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In vitro Antibacterial Activity on Some Sudanese *Combretum* Species

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Abstract: Based on ethno pharmacological literature, four medicinal plants from *Combretum* genus used in traditional medicine in Sudan were collected. Extracts of different polarities were tested in preliminary biological screening for their *invitro* antibacterial activity against 2 standard Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and three standard Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*). The active extracts were further tested against seventy clinical isolates and their Minimum Inhibitory Concentrations (MIC) were also determined. The results of the activity of standard antibiotics against the tested organisms were reported.

Key words: Folk medicine, antibacterial activity, *combretum*, *combretaceae*, Sudan

INTRODUCTION

The genus *Combretum* (family Combretaceae) is mainly tropical; with many species growing in tropical Africa consists of climbers, shrubs and trees and is readily characterized by fruits with wing- shaped appendages (El Amin, 1990). In Sudan, twenty seven species of genus *Combretum were* reported by Andrews (1950). Although traditional healers throughout Africa have used species of the *Combretum* for the treatment of a wide range of disorders, only about 25 out of the approximately 99 Africa species of *Combretum* have been subjected to any form of scientific studies (Hostettmann *et al*., 1996). Many *Combretum* species are used throughout Africa for the relief of pain of different origin. Combretum species were used in the traditional medicine for treatment of hepatic disease, skin ulcers, inflammatory, bilharziasis, against symptoms like diarrhea, hypertension and cancer and have medical applications against various bacterial infections, such as gonorrhea and syphilis (Hostettmann *et al*., 1996). Despite the wide use of species of this family by traditional healers, very little of pharmacological importance had been reported until recently. The first scientific study carried out was that on the West African drug Kinkeliba made from the leaves of *C. micranthum*. That drug which was used for the treatment of biliary fever, colic and vomiting had a cholagog and diuretic action and is antimicrobial (Paris, 1942). Studies indicated that some *Combretum* species have stable cyclooxygenase-inhibiting activity; anticancer activity (Asami *et al*., 2003; Simon *et al*., 2003; Ali *et al*., 2003). A similar stability in anti-bacterial activity was observed (Elegami *et al*., 2002; Kotze and Eloff, 2003; Afolayan *et al*., 2003; Kola *et al*., 2003; Masika *et al*., 2003; Eloff *et al*., 2001; Baba-Moussa *et al*., 1999; Eloff, 1999; Fyhrquist *et al*., 2002).

There is no phytochemical report encountered on these four *Combretum* species undertaken in this study. Series of combretastatins and their glycosides (Schwikkard *et al*. 2001); flavonoids (Banskota *et al*., 2001 and Katerere *et al*., 2003), tannins (Adnyana *et al*., 2002 and Asami *et al*., 2003) a series of acidic triterpenoids (Roger, 1989; Simon-G *et al*., 2003; Adnyana *et al*., 2001) substituted phenanthrenes and dihydrophenanthrenes (Pettit *et al*., 1982) stilbenes and dihydrostilbenes (Pettit *et al*., 1988) were isolated from other species of *Combretum*.

The major purpose of the present study, is to investigate the activity of some Sudanese *Combretum* species namely *C. adenogonium*, *C. glutinosum*, *C. aculeatum* and *C.* sp. *Aff. obovatum* which might have medicinal value as antimicrobial agents. The extracts which would show significant antibacterial effects are subjected for further investigations; they are to be tested against pathogenic clinical isolates and their MICs were determined.

