

Phytochemical and Biological Studies on *Combretum aculeatum* (Combretaceae) Leaves

Abdel Karim, M^{1*}, Maha, M¹, Nahla, A³ and Wafa, T³.

1-Sudan University of Science and Technology, Faculty of Science

2-National Research Centre, Cairo

Abstract – From ethyl acetate extract of the leaves of *Combretum aculeatum* a flavone was isolated using column and paper chromatography. The structure of the flavone was partially elucidated by a combination of spectral techniques (UV, ¹HNMR and MS). The methanolic and ethyl acetate fractions were evaluated for their antimicrobial and antioxidant properties. The methanolic extract showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. It also showed good activity against *Bacillus subtilis* and *Staphylococcus aureus*. This extract also exhibited significant antifungal activity against the fungi: *Candida albicans* and *Aspergillus niger*. However, the ethyl acetate fraction did not show any activity. *In vitro* antioxidant assay for the methanolic and ethyl acetate fractions was conducted. The assay was carried out by measuring the capacity of each extract against stable DPPH radical. The change in colour is measured spectrophotometrically at 516nm. Both methanolic and ethyl acetate fractions exhibited significant antioxidant capacity.

Keywords: *Combretum aculeatum*, Isolation, Flavone, Biological Activity.

Introduction

Combretum aculeatum belongs to the family of Combretaceae. The plant is sub-Saharan dry zone species. In Africa it is stretching from Senegal and Mauritania, to Somalia and Tanzania. Its distribution in Sudan is irregular and is locally common. In Sudan the plant is known as "Sheihheit". *Combretum aculeatum* is a climbing shrub growing up to 4 m, even taller if support is

available (Shanon et al., 2007). *C. aculeatum* is an important nutrient for animals, which consume the leaves, flower and young shoots (Shanon et al., 2007; Le Houérou, 1980). The plant is used traditionally throughout Africa for treating an array of human disorders (Shanon et al., 2007; Orwa, 2009). Biological studies of its leaves and roots extract showed antibacterial activity (Albagouri et al., 2014).

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 in Senegal to promote micturition in cases when venereal disease obstructs the urethra. In Burkina Faso and Senegal the plant is used for leprosy. In Senegal, the root is used traditionally against catarrha and eye troubles. Boiled roots are taken in Kenya for stomach upsets. Macerations of roots are used to enhance wounds healing. In Sudanese ethnomedicine, the aqueous extract of roots is used as a purgative and as a poultice for skin tuberculosis.

Some *Combretum* species are well known in traditional medicine and are used for treatment of many diseases, such as abdominal pain, back-pain, cough, cold, diarrhea, earache, fever, headache, fighting worms, infertility in women, leprosy, scorpion stings and snake bite (Awatif et al., 2007; El-Amin, 1990).

Several interesting constituents have also been isolated from *Combretum* species including: a substituted bibenzyl from *C. molle* (Ahmed et al., 2014). Some triterpenes and their glycosides were isolated from *C. laxum* (Pietrovski, 2006). The

alkaloids combretine and betonicine were isolated from the leaves of *C. micranthum*. Kaempferol and other flavonoids were reported from *C. erythrophyllum* (Angh, 2006), *C. apiculatum* and *C. rdamonin*. A Chalcone was also isolated from *C. apiculatum* (Rogers et al., 1996) and ellagic acid derivatives from *C. kraussii* (Banskota et al., 2000). Several *Combretum* species were reported to contain a group of stilbenes known as Combretastatins (Ogan, 1972).

Several phytochemical investigations on this genus focus mainly on pentacyclic triterpenoids, various polyphenols like flavonoids and stilbenoids (Martini et al., 2004). GC/MS showed presence of triterpenoids and stilbenoids in dichloromethane fractions of leaf and stem bark of *C. aculeatum*, *C. glutinosum* and *C. micranthum*. Ursolic acid was identified in the leaf extracts of all the three species whereas combretastatin was found in small amounts only in the bark extract of *C. glutinosum* species (Aderogba et al., 2012).

Oleanene-type of pentacyclic terpenoids containing 29-carboxyl-1 α -hydroxyl groups were isolated from various species of *Combretum* e.g. *C. molle* and *C. imberbe* confirming chemotaxonomically significant bifurcation in triterpenoids synthesis in *Combretum* species (Chlaabi, 2008).

