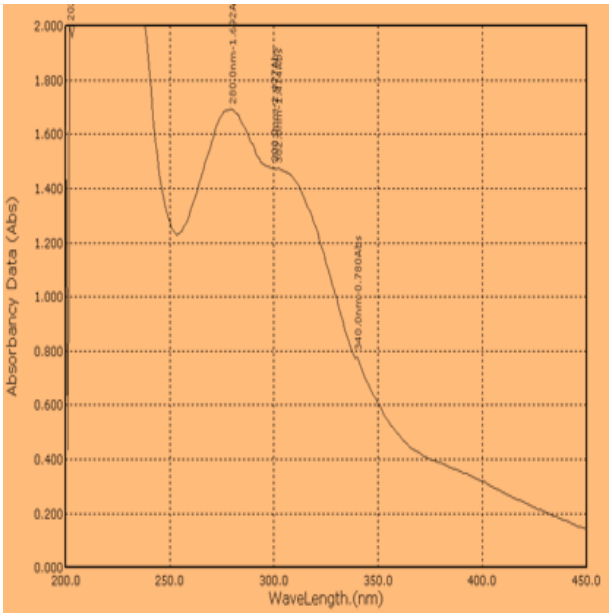


Fig. 4: Aluminium chloride spectrum of compound I.

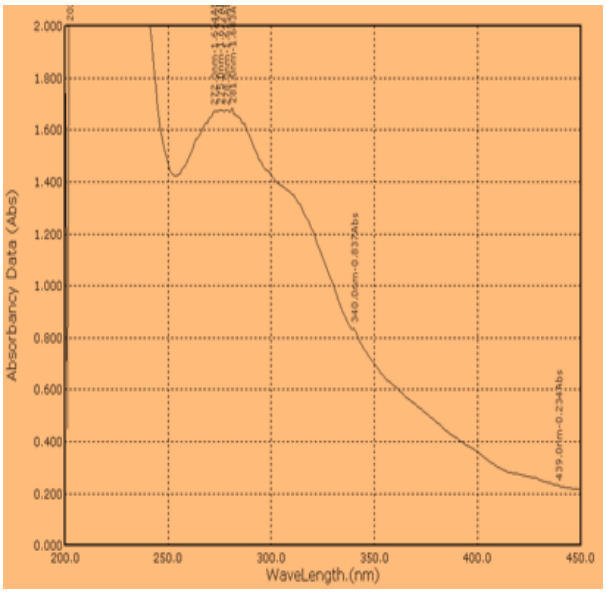


Fig. 5: Boric acid spectrum of compound I.

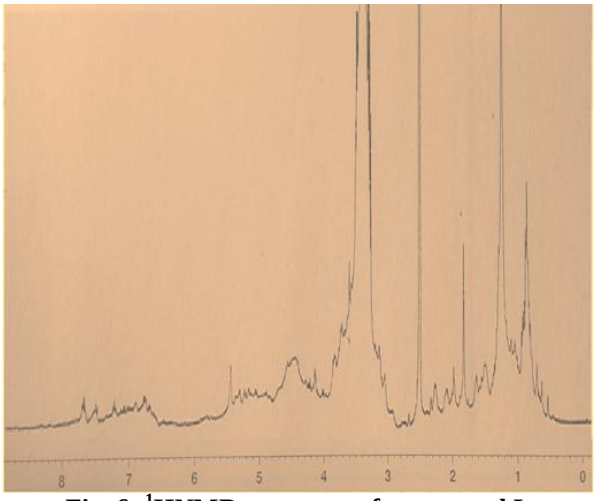
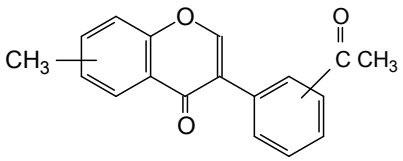


Fig. 6: [1]HNMR spectrum of compound I.

The [1]HNMR spectrum (Fig.6) showed δ(ppm): 1.25 (assigned for a methyl); 2.90(Acetyl); 4.00-5.50(assigned for a sugar residue-not identified in this study); 6.60 - 7.40 (multiplet, Ar. protons), 7.50 and 7.70 (Ar. protons). The signal at δ2.50 and δ3.50 is 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

Antimicrobial assay

The methanol extract and of *Combretum aculeatum* was screened for antimicrobial potential against six standard human pathogenic bacteria (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*). The methanolic extract showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. It showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. The extract also exhibited significant antifungal activity against the fungi: *Candida albicans and Aspergillus niger* (see Table 1).

Table 1: Inhibition zones of methanol extract.

	Inhibition zone diameter(mm); (100mg/ml sample)					
	*Ec	Pa	Bs	Sa	Ca	An
Methanoli Extract	22	30	17	17	19	22

*Ec= *Escherichia coli*
Bs= *Bacillus subtilis*
Sa= *Staphylococcus aureus*
Pa= *Pseudomonas aeruginosa*
Ca= *Candida albicans*
An= *Aspergillus niger*

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