MATERIALS AND METHODS

Plant materials: The plant materials were collected form Southern Kordofan and the identification was done by

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Table 1: Preliminary screening of different *combretum* extracts for antimicrobial activity against standard microorganisms

Botanical name/ Vernacular name	Part used	Solvent used	Yield (%)	Test organisms used M.D.I.Z.mm — *Bacteria* — *B.s.*	*S.a.*	*E.c.*	*Pr.v.*	*P.a.*
Combretum adenogonium Steud. Ex A .Rich GH 4/97	Leaf	$CHCl_3$	4.60	25	23	25	-	14
		MeOH	16.12	27	28	38	22	29
		H_2O	3.40	30	33	29	20	21
	Bark	$CHCl_3$	0.82	19	16	18	-	-
		MeOH	3.66	29	25	27	15	25
		H_2O	1.20	31	30	28	15	20
	Stem	$CHCl_3$	1.98	16	13	16	-	-
		MeOH	7.18	27	28	29	13	24
		H_2O	2.60	23	25	24	16	17
	Seed	$CHCl_3$	1.80	11	-	-	-	-
		MeOH	12.10	23	24	21	20	24
		H_2O	4.80	25	25	23	20	14
Combretum glutinosum Perrott. Ex DC. Vern. habeel al-gabal GH 6/97	Leaf	$CHCl_3$	2.94	18	17	17	-	-
		MeOH	22.52	40	41	32	23	27
		H_2O	6.00	19	23	23	20	16
	Bark	$CHCl_3$	2.98	27	26	-	-	17
		MeOH	17.32	25	36	23	17	23
		H_2O	3.80	29	22	24	20	20
	Stem	$CHCl_3$	1.66	24	22	21	-	20
		MeOH	8.60	26	26	25	20	25
		H_2O	3.20	16	13	-	17	21
	Seed	$CHCl_3$	4.20	15	-	-	13	12
		MeOH	13.20	24	25	23	18	24
		H_2O	1.33	13	13	-	-	-
Combretum aculeatum Vent. Vern. al shehalt Koko 9/96	Root	$CHCl_3$	1.82	22	25	-	15	16
		MeOH	10.68	23	23	19	21	29
		H_2O	5.80	19	15	-	-	20
	Bark	$CHCl_3$	2.40	26	27	17	15	25
		MeOH	13.06	31	42	25	24	28
		H_2O	6.00	35	35	30	24	28
	Stem	$CHCl_3$	0.96	15	14	-	14	-
		MeOH	6.86	25	24	24	16	22
		H_2O	6.20	26	25	30	19	27
	Seed	$CHCl_3$	13.65	17	14	-	-	-
		MeOH	8.00	15	14	13	11	-
		H_2O	1.33	25	27	26	16	26
Combretum sp. *Aff. obovatum* F.Hoffm GH 11/97	Leaf	$CHCl_3$	5.82	-	18	15	13	-
		MeOH	21.42	26	28	27	22	21
		H_2O	5.20	24	25	22	19	18
	Bark	$CHCl_3$	0.82	12	-	-	-	-
		MeOH	15.92	17	14	15	17	17
		H_2O	5.60	23	22	16	-	20
	Stem	$CHCl_3$	1.53	11	14	12	14	-
		MeOH	9.17	19	20	19	18	19
		H_2O	2.80	17	15	25	-	24
	Seed	$CHCl_3$	1.36	19	26	21	16	16
		MeOH	10.18	28	29	27	19	22
		H_2O	1.33	18	15	28	16	26

B.s. = Bacillus subtils ; S.a. = Staphylococcus aureus ; E.c. = Escherichia coli; Pr.v. = Proteus vulgaris ; P.a. = Pseudomonas aeruginosa, - = No inhibition zone ; M . D . I . Z mm = Mean Diameter of growth Inhibition Zones , in mm .concentration used in extract = 0.1g mL^{-1}

Table 2: Sources of clinical isolates

Clinical isolates (No)	Source — A	E.s	Ey.s	Pi	U	W.s
Staphylococcus aureus (20)	1	4	1	1	5	8
Escherichia coli (15)	-	-	-	-	15	-
Proteus vulgaris (15)	-	6	-	-	7	2
Pseudomonas aeruginosa (20)	-	5	-	-	4	11

A = Abscess; E.s = Ear swab; P.i = Pus inspiration; U = Urine; W.s = Wound swab; E.y.s = Eye swab

Ebtisam Gaber (Kordofan University) and Wail ELsadg (MAPRI). Voucher specimens were deposited in the Herbarium of the Institute.

