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Antioxidant and Antibacterial Activities of Two *Combretum* Species from Burkina Faso

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ABSTRACT

The aim of this study was to determine the phytochemical content and to evaluate the antioxidant and antibacterial activities of the acetone extract from *Combretum acutum* Laws (leaves) and *Combretum sericeum* G. Don (aerial part). Acetone extracts were fractionated with n-hexane, ethyl acetate and butanol successively and their bioactivities were also evaluated. Phenolic, tannin and flavonoid contents have been determined using spectrophotometric methods. The antioxidant activity of these plant extracts and fractions has been determined by ABTS, FRAP and DPPH methods. The phytochemical screening revealed the presence of tannins, steroids/triterpenoids, saponins and cardenolides and different contents of flavonoids and anthraquinones in the acetone extracts of both plants. Results obtained from this study also showed that the butanol fractions, with the highest phenolic and tannin contents, exhibited the best antioxidant and antibacterial activities and will be maintained for further investigations. In addition to that, the Minimum Inhibitory Concentrations (MICs) of the extracts and fractions against pathogenic bacteria (5) and serotyped bacteria (5) from American Type Culture Collection (ATCC) were also determined using the agar-well diffusion method. Both plants possess good antioxidant and antibacterial activities.

Key words: *Combretum*, phenolic, tannins, flavonoids, antioxidant, antibacterial

INTRODUCTION

Members of the Combretaceae family are widely traded in the traditional medicine market in Southern Africa. Species of the family are also used for medicinal purposes in the rest of Africa and Asia for close to 90 medicinal indications. Many of these indications are related to treating infections (Eloff *et al*., 2008). Species from the *Combretum* genus and to a lesser extent *Terminalia* are most

widely used for medicinal purposes and they are common and widely distributed throughout Western and Southern Africa (McGaw *et al*., 2001). *Combretum sericeum* is found in Western and Southern parts in Burkina Faso, while *Combretum acutum* is only localized in the Eastern of the country. Smoke from burning of *Combretum sericeum* is a remedy against cough while also roots decoction is used against diarrhoea and pneumonia (Abdullahi *et al*., 2003). Decoctions of *Combretum sericeum* and *Combretum nioroense* are used in Burkina Faso by local population as herbal tea for new born babies. No uses were been reported for *Combretum acutum*.

The *Combretum* genus is the source of a wide range of tannins, flavonoids, terpenoids and stilbenoids (Eloff *et al*., 2008). Several studies have demonstrated the antioxidant effect of these compounds (Robards *et al*., 1999; Lee *et al*., 2003; Jassbi, 2006). Recently, a great interest has been given to naturally occurring antioxidants, which may play important roles in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes (Carini *et al*., 1990). Therefore, screening plant materials on the basis of their antioxidant potency seems to be of central importance in order to identify extracts or fractions possessing the ability either in scavenging both free radicals and chain-reactions initiation or in binding with catalysts of the oxidative reactions, such as some metal ions (Dorman *et al*., 2003). From the viewpoint of their high antioxidant potency, the consumption at high scale of many plants has been recommended (Kitts *et al*., 2000). Therefore, the evaluation of antioxidant activities of extracts and fractions is considered as an important step prior to the isolation of antioxidant phytochemicals that they contain.

Antibiotic-resistant bacteria is still of world-wide concern. Since the use of antibiotics became widespread over 50 years ago, bacteria have progressively developed resistance (Hsueh *et al*., 2005). Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic therapy. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (Arias *et al*., 2004; Kloucek *et al*., 2005).

The aim of this study was then to evaluate the antioxidant properties and antibacterial activities of extracts and fractions from the two plants concerned. Furthermore, correlation between total phenolic content and biological activities were examined in order to give an orientation to the search of antioxidant and antibacterial compounds. Such study would contribute to further knowledge related to the screening of antioxidant and antibacterial compounds into these two *Combretum* species.

MATERIALS AND METHODS

Plant materials, extractions and fractionations: *Combretum acutum* Laws was harvested in June 2008 near Pendjari and *Combretum sericeum* G. Don was collected in May 2008 near Nasso (Bobo-dioulasso), respectively in the eastern and Western part of Burkina Faso (West Africa). Plants were identified by Prof Millogo-Rasolodimby, a botanist from the University of Ouagadougou. Voucher specimens were deposited in the Herbarium of Laboratoire de Biologie et d'Ecologie Végétales UFR/SVT, University of Ouagadougou.