Materials and methods

Materials

Analytical grade reagent were used. The UV spectra were recorded on a Shimadzu UV – 2401PC Spectrophotometer and a UV lamp was used for localization of spots on TLC plates. Nuclear Magnetic Resonance spectra were run on a Joel ECA 500MHZ NMR Spectrophotometer. Mass spectra were measured on a Joel MS Spectrometer (JMS- AX500).

Plant Material

The leaves of *Combretum aculeatum* were collected from Basunga, Gadaref State during June, 2016. The plant was authenticated by the Department of

Phytochemistry and Taxonomy, National Research Center, Khartoum.

Methods

Extraction and isolation of flavonoids

Powdered shade-dried leaves of *Combretum aculeatum* (1 kg) were macerated with 5 liter 80% aqueous methanol for 48hr at ambient temperature with occasional stirring and then filtered off. The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated under reduced pressure, yielding a crude product, which was suspended in 300 ml water and partitioned successively with chloroform, ethyl acetate and n-butanol to afford four fractions: methanolic, chloroform, n-butanol and ethyl acetate fractions which were subjected to TLC experiments for comparison.

The ethyl acetate fraction (5 g) - being rich in phenolics - was chromatographed on a silica gel column (100-200 mesh) using (CH₂Cl₂: MeOH) as eluent. Elution commenced by CH₂Cl₂:MeOH (95:5, v:v) in increasing order of polarity stepwise until 100% methanol. Fractions of 10 ml were collected, and then investigated by thin layer chromatography. The spots were visualized under UV light using detecting reagents; NH₃ and NA. Similar fractions were combined (F35-F65), concentrated and applied on sheets of Whatman paper 3mm (40× 57 cm). The sheets were irrigated with; butanol: acetic acid: water; 4:1:5 (v:v:v) upper layer. Bands with R_f 0.62 from each paper were cut out into small strips and slurred with methanol. After several hours of contact with occasional shaking, the liquid was filtrated and evaporated under reduced pressure to give compound I.

Antibacterial activity

The methanolic and ethyl acetate fractions were screened for their antimicrobial activity against six

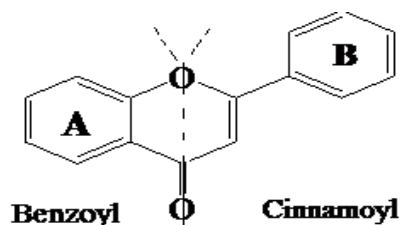
standard human pathogens (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*) using the cup plate agar diffusion method with some minor modification(Kavanagh,1972).

Antioxidant assay

Evaluation of the antioxidant capacity of the methanolic and ethyl acetate extracts was carried out by measuring the decolorizing capacity of each extract against stable DPPH radical. The change in colour was measured by a UV spectrophotometer at λ_{max} 516 nm(Sekiwa et.al.,2000).

Results and Discussion

Most flavonoids show two absorption bands; band I and II. Band I is considered to be associated with the absorption of the cinnamoyl system, while band II originates from the benzoyl system. Due to conjugation between benzoyl and cinnamoyl chromophores, flavones, flavonols, chalcones and aurones give both bands I and II, while isoflavones, dihydroflavonols, dihydrochalcones and flavanones give only band II due to loss of conjugation between the carbonyl function and ring B(Harborne et.al.,1974).



The UV spectrum compound I (Fig.1) showed λ_{max} 271,336 nm which is characteristic of flavones (Harborne et.al.,1974).

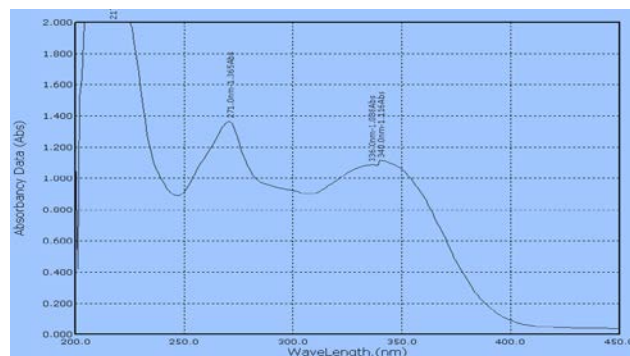


Fig.1: UV spectrum of compound I

When compound I was treated with the shift reagent sodium methoxide a bathochromic shift -with increase in intensity- was observed indicating (Harborne et.al.,1974) a free OH group at C₄ (Fig.2)