Methods of extraction: The Shade dried, coarsely powdered plant materials (200 g) were successively extracted by soxhlet using chloroform and methanol. Each extract was filtered and evaporated to dryness under vacuum at (40°C) using a rotatory evaporator. The dried chloroform concentrate was redissolved in a mixture containg methanol: Petroleum ether (2: 1), the methanol concentrate was redissolved in methanol. Water extract was prepared from fresh material (20 gm) by infusion method with occasional shaking for 3 h. The final volume of each extract was adjusted to give a concentration of 50 mg mL^{-1}. The yield % was presented in Table 1.

Test organismsL: The plant extracts were tested against two Gram positive bacteria *(Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 25923, three Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus vulgaris* ATCC 6380). Seventy clinical isolates were collected randomly form patients attending Khartoum Teaching Hospital and National Health Laboratory (Table 2).

Antimicrobial test: The cup-plate agar diffusion method was adopted, with some minor modifications, to asses the antibacterial activity of the prepared extract (Kavanagh, 1972). The (MICs) of aqueous and methanolic extracts against standard organisms were determined using agar dilution method (Blair, 1970).

RESULTS AND DISCUSSION

In the present researcha total of 48 extracts belonging to four Sudanese medicinal plant species from genus *Combretum* (namely: *C.adenogonium*, *C.glutinosum*, *C. aculeatum* and *C.sp. Aff. obovatum*); were investigated for their antibacterial activity against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and three gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* standard bacterial organisms and 70 clinical isolates. The results are shown in Table 1 and 3.

The results of the activity of the standard reference antibiotics against the four tested organisms are included in Table 1.

The most commonly inhibited bacterium was *Bacillus* subtilis (being inhibited by 38 extracts, 79.17%) and the most resistant was *Proteus vulgaris* (being inhibited by 14 extracts, 29.17%).

Concerning the solvent used for extraction, methanol extracts showed the highest antibacterial activity against the five tested organisms (41.34%); followed by aqueous extracts (37.99%) and finally the chloroform extracts (20.67%).

The chloroformic extracts of *Combretum adenogonium* exhibited variable activity against the five tested organisms. Methanolic and aqueous extracts showed high activity against all tested organisms, with large inhibition zones, which is in agreement with Elegami (2002) who found that the chloroformic extract of all parts of *C. hartmannianum* was inactive against gram-negative but showed some activity against gram-postive standard organisms. The highest activity was exhibited by methanolic and aqueous extracts of leaves, bark and fruit.

In the MICs of methanolic extracts of leaf and bark showed lower minimum inhibitory concentration against Gram-postive in the range of 1.17-2.35 mg mL^{-1} than those of stem and seed against most of the standard bacterial organisms,while in the range of 9.38-18.75 mg mL^{-1}. On the other hand, the minimum inhibitory concentration of aqueous extracts of stem and seed were lower against *S. aureus*, *Pr. vulgaris* and *Ps. aeruginosa* in the range of 1.17-4.69 mg mL^{-1}. In the case of other standard organisms the minimum inhibitory concentration of aqueous extracts were higher in the range of 18.75-37.5 mg mL^{-1} (Table 4).

The methanolic extracts of leaf and seed showed high activity against all clinical isolates, bark extract showed moderate activity, while stem extract showed low activity. The aqueous extract of leaf and stem showed moderate activity against all clinical isolates. The aqueous extract of bark showed no activity against all clinical isolates, while seed extract showed high activity against all clinical isolates (Table 3).