For this study, leaves of *Combretum acutum* and aerial parts of *Combretum sericeum* were used. Ground air-dried plant materials were macerated in acetone (1/10, m/v) at room temperature during 48 h. Preparations were filtered through Whatman N° 1 filter paper. They were concentrated under reduced pressure at 40°C to obtain crude extracts. Each dry residue (1 g) was subjected to successive liquid-liquid fractionation (Eloff, 1998). Four fractions were obtained: the

n-hexane fraction, the ethyl acetate fraction, the butanol fraction and the water fraction. Solvents have been eliminated and the different residues obtained were used for different biological activities.

Chemicals and instruments: Folin Ciocalteu-reagent, NaH_2PO_4, Na_2HPO_4, sodium carbonate, aluminium trichloride ($AlCl_3$), gallic acid and quercetin were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS, 2,2-Dipheny-1-picrylhydrazyl (DPPH), trichloroacetic acid, potassium persulfate, acetone, methanol, n-hexane, ethyl acetate and n-butanol were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [$K_3Fe(CN)_6$] was from Prolabo (Paris, France); ascorbic acid and iron trichloride ($FeCl_3$) were supplied by Labosi (Paris, France). The experiments were performed using a Cecil CE 2041 spectrophotometer (Cecil Instruments, England).

Phytochemical screening: The phytochemical screening was conducted with the acetone extracts for alkaloids, tannins, flavonoids, saponins, steroids/triterpenoids, anthraquinones, coumarins and cardenolids using the method described by Ciulei (1982).

Estimation of total phenolic, total flavonoid and total tannin contents: The total phenolics of plant extracts were determined by the Folin-Ciocalteu method (Lamien-Meda *et al*., 2008). The diluted aqueous solution of each extract (0.5 mL) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 mL). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g L^{-1} in water, 2 mL) was added. After 2 h incubation, the absorbencies were measured at 760 nm against water blank. A standard calibration curve was plotted using gallic acid (0-200 mg L^{-1}). The results were expressed as mg of Gallic Acid Equivalents (GAE) per gram of extracts or fractions.

The total flavonoids were estimated according to the Dowd method as adapted by Lamien-Meda *et al*. (2008). A diluted methanolic solution (2 mL) of each extract was mixed with 2 mL of aluminium trichloride ($AlCl_3$) in methanol (2%). The absorbance was read at 415 nm after 10 min against a blank consisting of 2 mL of methanol and 2 mL of plant extract (without $AlCl_3$). Quercetin was used as reference to produce the standard curve and the results were expressed as mg of Quercetin Equivalents (QE) per gram of extracts or fractions.

Européenne Commission (2000) reference method was used to determine the total tannins content using tannic acid as standard curve. Briefly, 200 µL of extracts or fractions were mixed with 1000 µL of water, 200 µL of ferric ammonium citrate (3.5 g L^{-1}) prepared freshly and 200 µL of ammoniac (8 g L^{-1}). The absorbance of the mixture was measured at 525 nm. The results were expressed as mg of Tannic Acid Equivalent (TAE) per gram of extracts or fractions.

Antioxidant activity

Iron (III) to iron (II)-reducing activity (FRAP): The total antioxidant capacity of the plant extract was determined using the iron (III) reduction method (Hinneburg *et al*., 2006). The diluted aqueous solution of plant extract (1 mL at a concentration of 100 µg mL^{-1}) was mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (2.5 mL). After 30 min incubation at 50°C, 2.5 mL of a trichloroacetic acid 10% was added and the mixture was centrifuged at 3000 rpm for 10 min. Then, the upper layer solution (2.5 mL)

was mixed with water (2.5 mL) and aqueous $FeCl_3$ (0.1%) solution (0.5 mL). The absorbance was read at 700 nm and ascorbic acid was used to produce the calibration curve. The iron (III) reducing activity determination was expressed in mmol Ascorbic Acid Equivalents (AAE) per gram of extract or fractions.

DPPH radical scavenging activity: The scavenging activity of extracts and fractions for the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Velázquez *et al.* (2003). Extracts or fractions dissolved in methanol (0.75 mL) were mixed with 1.5 mL of DPPH methanolic solution (0.02 mg mL^{-1}). After 15 min incubation in the darkness, the absorbance was read at 517 nm. The antioxidant content was determined using a standard curve of ascorbic acid. The results were expressed as mmol ascorbic acid equivalent (AAE) per gram of extracts or fractions.