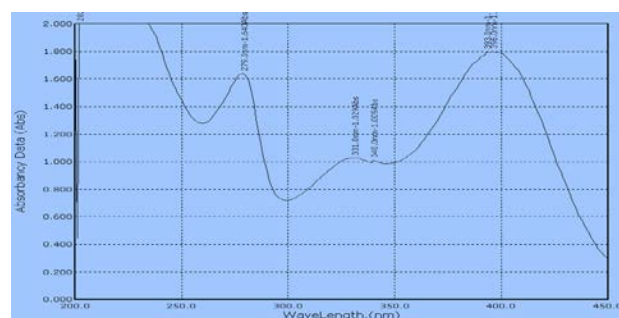


Fig.2: Sodium methoxide spectrum of compound I

The sodium acetate spectrum(Fig.3) revealed a bathochromic shift which is diagnostic (Harborne et.al.,1974) of a free 7-OH group. The aluminium chloride spectrum (Fig.4) also showed bathochromic shift. However, the spectrum degenerated(Fig.5) on addition of acid indicating a catechol system. The boric acid spectrum- which revealed a bathochromic shift cited (Harborne et.al.,1974) additional evidence in favor of the catechol moiety(Fig.6).

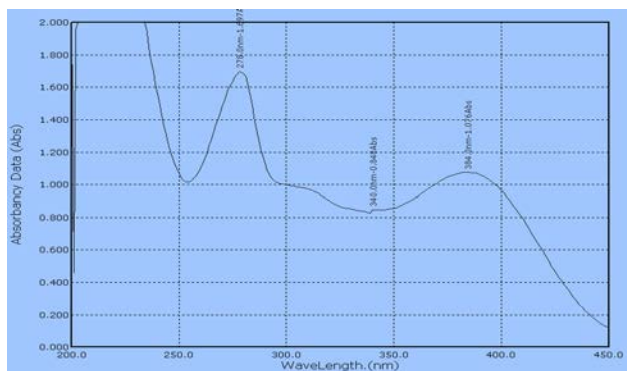


Fig.3: Sodium acetate spectrum of compound I

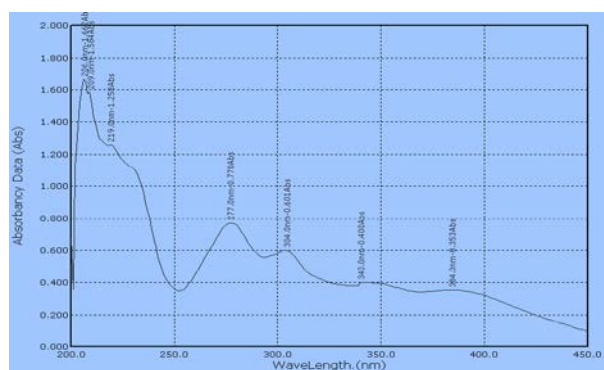


Fig.4: Aluminium chloride spectrum of compound I

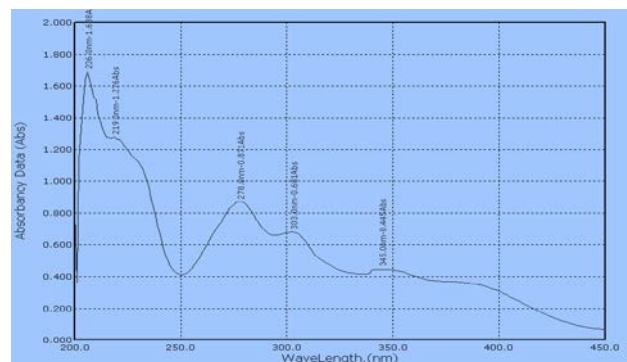


Fig.5: Aluminium chloride /HCl spectrum of compound I

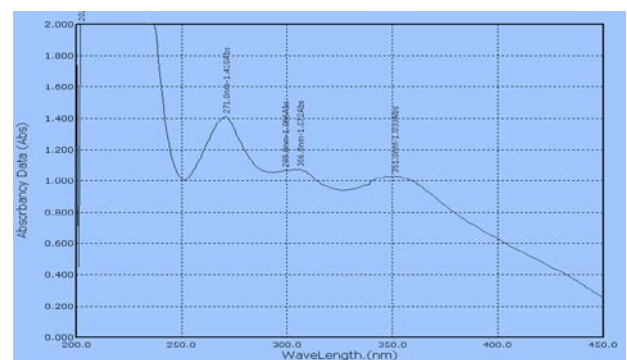


Fig.6: Boric acid spectrum of compound I

The ¹HNMR spectrum (Fig.7) revealed a pattern characteristic of flavones. The signals at δ 6.21 and δ 6.87 ppm were assigned for C₆ – and C₈ – proton respectively (Harborne et.al.,1974). Usually C₆ – H resonates at higher field relative to the C₈ – proton due to the deshielding effect of the oxygen atom at position 1. The singlet at δ 6.56 ppm accounts for the olefinic proton at the heterocyclic C ring, while the resonances at δ 7.36, 7.67 ppm account for B ring