The chloroformic extract of leaf of C. *glutinosum* showed moderate activity against *S.a*, *B.s* and *E.coli*; bark extract showed high activity against *B. subtilis*, *S. aureus* and *Ps. aeruginosa*, while showed no activity against *E. coli* and *Pr. vulgaris*; stem extract showed high activity against four standard organisms except *Pr. vulgaris* showed no activity, while seed extract was completely inactive. The methanolic extract of leaf of *C. glutinosum* showed low minimum inhibitory concentration against *S. aureus*, *B. subtilis* and *Ps. aeruginosa* in the range of 0.3- 1.17 mg mL^{-1}. In the case of *E.coli* and *Pr. vulgaris*, The MICs were high (9.38 mg mL^{-1}). The methanolic extract of bark showed low minimum inhibitory concentration against *B. subtilis*, *E. coli* and *Ps. aeruginosa* (2.35 mg mL^{-1}). In the case of *S. aureus* and *Pr. vulgaris* the MICs were high (4.69 mg mL^{-1}). The

Table 3: Effect of *Combretum* methanolic and aqueous extract on different clinical microbial isolates

				No.of clinical isolates					
				S		I		R	
Name of plant	Part used	Organisms	No.	Me	H_2O	Me	H_2O	Me	H_2O
C. adenogonium	Leaf	*S.a*	20	17	3	3	6	0	11
		E.c	15	14	1	1	6	0	8
		Pr.v	15	14	4	1	10	0	1
		P.a	20	19	6	1	9	0	5
	Bark	*S.a*	20	5	0	14	2	1	18
		E.c	15	12	0	1	1	2	14
		Pr.v	15	7	1	7	3	1	11
		P.a	20	10	0	9	0	1	20
	Stem	*S.a*	20	0	2	10	6	10	12
		E.c	15	1	3	5	4	9	8
		Pr.v	15	0	4	3	9	12	2
		P.a	20	0	3	0	13	20	4
	Seed	*S.a*	20	20	9	0	11	0	0
		E.c	15	15	13	0	1	0	1
		Pr.v	15	15	15	0	0	0	0
		P.a	20	20	19	0	1	0	0
C. glutinosum	Leaf	*S.a*	20	18	2	2	17	0	1
		E.c	15	15	0	0	15	0	0
		Pr.v	15	15	4	0	10	0	1
		P.a	20	20	3	0	12	0	5
	Bark	*S.a*	20	10	9	9	11	1	0
		E.c	15	15	3	0	12	0	0
		Pr.v	15	15	5	0	9	0	1
		P.a	20	18	5	2	14	0	1
	Stem	*S.a*	20	0	1	12	4	8	15
		E.c	15	4	0	10	3	1	12
		Pr.v	15	2	0	13	5	0	10
		P.a	20	2	1	7	8	11	11
	Seed	*S.a*	20	16	10	4	10	0	0
		E.c	15	15	14	0	1	0	0
		Pr.v	15	15	13	0	2	0	0
		P.a	20	20	20	0	0	0	0
C. aculeatum	Root	*S.a*	20	5	2	15	18	0	0
		E.c	15	15	5	0	10	0	0
		Pr.v	15	14	10	1	5	0	0
		P.a	20	16	11	4	9	0	0
	Bark	*S.a*	20	18	15	2	5	0	0
		E.c	15	15	14	0	1	0	0
		Pr.v	15	15	14	0	1	0	0
		P.a	20	20	18	0	2	0	0
	Stem	*S.a*	20	4	2	15	15	1	3
		E.c	15	4	4	10	11	1	0
		Pr.v	15	2	9	11	6	2	0
		P.a	20	3	11	17	9	0	0
	Seed	*S.a*	20	1	1	13	6	6	13
		E.c	15	1	0	8	4	6	11
		Pr.v	15	9	1	4	7	2	7
		P.a	20	8	2	9	7	3	11
C. sp. Aff. obovatum	Leaf	*S.a*	20	15	4	5	11	0	5
		E.c	15	13	1	2	9	0	5
		Pr.v	15	15	7	0	7	0	1
		P.a	20	20	7	0	12	0	1
	Bark	*S.a*	20	3	3	17	16	0	1
		E.c	15	11	3	4	8	0	4
		Pr.v	15	15	4	0	10	0	1
		P.a	20	18	3	2	16	0	1
	Stem	*S.a*	20	12	8	8	11	0	1
		E.c	15	13	7	2	8	0	0
		Pr.v	15	14	13	1	2	0	0
		P.a	20	18	16	2	4	0	0
	Seed	*S.a*	20	20	4	0	11	0	5
		E.c	15	15	4	0	9	0	2
		Pr.v	15	15	11	0	4	0	0
		P.a	20	20	9	0	10	0	1