ABTS radical cation decolorization assay: The radical scavenging capacity of antioxidants for the ABTS (2,2'-azinobis-3-ethyl-benzothiazoline-6-sulphonate) radical cation was determined as described by Lamien-Meda *et al.* (2008). The $ABTS^{\bullet+}$ was generated by mixing a 7 mM aqueous solution of ABTS with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 12 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70±0.02 units at 734 nm using spectrophotometer.

The diluted methanol solution of the extract (10 μL) was allowed to react with fresh $ABTS^{\bullet+}$ solution (990 μL) and then the absorbance was measured 6 min after initial mixing. Ascorbic acid was used as a standard and the capacity of free radical scavenging was expressed as mmol Ascorbic Acid Equivalents (AAE) per gram of extract or fractions. Quercetin and gallic acid were used as positive controls.

Antibacterial activity

Microorganisms: The microorganisms used in this study consisted of clinical isolates and collection/serotyped strains. The clinical isolates were obtained from biomedical laboratories. They were: *Escherichia coli*; *Salmonella typhimurium*; *Klebsiella pneumoniae*; *Staphylococcus aureus; Streptococcus faecalis*. The following serotyped strains used in this study are: *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 13311; *Staphylococcus aureus* ATCC 6538; *Staphylococcus epidermidis* ATCC 12228 and *Proteus mirabilis* ATCC 35659. Before testing, pure cultures were realized with all the strains in Mueller Hinton Agar and Tryptic Soy Broth. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 Mc Ferland standard.

Antibacterial tests: The agar-well diffusion method (Ojala *et al.*, 2000) was used to evaluate the antibacterial activity. Minimum Inhibitory Concentrations (MICs) of the extracts and fractions of the two species were determined using the agar-well diffusion method. All the extracts and fractions were diluted in Dimethylsulfoxide (DMSO) 10% and sterile distilled water to obtain series of concentrations of 20, 10, 5, 2.5, 1.25, 0.625 and 0.312 mg mL^{-1}. The MIC was taken as the lowest concentration of extracts or fractions that caused a clear to semi-clear inhibition zone around the hole after 24 h incubation at 37°C.

Sterile Petri dishes (d = 10 cm, Bibby Sterilin, UK) were prepared with a base layer of Müller-Hinton agar (Difco). Bacteria at density of 10^6-10^7 CFU were inoculated on solid agar. Holes (6 mm)

were made in the agar with a sterile cork borer and filled with 50 µL of different dilutions of the extracts and fractions. Petri dishes were incubated at 37°C for 24 h. The diameters of the circular inhibition zones obtained were measured. Commercial antibiotic discs of Gentamicin and Ampicillin were used as positive controls. DMSO 10% was used as a negative control.

Statistical analysis: All assays were carried out in triplicates and results are expressed as Mean±SD calculated with Excel 2007. Statistical comparisons were done with the XLSTAT 7.5, using Spearman correlation. Differences were considered to be significant at $p<0.05$.

RESULTS

Table 1 shows that both plants contain tannins, steroids/triterpenoids, saponins and cardenolides with variable amounts of flavonoids and anthraquinones.

To check if there is a relationship between these compounds and different biological activities observed in traditional medicine, we have determined the contents of phenolic compounds in the various extracts and fractions tested. From the results summarized in Table 2, we can easily conclude that *Combretum sericeum* is rich in tannins and in flavonoids, whereas *Combretum acutum* is poor in flavonoids, but rich in tannins. The acetone extract of *Combretum acutum* showed higher level of total phenolic (664.4±56.4 mg GAE g^{-1}) than acetone extract of *Combretum sericeum* (459.5±12.4 mg GAE g^{-1}) and the difference is significant ($p<0.05$). Four solvents having different polarities, n-hexane, ethyl acetate, butanol and water, were used to extract phenolic compounds from acetone extracts of both plants and the phenolic compounds were separated into different solvents based on the polarity. The water fraction was not been investigated. As the Table 2 indicates, the butanol fractions of both plants showed the highest content in total phenolics

Table 1: Phytochemical components of acetone extracts of *C. acutum* and *C. sericeum*

Phytochemical components	*Combretum acutum* Laws (leaves)	*Combretum sericeum* G. Don (aerial part)
Tannin	++	++
Flavonoid	+	++
Anthraquinone	++	+
Steroid/triterpenoid	++	++
Coumarin	-	-
Alkaloid	nt	nt
Saponin	++	++
Cardenolide	++	++