protons. Such protons resonate at lower field relative to A ring protons due to the deshielding influence of the heterocyclic C ring (Harborne et.al.,1974). The C₅- proton resonates well downfield at δ 7.98 ppm due to the deshielding influence of the neighboring 4 keto function.

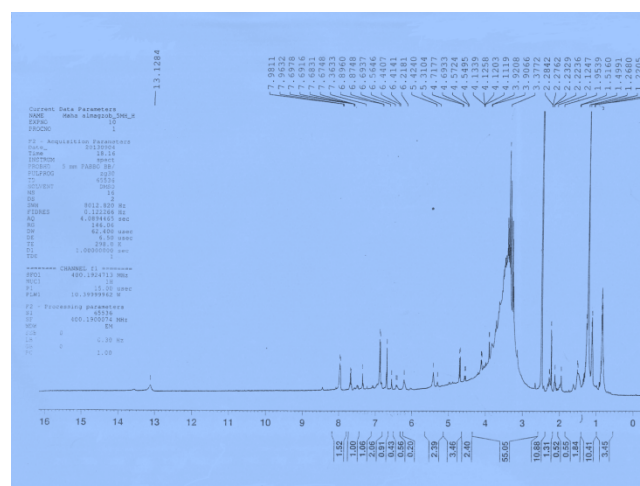
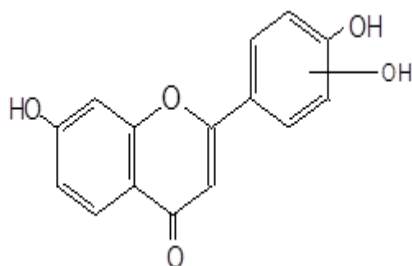


Fig.7: ¹HNMR spectrum of compound I

The mass spectrum gave m/z 288 for M⁺ + 2H and the retro Diels – Alder fission (Scheme I) – which revealed (Harborne et.al.,1974) a fragment for intact B ring at m/z 149 – cites evidence for the substitution pattern of this ring.

On the basis of the above argument, the following partial structure was suggested for compound I.



Compound I

antimicrobial potential against six standard human pathogens (*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 also showed moderate activity against *Bacillus subtilis* and *Bacillus subtilis*. This extract also exhibited significant antifungal activity against the fungi:

Candida albicans and *Aspergillus niger*. However, the ethyl acetate extract was devoid of activity (Inhibition zones are depicted in Table 2).

Table 2: Inhibition zones for methanolic and ethyl acetate extracts

Extract	Inhibition zone diameter (mm/mg sample)					
	*Ec	Pa	Bs	Sa	Ca	An
Methanolic	22	30	17	17	19	22
Ethyl acetate	–	–	–	–	–	–

