

ANTIBACTERIAL ACTIVITY OF *COMBRETUM ACULEATUM* VENTOMER MOHAMMED KHALIL<sup>1</sup>, AISHA ZOHEIRALMAGBOUL<sup>2</sup>, MOHAMMED ELFATIH AHMED OMER<sup>2</sup> ANDAWATIF ABDELBAGI ELEGAMI<sup>2</sup>

## ABSTRACT

Leaves extracts of *Combretum Aculeatum* Vent were subjected to a preliminary antibacterial screening against two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), and two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

The petroleum ether extract of the leaves was completely inactive towards the organisms tested while methanol and aqueous extracts showed high activity against the two gram-positive as well as gram-negative bacteria. The active extracts were further tested against 38 clinical isolates, isolated from different sources (abscess, ear swabs urinary tract infection and wounds), and their minimum inhibitory concentrations were determined. The leaves of *C. aculeatum* Vent were found to contain tannins in high concentration and flavonoids in low concentration to which the antibacterial activity may be attributed. The standard bacteria were tested against two antibiotics and the results were compared with the activity of the plant extracts.

## الملخص

تم اختبار خلاصة نبات الكمبريتيم بالإيثر البترولي، الميثانولي والمائي ضد أربعة أنواع من البكتريا المعيارية وهي العسوية الرقيقة، العنقودية الذهبية، الإشريكية القولونية والزانفة الزنجارية. وقد أظهرت الخلاصة أن المستخلص الإيثر البترولي ليس له أي فعالية ضد الأنواع الأربعة من البكتريا. أما الخلاصة الميثانولية والمائية فقد أظهرت فعالية عالية ضد البكتريا موجبة الجرام وسالبة الجرام. كما تم اختبار الخلاصات أعلاه ضد 38 عينة من البكتريا معزولة من مرضى من مصادر مختلفة (خراج ومسحات من الأنف وجمع المجاري البولية والجروح).

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وتم تحديد أقل جرعة تشبّه نمو البكتريا القياسية، كما تم التحليل الكيميائي الأولي لمعرفة المكونات الكيميائية للنبات حيث وجد أن الأوراق تحتوى على نسبة عالية من مادة الفينول وقليل من الفلافونويد، وعزى النشاط المضاد للبكتريا لهذين المركبين. كما اختبرت البكتريا القياسية ضد اثنين من المضادات الحيوية وتمت مقارنتهما مع فعالية الخلاصات النباتية.

## INTRODUCTION:

*Combretum aculeatum* Vent, locally known as Alsheheit belongs to the family, Combretaceae. Its habitat is lowland places and it is widely distributed. Samples were collected from Nuba mountains (Southern Kordofan province).

World wide there are no phytochemical reports on *Combretum aculeatum*. Flavones, flavonoids, tannins, hydrocyanic acid and phenanthrene derivatives were reported to be separated from other related species<sup>(1)</sup>

Combretine and Betaine alkaloids were separated from the leaves of *Combretum microanthum*<sup>(2)</sup>. The roots are used for wounds healing, skin tuberculosis and as a purgative (Information by folk medicine herbalist from Nuba mountains).

## PLANT MATERIAL

The dried leaves of *Combretum aculeatum* Vent (Combretaceae), collected from Nuba mountains, Western Sudan, were authenticated by Dr. G.E.B El Ghazali, Medicinal and Aromatic Plants Research Institute, National Center for Research. Avoucher specimen was deposited at the herbarium of the institute.

## METHOD OF EXTRACTION:

A coarsely powdered dry leaves (20 gm) of *Combretum aculeatum* Vent were successively Soxhlet extracted using petroleum ether, and methanol. Each extract was separately evaporated under vacuum. Each residue was redissolved or suspended in the same extracting solvent, then the final volume was adjusted to 20 ml and kept in refrigerator till used. Leaves water extract was also prepared by infusion using boiled distilled water with occasional shaking for four hours.

The aqueous extract was then filtered and the final volume was adjusted to 20 ml with boiled distilled water and used immediately.

## TEST ORGANISMS

The plant extracts were tested against two Gram positive (*Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* NCTC 6447, two Gram negative bacteria (*Escherichia coli* NCTC 8196, and *Pseudomonas aeruginosa* NCTC 6750). The tested organisms were obtained from the Department of

Microbiology, Faculty of Pharmacy, University of Khartoum. Thirty eight clinical isolates, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were collected randomly from Sudanese patients attending Soba Hospital; Khartoum Teaching Hospital and the National Health laboratory. The bacterial cultures were maintained on nutrient agar (Oxoid) and incubated at 37 °C for 18 hours and then used for the tests.