++: Present in appreciable amount, +: Present in low amount, -: Absent, nt: Not tested

Table 2: Total phenolic, total tannin and total flavonoid contents in extracts/fractions from *C. acutum* and *C. sericeum*

	Combretum acutum Laws				*Combretum sericeum* G. Don			
Extract and fractions	Yields (%)	Total phenolics (mg GAE g^{-1})	Total flavonoids (mg QE g^{-1})	Total tannins (mg TAE g^{-1})	Yields (%)	Total phenolics (mg GAE g^{-1})	Total flavonoids (mg QE g^{-1})	Total tannins (mg TAE g^{-1})
AE	18.32	664.4±56.4^{a}	25.2±1.8^{d}	327.7±8.1^{a}	10.69	459.5±12.4^{c}	186.1±4.6^{b}	306.7±10.4^{b}
BF	25.00	552.0±17.3^{b}	57.8±0.7^{b}	282.0±7.2^{b}	21.00	692.7±26.6^{a}	147.0±3.6^{c}	530.7±15.5^{a}
EF	9.0	201.2±8.7^{c}	38.5±1.7^{c}	31.2±2.0^{c}	12.00	555.7±12.3^{b}	474.8±5.0^{a}	110.4±7.2^{c}
HF	4.2	68.3±7.8^{d}	66.5±1.2^{a}	-	7.20	170.3±2.8^{d}	114.1±1.2^{d}	20.7±1.0^{d}

AE: Acetone extract; BF: Butanol fraction; EF: Ethyl acetate fraction; HF: n-hexane fraction; -: Very low amount. GAE: Gallic acid equivalent; QE: Quercetin equivalent and TAE: Tannic acid equivalent. Different letters in the same column indicate significant difference ($p<0.05$)

Table 3: Antioxidant activity of extracts/fractions from *C. acutum* and *C. sericeum*

Extract and Fractions	*Combretum acutum* Laws			*Combretum sericeum* G. Don		
	FRAP	ABTS	DPPH	FRAP	ABTS	DPPH
	(mmol AAE g^{-1})					
AE	5.30±0.16^{b}	7.80±0.28^{a}	10.88±0.12^{b}	4.93±0.20^{b}	5.16±0.04^{b}	9.67±0.13^{b}
BF	7.08±0.13^{a}	5.55±0.61^{b}	12.20±0.16^{a}	6.02±0.07^{a}	7.38±0.22^{a}	11.28±0.23^{a}
EF	4.50±0.11^{c}	3.13±0.19^{d}	7.02±0.08^{c}	4.05±0.20^{b}	4.80±0.44^{b}	6.17±0.09^{c}
HF	1.43±0.22^{d}	4.14±0.42^{c}	3.61±0.07^{d}	1.49±0.12^{d}	2.05±0.14^{d}	3.02±0.04^{d}

Gallic acid: FRAP (18.46±1.51 mmol AAE g^{-1}); ABTS (13.4±0.11 mmol AAE g^{-1}). Quercetin: FRAP (13.19±2.17 mmol AAE g^{-1}); ABTS (7.81±0.21 mmol AAE g^{-1}); DPPH (14.33±1.22 mmol AAE g^{-1}). AE: Acetone extract; BF: Butanol fraction; EF: Ethyl acetate fraction; HF: n-hexane fraction and AAE: Ascorbic acid equivalent. Different letters in the same column indicate significant difference ($p<0.05$)

(692.7±26.6 mg GAE g^{-1} and 552.0±17.3 mg GAE g^{-1} respectively for *C. sericeum* and *C. acutum*), whereas it is the ethyl acetate fraction of *Combretum sericeum* and the n-hexane fraction of *Combretum acutum* which showed the highest content in total flavonoids (474.8±5.0 mg QE g^{-1} and 66.5±1.2 mg QE g^{-1}, respectively). The majority of total phenolics in butanol fraction of *C. sericeum* are constituted by tannins (530.7±15.5 mg TAE g^{-1}, representing 76.61%).

The principle of the antioxidant activity is the availability of electrons to neutralize any so-called free radicals. In this work, three methods have been used to measure the antioxidant activities of both plants extracts and fractions: DPPH, FRAP and ABTS. For this study Gallic acid and Quercetin were used as standards (Table 3) in order to compare their antioxidant activities with those of extracts.