Ec= *Escherichia coli*

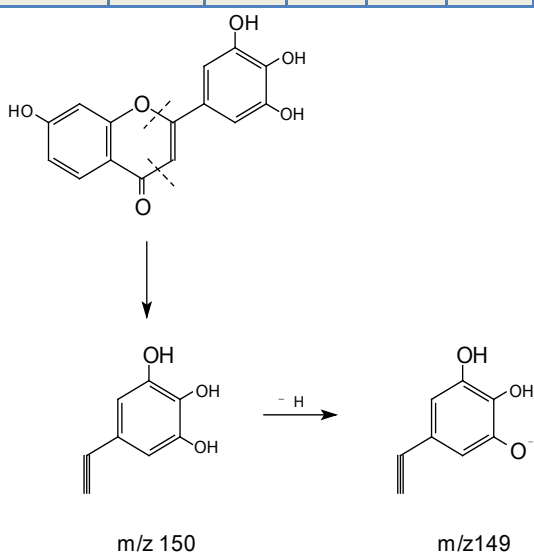
Bs= *Bacillus subtilis*

Sa= *Staphylococcus aureus*

Pa= *Pseudomonas aeruginosa*

Ca= *Candida albicans*

An= *Aspergillus niger*



Retro Diels-Alder fission of compound I

Antimicrobial assay

The methanolic and ethyl acetate extracts of *Combretum aculeatum* were evaluated for their

Antioxidant assay

In vitro antioxidant assay for the methanolic and ethyl acetate extracts of *Combretum aculeatum* was conducted. Evaluation of the antioxidant activity was carried out by measuring the capacity of each extract against stable DPPH radical. The change in colour is measured spectrophotometrically at 516nm. As depicted in Table (3). Both methanolic and ethyl acetate extracts exhibited significant antioxidant activity.

Table 3: Radical scavenging activity of *combretum aculeatum* extracts

Samlpe	Mean	Antioxidant
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	absorbance	activity(%)
Trolox	0.0275	96.50
Methanolic extract	0.050	93.70
Ethyl acetate extract	0.0525	93.50

References

Angeh J.E. Isolation and Characterization of Antibacterial Compounds Present in Members of Combretum , PhD Thesis, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Peritoria (2006).

Albagouri,H.A., Awatif,A., Koko,S.W., *Journal of Forest Product and Industries*, **3**,93(2014).

Awatif ,A., Sara ,M.O. ,Mohammed E.O., *International Journal of Tropical Medicine* , **2**(2):45 (2007).

Ahmed H. , Awatif A. Elegami1, Waleed S. Koko ,I,Elbadri, I., Osman, E.,, Mahmoud, M. D., . *Journal of Forest Products and Industries* , **3**(2), 93(2014).

Aderogba, M.A., Kgatele, D.T., McGaw, L.J., Eloff, J.N.. *South Afr. J. Bot.*, **79**,125(2012)

Banskota, A.H., Tezuka, Y., Kim, Q.T.; Tanaka, K., Saiki, L., Kadota, S. , *J. Nat. Prod.*,**63**, 57(2000).

Ch1aabi, M.; Benayache, S.; Benayache, F.; N’Gom, S.; Koné, M.; Anton, R.; Weniger, B.; Lobstein, A. , *Biochem. Systemat. Ecol.*, **36**, 59(2008).

El Amin H. E. "Trees and Shrubs of the Sudan" , Khartoum University Press, Sudan (1990).

Harborne,J.B., Mabry,T.J.,Mabry,H., " The Flavonoids" , Vol.I , Chapman and Hall, London (1974)

Kavanagh,F., "Analytical Microbiology" , Academic Press,New York andLondon(1972).

Le Houérou, H. N. In : "International.Livestock Center for Africa" , Addis Ababa, Ethiopia,P 491 (1980).

Martini, N.D.; Katerere, D.R.P.; Eloff, J.N. *J. Ethnopharmacol.* , **93**, 207(2004)

Ogan, A.U. , *Planta Med.* , **21**, 210(1972).

Orwa, C, A. Mutua, Kindt, R. and Jamnadass, R. (2009). *Agroforestry Database Version 4:* (www.worldagroforestry.org/sites/treedbs/treedatabases) .

Pietrovski, E.F., Rosa, K.A., Facundo, V.A. , Rios, K., Marques, M.C.A.,Santos, A.R.S., *Pharmacol. Biochem. Behav.* , **83**, 90(2006).

Rogers, C.B.; Verotta, L. *Chemistry and Biological Properties of the African Combretaceae.* In : "Chemistry, Biological and Pharmacological Properties of African Medicinal Plants"; K., Hostettman, Chinyanganga, F., Maillard, M., Wolfender, J.L., Eds.; University of Zimbabwe Publications: Harare, Zimbabwe(1996).

Sekiwa, Y., Kubota, K. and Kobayashi, A. , *Journal of Agriculture and Food Chemistry*, **48**, 373 (2000).

Sanon, M.,Sacande,M.,Schmidt, *Seed Leaf Lett*, 127(2007)