

Full Length Research Paper

Antibacterial, antioxidant, cytotoxic activities and preliminary phytochemical screening of extracts from *Combretum schumannii* Engl.

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Combretum schumannii Engl. is a plant documented in traditional medicine system for treatment of headache, oedema and pneumonia in some areas of North Eastern Tanzania. In the present study, extracts from parts of this plant were assessed for antibacterial, antioxidant capacity and cytotoxic activities to establish rationale for its use in traditional medicine. Dried leaves, fruits, stem bark and roots of *C. schumannii* were extracted using dichloromethane:methanol (1:1), acetone (100%) and aqueous ethanol (80%), to afford dry extracts that were tested for antibacterial activity against eleven standard strains of bacteria. Portions of the same extracts were further subjected to *in vivo* brine shrimp lethality test and antioxidant activity test using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. Phytochemical screening of extracts was done by using standard chemical tests. Extracts of leaves, fruits, stem bark and roots of *C. schumannii* showed antibacterial activity against all test organisms, namely, *Bacillus anthracis*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhi*, *Vibrio cholera*, *Mycobacterium madagascariense* and *Mycobacterium indicuspranii*. All extracts that were tested exhibited no significant toxicity against brine shrimp larvae with LC_{50} values ≥ 253.30 $\mu\text{g/ml}$. Cyclophosphamide was used as a positive control with LC_{50} of 16.37 $\mu\text{g/ml}$. Furthermore, all the extracts showed mild to strong DPPH scavenging activity in which case ascorbic acid was used as a standard antioxidant. The present study indicated that extracts from the leaf, stem bark and roots of *C. schumannii* are safe for human use and that, this plant may be a good source of antimicrobial compounds worth further development.

Key words: *Combretum schumannii*, antibacterial, antioxidant, cytotoxicity, phytochemical screening.

INTRODUCTION

Application of medicinal plants for treatment of human ailments is a well-known phenomenon embedded in cultures of different communities of the developing world. Currently, there is resurgence in the use of herbal medicines globally; hence, implying increased efforts in

evaluating these medicinal plants to establish their safety and efficacy. In developing countries, dependence on traditional medicines for primary healthcare needs has obvious reasons including, connections to cultural preference, but also largely due to limited modern health

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care facilities for at least up to 65% of the world population (Daniel and Norman, 2001). Acceptance of traditional medicines globally, as complementary and/or alternative medicines has been driven largely by the fact that these medicines are effective and safe, usually based on ethnobiomedical information on their use by local communities. However, many concerns have been raised on lack of scientific evidence to support the medicinal values for many plants among those widely used for treatment of human diseases. This situation poses major challenge in the efforts directed towards mainstreaming traditional medicines healthcare system to modern health care system and therefore raising the demand for screening of these plant extracts and their evaluation for justifying their respective pharmacological activities.

Plants of the genus *Combretum* constitute majority of the family Combretaceae. The genus comprises about 370 species of trees, shrubs and lianas, roughly 300 of which are native to tropical and southern parts of Africa. At least 55 species out of those are reported to be growing in Tanzania, few of which are considered endemic (Wickens, 1973). Recently, there have been immense ethnobiomedical records indicating increasing use of medicinal plants of the Combretaceae family for the treatment of especially various HIV-1 related diseases including tuberculosis, venereal diseases, fever, cough, diarrhea, malaria, pneumonia, general weakness, mycotic infections, tumors and wasting (De Morais et al., 2012; Mushi et al., 2012). Subsequently, this trend has spurred substantial scientific investigations aimed at validating the claimed traditional uses and hence establishing margins of safety and efficacy for plants of this genus (McGaw et al., 2001).

Combretum schumannii Engl. (syns: *Combretum copaliferum* Chiov. or *Combretum macrostigmatum* Engl. & Diels) is a tree which grows up to 20 to 30 m high, occasionally described as scandent. Its bark peeling or flaking shows a paler underbark and the fruits are characterised by the wing-shaped appendages as is the case with other members of the Combretaceae. The tree is widely distributed in countries of Eastern and Southern Africa, including Somalia, Kenya, Tanzania, Zambia, Zimbabwe and Botswana (Carr, 1988). In Tanzania, the plant is locally known as Mpera-mwitu in Kiswahili, with several vernacular names within the region, for example, Mgongolo (Digo), Mgurure (Giryama), Mlanyika, Mgurulu from other tribes in Tanzania (Kokwaro, 1976; Martin, 1992). In traditional medicine, fresh root bark is usually ground and rubbed on swollen parts of the body to reduce oedema. Additionally, fresh leaves are heated over the fire and then applied to the chest as a poultice for half an hour to treat pneumonia and headache (Kokwaro, 1976). The present study aimed at evaluating different extracts from the leaves, stem bark, fruits and roots of *C. schumannii* for antibacterial, antioxidant and cytotoxic activities to ascertain whether there is relevance

in the use of this plant by the local population against HIV/AIDS-related secondary infections.