In the Table 3 the DPPH free radical scavenging activity results are shown as relative activities against the control. The acetone extracts of both plants showed the best radical scavenging with 10.88±0.12 mmol AAE g^{-1} for *Combretum acutum* and 9.67±0.13 mmol AAE g^{-1} for *Combretum sericeum*. The results revealed that for *Combretum acutum*, the fraction with the highest effective radical scavenging activity was the butanol fraction, followed by the ethyl acetate fraction while lower activity was found with the n-hexane fraction. The same thing was observed with *Combretum sericeum*. Compared to the Quercetin activity (14.33±1.22 mmol AAE g^{-1}) that is a standard, we can say that the butanol fractions had appreciable activities. Correlations (r^2) between the antioxidant activity by DPPH assay and phenolics, tannins and flavonoids were 0.78 ($p<0.05$), 0.92 ($p<0.05$) and 0.37, respectively for *C. sericeum* and 0.78 ($p<0.05$), 0.75 ($p<0.05$) and -0.38, respectively for *C. acutum*.

The acetone extracts of both plants had reducing power (5.30±0.16 mmol AAE g^{-1} and 4.93±0.20 mmol AAE g^{-1}, respectively for *Combretum acutum* and *Combretum sericeum*) (Table 3). The reducing power of fractions of both plants mentioned in the Table 3 was in the same order than their radical scavenging activity. Indeed, it is the butanolic fractions of *C. acutum* (7.08±0.13 mmol AAE g^{-1}) and *C. sericeum* (6.02±0.07 mmol AAE g^{-1}) which have shown best reducing powers, while the hexane fractions have presented the lowest activities (1.43±0.22 mmol AAE g^{-1} and 1.49±0.12 mmol AAE g^{-1}, respectively for *C. acutum* and *C. sericeum*). In this assay, there is some significant difference ($p<0.05$) between the antioxidant activities of extracts and fractions and reference compounds used. With this method, correlations (r^2) between antioxidant activity and phenolics, tannins and flavonoids were 0.75 ($p<0.05$), 0.97 ($p<0.05$) and 0.38, respectively for *C. sericeum* and 0.80 ($p<0.05$), 0.75 ($p<0.05$) and -0.37, respectively for *C. acutum*.

Table 4: Diameters (mm) of zones of inhibitions produced by the acetone extracts and fractions of *C. acutum* and *C. sericeum* at 20 mg mL^{-1}

	Combretum acutum Laws				*Combretum sericeum* G. Don				Reference antibiotics	
Microorganisms	AE	BF	EF	HF	AE	BF	EF	HF	Gentamicin	Ampicillin
E. coli	14.5±0.5	13.5±0.5	R	11.0±0	12.0±0	15.0±0	10.5±0.5	9.00±0	21	R
S. typhimurium	14.5±0.5	14.5±0.5	12.0±1	13.0±0	14.0±0	16.5±0.5	12.0±0	12.0±0	22	18
S. aureus	16.0±0	17.5±0.5	14.0±0	14.5±0.5	15.0±0	16.0±0	12.5±0.5	12.0±0	-	-
K. pneumoniae	12.0±1	13.0±0	8.50±0.5	10.0±0	10.0±0	13.0±0	R	R	8	R
S. faecalis	14.0±0	15.5±0.5	12.0±0	12.0±0	15.0±0	18.5±0.5	11.5±0.5	11.0±0	-	-
E. coli ATCC 25922	15.0±0	15.5±0.5	R	13.0±0	14.0±0	16.0±0	12.0±0	10.5±0.5	21	R
S. typhimurium ATCC 13311	16.0±0	16.0±0	14.0±0	14.0±0	16.5±0.5	17.0±0	14.5±0.5	14.5±0.5	25	-
S. aureus ATCC 6538	19.0±1	20.0±1	15.0±0	16.0±0	19.5±0.5	20.5±0.5	15.0±0	13.0±0	18	20
S. epidermidis ATCC 12228	17.5±0.5	18.0±0	15.0±1	14.5±0.5	17.5±0.5	16.0±0	14.5±0.5	14.0±0	22	22
P. mirabilis ATCC 35659	16.0±0	14.0±0	R	R	15.5±0.5	15.0±0	13.5±0.5	12.5±0.5	23	20

AE: Acetone extract; BF: Butanol fraction; EF: Ethyl acetate fraction; HF: n-hexane fraction; R: Resistant, -: Not tested. The diameters included with the diameter of holes (6 mm)