S.a. = Staphylococcus aureus; E.c. = Escherichia coli; Pr.v. = Proteus vulgaris; P.a. = Pseudomonas aeruginosa. concentration used in extract = 0.1g mL^{-1}.Gram positive bacteria (*B.s* and *S. a*),Gram-negative(*E .c.; K.p; P.v* and *Ps.a*),>8mm(M.I.Z.D)=Sensitive (S), >6mm (M.I.Z.D) = Sensitive (S), 14-18 mm (M.I.Z.D) = intermediate (I) 13-16mm (M.I.Z.D) = intermediate (I), <14 mm (M.I.Z.D) = Resistant (R) <13 mm (M.I.Z.D)= Resistant (R)

Table 4: Minimum Inhibitory Concentration (MIC) mg mL^{-1} of the plants extracts against standard bacterial organisms minimum inhibitory concentration mic mg mL^{-1} of the plants extracts against standard bacterial organisms

Botanical name	Part used	Solvent	*B.s.*	*S.a.*	*E.c.*	*P.s.*	*Pr.v.*
C.adenogonium	L	MeOH	1.17	1.17	4.69	2.35	4.69
		H_2O	2.35	2.35	9.38	9.38	9.38
	B	MeOH	1.17	2.35	2.35	4.69	9.38
		H_2O	4.69	2.35	9.38	9.38	9.38
	S.t	MeOH	9.38	9.38	18.75	37.50	18.75
		H_2O	18.75	4.69	37.50	18.75	18.75
	S	MeOH	2.35	2.35	4.69	9.38	9.38
		H_2O	4.69	1.17	4.69	4.69	4.69
C.glutinosum	L	MeOH	0.3	1.17	9.38	1.17	9.38
		H_2O	1.17	1.17	2.35	2.35	2.35
	B	MeOH	2.35	4.69	2.35	2.35	4.69
		H_2O	2.35	0.3	2.35	4.69	2.35
	S.t	MeOH	0.6	2.35	4.69	4.69	4.69
		H_2O	9.38	4.69	9.38	4.69	9.38
	S	MeOH	1.17	2.35	2.35	4.69	2.35
		H_2O	4.69	4.69	18.75	18.75	18.75
C.aculeatum	R	MeOH	0.3	0.6	2.35	4.69	2.35
		H_2O	1.17	0.3	9.38	4.69	4.69
	B	MeOH	0.3	1.17	1.17	4.69	4.69
		H_2O	2.35	0.3	4.69	9.38	9.38
	S.t	MeOH	4.69	4.69	4.69	9.38	9.38
		H_2O	9.38	4.69	18.75	18.75	18.75
C. sp.Aff.obovatum F.Hoffm	L	MeOH	0.3	1.17	2.35	4.69	4.69
		H_2O	4.69	2.35	18.75	18.75	18.75
	B	MeOH	2.35	2.35	4.69	18.75	9.38
		H_2O	2.35	2.35	4.69	4.69	9.38
	S.t	MeOH	2.35	4.69	18.75	18.75	9.38
		H_2O	2.35	4.69	18.75	18.75	18.75
	S	MeOH	0.3	0.3	1.17	2.35	4.69
		H_2O	4.69	4.69	9.38	9.38	9.38
T.laxiflora	L	MeOH	1.17	1.17	2.35	2.35	2.35
		H_2O	2.35	2.35	4.69	4.69	9.38
	S	MeOH	1.17	2.35	4.69	4.69	4.69
		H_2O	2.35	1.17	4.69	9.38	9.38

L = Leaf ; B = Bark; st = Stem ; S = Seed ; R = Root, *S.a = Staphylococcus aureus ; B.s = Bacillus subtilis; E.c = Escherichia coli ; P.a =Pseudomonas aeruginosa ; Pr.v = Proteus vulgaris ;* MIC = Minimum Inhibtory Concentration