#### ANTIMICROBIAL TEST

The cup-plate agar diffusion method<sup>(3)</sup> was adopted, with some minor modifications, to assess the antibacterial activity of the prepared extracts.

Two ml of the standardized bacterial stock suspension ( $10^8 - 10^9$  colony forming units per ml) were thoroughly mixed with 250 ml of sterile melted nutrient agar which was maintained at 45 °C.

Twenty ml aliquots of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set, and in each of these plates, five cups (10mm in diameter) were cut using a sterile cork borer (NO.4) and the agar discs were removed. Alternate cups were filled with 0.1 ml of each of the extracts, using standard Pasteur pipettes, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position, at 37 °C for 18 hours. Three replicates were carried for each extract against each of the test organisms. Simultaneously, positive controls, involving addition of respective solvents and antibiotics (ampicillin and neomycin), instead of the extracts, were carried out. After incubation, diameters of resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

The minimum inhibitory concentration (MIC) of aqueous and methanolic extracts against standard organisms was determined using agar dilution method<sup>(4)</sup>.

Phytochemical screening of *Combretum aculeatum* was carried out using methods adopted by similar survey<sup>(5)</sup>.

#### RESULTS

Table (1) represents antibacterial activity against standard organisms and Table (2) represents antibacterial activity against clinical isolates.

Table 1: Antibacterial activity of *Combretum aculeatum* Vent against four standard organisms

Plant part	Extract	Yield %	Phytochemical Screening	Inhibition zone (mm)					*MIC (mg/ml)				
				B.s	S.a	E.c	P.s.a	B.s	S.a	E.c	P.s.a		
Leaf	Petroleum ether	4.32	T+	0.0	0.0	0.0	0.0	N.d	N.d	N.d	N.d		
	MeOH	9.58	F+, Tan+++	26.0	21.0	19.0	28.0	5.99	2.99	11.98	5.99		
	H <sub>2</sub> O	20.01	Tan+++	20.0	20.0	20.0	26.0	12.26	25.13	>50.25	50.25		
Ampicillin (40µg/ml)		004		21.0	23.0	17.0	16.0	N.d	N.d	N.d	N.d		
Neomycin (40µg/ml)		004		17.0	21.0	18.0	17.0	N.d	N.d	N.d	N.d		

• MIC = minimum inhibitory concentration. B.s = *Bacillus subtilis*, S.a = *Staphylococcus aureus*, E.c = *Escherichia coli*, P.s.a = *Pseudomonas aeruginosa*. F = Flavonoids, Tan = Tannins, T = Terpenes; +, low concentration; +++, high concentration; N.d not done. Test concentration of extract, 45 mg/ml, 0.1 ml cup, inhibition zones are the mean of 3 replicates.

**Table 2 : Sensitivity of clinical isolates towards *Combretum aculeatum* Vent leaves extracts:**

Clinical isolates (n)		Sensitive strains(n) Leaf extracts	
Microorganisms	Source	MeOH	H <sub>2</sub> O
<i>E.coli</i>	Urinary tract(13)	12	12
<i>S.aureus</i>	Acess(1)	1	1
	Ear(1)	1	1
	Urinary tract (5)	4	3
	Wound	3	0
<i>Ps. aeruginosa</i>	Ear (2)	2	2
	Urinary tract (2)	2	1
	Wound (3)	2	1
<i>Pr.vulgaris</i>	Ear(1)	8	6
	Urinary tract (5)	5	5
<i>K.pneumoniae</i>	Wound (3)	3	2

The sensitive organisms are those exhibiting 15 mm inhibition zone or more. Test concentration of extracts 45 mg/ml, 0.1 ml/cup

## DISCUSSION

*Combretum aculeatum* Vent was screened against four standard organisms i, e *B. subtilis*, *S. aureus*, *E. coli* and *Ps. aeruginosa*. The leaves petroleum ether extract was completely inactive against the organisms tested. The methanolic and aqueous extract exhibited high antibacterial activity against the two Gram positive and the two Gram negative organisms. It may be concluded that the methanolic and aqueous extracts inhibited both Gram positive and Gram negative organisms with inhibitory action almost similar to 40 ug/ml ampicillin and neomycin and these results may justify its traditional uses in folk medicine.

Testing leaves methanolic and aqueous extracts of *C. aculeatum* against different clinical isolates, it was found that methanolic extract showed higher activity than aqueous extract. Among the clinical isolates tested, *Pseudomonas aeruginosa* was found to be more resistant to the extract than the rest of the organisms.

The minimum inhibitory concentration of *C. aculeatum* against the four organisms showed that the methanolic extract against both Gram positive organisms is more active than that of the Gram negative organisms. The

antibacterial activity of *C. aculeatum* might be due to the presence of tannins detected by phytochemical screening.

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