The ABTS assay results presented in Table 3 showed that acetone extract of *Combretum acutum* had higher antioxidant activity (7.80±0.28 mmol AAE g^{-1}) than its fractions. But for *Combretum sericeum*, it is the butanol fraction that showed the highest activity (7.38±0.22 mmol AAE g^{-1}). There is no significant difference ($p>0.05$) between antioxidant activity of the butanol fraction of *C. sericeum* and that of Quercetin. Correlations (r^2) evaluate in this assay between antioxidant activity and phenolics, tannins and flavonoids were 0.83 ($p<0.05$), 0.87 ($p<0.05$) and 0.43, respectively for *C. sericeum* and 0.76 ($p<0.05$), 0.75 ($p<0.05$) and -0.40, respectively for *C. acutum*.

As we can see in Table 4, all tested bacterial strains were susceptible to gentamicin, whereas, *E. coli*, *E. coli* ATCC 25922 *and K. pneumoniae* showed to be resistant to ampicillin. The extracts and fractions were used at a concentration of 20 mg mL^{-1} in DMSO 10%. The diameters of zone inhibition are ranged from 0 to 20±1 mm for *Combretum acutum* and from 0 to 20.5±0.5 mm for *Combretum sericeum*. All the assayed bacterial species were susceptible to acetone extract and fractions of *Combretum sericeum* with exception of *K. pneumoniae* which showed resistance to ethyl acetate fraction and n-hexane fraction. For *Combretum acutum*, all tested extract and fractions possessed antibacterial activity against bacterial strains; but pathogenic *E. coli* and *E. coli* ATCC 25922 strains showed resistance to ethyl acetate fraction and *P. mirabilis* ATCC 35659 also showed resistance to n-hexane fraction. The butanol fractions showed the most potency in terms of zones of inhibition sizes for both plants in all the test microorganisms used in this study. No inhibition growth was been observed for the negative control (DMSO 10%).

Table 5 shows that ethyl acetate and n-hexane fractions of both plants showed no activity to moderate activity depending on the bacterial strains, whereas acetone extracts and butanol fractions of both plants displayed the best activity with the MIC values ranging from 2.5 to less than 0.325 mg mL^{-1}. In this study, the *Staphylococcus* species showed to be the most susceptible

Table 5: Minimum inhibitory concentrations (MIC) in mg mL^{-1}

	Combretum acutum Laws				*Combretum sericeum* G. Don			
Microorganisms	AE	BF	EF	HF	AE	BF	EF	HF
E. coli	1.25	2.5	>20	5	2.5	1.25	5	10
S. typhimurium	1.25	1.25	5	2.5	0.625	0.312	2.5	2.5
S. aureus	0.312	0.312	2.5	2.5	<0.312	<0.312	2.5	2.5
K. pneumoniae	2.5	2.5	10	5	5	2.5	>20	>20
S. faecalis	1.25	0.625	5	5	0.312	1.25	5	5
E. coli ATCC 25922	0.625	1.25	>20	2.5	1.25	0.625	2.5	5
S. typhimurium ATCC 13311	1.25	0.625	2.5	2.5	0.312	0.312	1.25	1.25
S. aureus ATCC 6538	<0.312	<0.312	1.25	1.25	<0.312	<0.312	1.25	1.25
S. epidermidis ATCC 12228	<0.312	0.312	5	1.25	<0.312	<0.312	0.625	0.625
P. mirabilis ATCC 35659	1.25	2.5	>20	>20	0.625	0.625	1.25	2.5

AE: Acetone extract; BF: Butanol fraction; EF: Ethyl acetate fraction; HF: n-hexane fraction

with the MIC values = 0.325 mg mL^{-1}. We can also notice that *K. pneumoniae* was the less susceptible strain to the extracts and fractions of both plants tested.

DISCUSSION

In this study tannin, flavonoid, triterpène/steroid, anthraquinone, saponin and cardenolide have been found in the extracts of leaves and aerial part of the plants that have been studied. We also found high content of polyphenols in the extracts. This may be explain by the plant aerial parts used. It is well known that high levels of UV radiation increases the concentrations of total phenols and the main flavonoids (Garcia-Macias *et al*., 2007). Previous works have reported high leaf/stem polyphenol proportions in Plantogo species (Grubesic *et al*., 2005), thus confirming that leaf function serves as defence mechanism against UV damage (Harborne and Williams, 2000). Having used several solvents for fractionation, we found that the best yields of phenolic contents, especially tannins, were obtained in butanol fractions. This is in agreement with the results of a study on *Pistacia vera*, where it was found that the yield in total phenols depended on the method and the choice of solvent (Amir *et al*., 2005).