Table 5: Antibacterial activity of reference drugs against standard organisms

		Test organisms used M . D . I . Z mm				
Drug	Conc.µg mL^{-1}	*B.s .*	*S.a.*	*E.c.*	*Pr.v.*	*Ps.a.*
Tetracycline	40	23	31	-	16	16
	20	21	27	-	-	13
	10	20	25	-	-	12
	5	18	17	-	-	-
Ampicillin	40	15	25	-	-	-
	20	14	20	-	-	-
	10	13	18	-	-	-
	5	12	15	-	-	-
Gentamicin	40	29	35	32	25	23
	20	22	33	30	24	22
	10	20	30	17	23	22
	5	17	28	-	22	19

B.s = Bacillus subtilis ; S.a = Staphylococcus aureus ; E.c = Escherichia coli ; P.a Pseudomonas aeruginosa ; Pr.v = Proteus vulgaris,M . D . I . Z mm = Mean Diameter of growth Inhibition Zones, in mm, Average of 2 replicates, - = No inhibition zones

minimum inhibitory concentration of aqueous extracts of stem and seed were higher than the methanolic against all standard organisms in the range of 9.38-18.75 mg mL^{-1} (Table 4).

The methanolic extracts exhibited the highest activity against all clinical isolates except the stem extract which showed moderate activity. The aqueous extracts of seed showed high activity against all clinical isolates; the leaf and bark extracts showed moderate activity and the stem extract showed very low activity (Table 3).

The chloroformic extracts of root and bark of *C. aculeatum* showed high activity against Gram+ve than Gram-ve, whereas stem and seed extracts showed variable activity. The methanolic extracts of root, bark and stem

showed high activity against all standard organisms. The aqueous extracts of root showed high activity against *B. subtilis* and *Ps. aeruginosa,* while the bark, stem and seed showed high activity against all standard organisms.

The MIC of methanolic extract of root were low against *S. aureus B. subtilis*, *E. coli* and *Pr. vulgaris* in the range of 0.3-2.35 mg mL^{-1}.In the case of *S. aureus* the MICs of aqueous extract were also low (MIC value 0.3 mg mL^{-1}). The methanolic extracts of bark and stem showed low minimum inhibitory concentration against all standard organisms in the range of 0.3-9.38 mg mL^{-1}. However, MIC of gram-positive were lower than gram-negative, which agrees with the findings of Khalil *et al.* (2001) and Elegami *et al.* (2002) (Table 4).

The methanolic and aqueous extracts of root and bark showed high activity against all clinical isolates, while stem extract showed moderate activity and seed extract was completely inactive (Table 3).

The chloroformic extracts of leaf, bark and stem of *C. sp. Aff. Obovatum* was completely inactive against all standard organisms, while seed extracts showed high activity against *S. aureus* and *E. coli.* The methanolic and aqueous extracts of leaf and seed showed high activity against all standard organisms, while bark and stem showed moderate activity against different standard organisms.

With regard to *C. sp. Aff. obovatum*, the methanolic and aqueous extracts of bark and stem exhibited the same MIC against *S. aureus* (2.35 mg mL^{-1}). The methanolic and aqueous extracts of bark showd low MIC against *S. aureus* and *E. coli* in the range of 2.35-18.75 mg mL^{-1}, whereas the methanolic extracts of leaf and seed showed low MIC against all standard organisms in the range of 0.3-4.69 mg mL^{-1} (Table 4).

The methanolic extracts of all parts showed high activity against all clinical isolates. The aqueous extracts showed moderate activity against all isolates except six isolates of *E.coli* (Table 3).

The results of the present study indicate that there are promising extracts with high and broad antimicrobial activity, when compared with some antimicrobial drugs in current use (Table 5). This verified the claimed bioactivity of the plants employed in traditional medicine in Sudan. This genus thus is of economic importance as a reservoir of potentially useful medicinal compounds. Work is in progress to identify the active constituent (s) from the most promising extracts.

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