All extracts and fractions of both plants exhibited antioxidant activities. The antioxidant activity of *C. sericeum* could be attributed to tannins. As for *C. acutum*, In addition to tannins, others phenolic compounds as anthraquinones identified in the extract screening could contribute to the antioxidant activity of the plant. The butanol fractions of both plants displayed excellent antioxidant activities, implying high polarity of active compounds. Several studies have reported the antioxidant activities of some *Combretum* species (Masoko and Eloff, 2007; Coulidiati *et al*., 2009). The results showed that there is correlation between the phenolic compounds contents and the antioxidant activity of the investigated plant species which means that the total phenolic contents contributed significantly to the antioxidant activity. Among these phenolic compounds, tannins were of a great contribution. These results confirmed the findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity (Cai *et al*., 2004; Djeridane *et al*., 2006; Li *el al*., 2008). Very weak correlation was noticed between flavonoids and antioxidant activities in our study. Others authors have also found a low correlation between plant flavonoids levels and antioxidant activity (Miliauskas *et al*., 2004; Vundac *et al*., 2007). It is known that only flavonoids of a certain molecular structure, particularly

those with a certain hydroxyl position, could determine the antioxidant property. In general, these properties depend on the ability to donate hydrogen or electrons to a free radical (Meda *et al.*, 2005).

Phenolic compounds such as flavonoids, phenolic acids and tannins are considered as the major contributors to the antioxidant capacity of plants. These phenolic compounds also possess diverse biological activities (anti-inflammatory, anti-atherosclerotic and anticarcinogenic activities) that may be related to their antioxidant property (Chung *et al.*, 1998). Thus, the total phenolic, total tannin and flavonoid contents in the extracts and fractions of these plant species were also evaluated. The results obtained in this study could explain the use of these species as tisane for the newborn babies for their protection.

The results of the antibacterial study showed that the acetone extracts of *C. acutum* and *C. sericeum* produced zones of inhibition against all microorganisms tested, with the lowest MIC of = 0.312 mg mL^{-1} for the most susceptible bacteria. This indicates the presence of potent antibacterial activity, which confirms their use as anti-infective. Butanol fractions showed more inhibitory effects and low MIC values than the ethyl acetate fractions and n-hexane fractions. This tends to show that the active ingredients in the leaves were better extracted with butanol. In this study, *Escherichia coli* and *Klebsiella pneumoniae*, some of which showed resistance to ampicillin, were found to be susceptible to the tested acetone extracts and butanol fractions obtained from both plants. The antibacterial activity of these plants could be due to their phytochemical components. Indeed, the antibacterial activity of crude extracts has been attributed to the presence of some of the phytochemical components like, saponins, flavonoids and tannins (Musa *et al.*, 2008; Adebayo-Tayo and Ajibesin, 2008) which is in agreement with our results. Extracts from the leaves of *Combretum* species were found to contain tannins, flavonoids, alkaloids, triterpenoids and saponins and were effective against some strains of *E. coli*, *S. aureus* and *S. typhimurium* (Martini *et al.*, 2004; Angeh *et al.*, 2007; Coulidiati *et al.*, 2009). Sini *et al.* (2008) have reported that phytochemical screening of the aqueous extract of *C. sericeum* roots revealed the presence of tannins, flavonoids, glycosides, anthraquinones and alkaloids. The same authors have reported that the water extract of *C. sericeum* roots may be active against diarrhea. All these previous studies support results obtain in this study. The presence of bioactive components in the crude drugs has been linked to their activities against disease caused by microorganisms (Farnsworth, 1990) and also offering the plants them-selves protection against infection by pathogenic microorganisms (De and Ifeoma, 2002).

CONCLUSION

The results obtained in this study appear to confirm the antioxidant property and the antibacterial potential of *Combretum acutum* and *Combretum sericeum*, thus justifying their successful use in the treatment of infectious diarrhea. The efficacy of the acetone extracts and fractions of both plants could be attributed to the phenolic compounds such as tannins. Further investigations will be conducted on the butanol fractions for the isolation and identification of active principles.

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