MATERIALS AND METHODS

Dichloromethane was purchased from UNILAB (UNILAB[®], Nairobi, Kenya), ethanol (absolute) from FlukaChemie GmbH (Sigma-Aldrich[®], Zwijndrecht, Netherlands), methanol and acetone from Sigma-Aldrich GmbH (Germany), whereas dimethyl sulfoxide (DMSO) was from Sigma[®] (Poole, Dorset, UK). Tryptone Soya broth was obtained from HIMEDIA[®] (Himedia Laboratories Pvt Ltd, Mumbai, INDIA). *Mycobacterium madagascariense* (DSM 44641) and *Mycobacterium indicuspranii* (DSM 45239) were obtained from the Germany Resource Centre for Biological materials (DSMZ, Braunschweig, Germany). *Staphylococcus aureus* (NCTC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 29953), *Salmonella typhi* (NCTC 8385), *Vibrio cholerae* (clinical isolate), *Bacillus anthracis* (NCTC10073), *Bacillus subtilis* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate) and *Enterococcus faecalis* (clinical isolate) were obtained from the Department of Microbiology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania. Iodonitrotetrazolium chloride was bought from SIGMA[®] (Sigma-Aldrich[®], St Louis, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from SIGMA[®] (Sigma-Aldrich[®], South Africa). The Brine Shrimps eggs were purchased from Aquaculture Innovations (Grahamstown 6140, South Africa) and sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam coast.

Collection, preparation and extraction of plant

Roots, stem bark, fruits and leaves of *C. schumannii* were collected from Handeni, Tanga, Tanzania in May, 2010 and a voucher specimen (collection No. 3614) have been deposited at the herbarium, Department of Botany, University of Dar es Salaam. All plant parts were air-dried, pulverized and extracted by maceration sequentially using dichloromethane:methanol (DCH:MeOH, 1:1), acetone (100%) and aqueous ethanol (80%). The extracts were dried under *vacuo* using rotary evaporator and/or freeze-dried to complete dryness before testing.

Antimicrobial test

Antibacterial activities of extracts were assayed by using two fold microdilution method (Eloff, 1998), whereas antimycobacterial activity was assayed according to previous related research (Erasto et al., 2011). Gentamycin was used as a positive control and the MIC-value of each extract was read at the concentration where a marked reduction in colour formation due to bacterial growth was marked.

Determination of DPPH radical scavenging activity

The antioxidant activity of leaf, root and fruit extracts was determined using DPPH assay as described by Liyana-Pathirana and Shahidi (2005).

Brine shrimps lethality test

Brine shrimps lethality test (BST) was used to predict the presence of cytotoxic compounds in the extracts as previously reported (Meyer et al., 1982). Cyclophosphamide was used as a standard

Table 1. *In vitro* antibacterial activity of extracts from roots, stem barks and leaves of *Combretum schumannii*.

Test organisms	Minimum Inhibitory Concentrations, MIC (mg/ml)									
	SBE	SBA	SBDM	RE	RA	RDM	LE	LA	LDM	Gentamycin
<i>M. madagascariense</i>	0.625	1.25	-	0.625	0.625	-	1.25	0.313	0.625	NT
<i>M. indicuspranii</i>	1.25	5.0	0.313	0.313	0.625	5.0	0.625	1.25	1.25	NT
<i>E. faecalis</i>	5.0	5.0	5.0	1.25	0.625	1.25	1.25	5.0	2.5	0.000224
<i>S. aureus</i>	5.0	5.0	0.625	1.25	1.25	1.25	0.625	-	5.0	0.000781
<i>E. coli</i>	5.0	5.0	5.0	1.25	2.5	2.5	-	2.5	2.5	0.00156
<i>P. aeruginosa</i>	5.0	2.5	5.0	1.25	2.5	2.5	0.625	1.25	1.25	0.00156
<i>B. anthracis</i>	0.625	5.0	0.625	1.25	1.25	1.25	0.313	0.625	1.25	0.0025
<i>B. subtilis</i>	5.0	5.0	5.0	1.25	1.25	1.25	1.25	1.25	2.5	0.00125
<i>V. cholerae</i>	5.0	5.0	5.0	1.25	1.25	1.25	1.25	2.5	1.25	0.00156
<i>K. pneumoniae</i>	5.0	5.0	5.0	2.5	2.5	2.5	0.313	2.5	5.0	0.0025
<i>S. typhi</i>	5.0	5.0	5.0	2.5	1.25	2.5	1.25	2.5	2.5	0.00625

- = No inhibition; NT = not tested. SBE= Stem bark ethanol (80%) extract; SBA = Stem bark acetone (100%) extract; SBDM= Stem bark dichloromethane:methanol (1:1) extract; RE= Root ethanol (80%) extract; RA = Root acetone (100%) extract; RDM= Root dichloromethane:methanol (1:1) extract; LDM= Leaves dichloromethane:methanol (1:1) extract; LA = Leaves acetone (100%) extract; LE= Leaves ethanol (80%) extract.

test drug.

Preliminary phytochemical screening test

Qualitative chemical test was carried out to identify the constituents of each extract using standard methods (Trease and Evans, 1989; Harbone, 1973).

Data analysis

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Fig P computer program (BiosoftInc USA), which also gives regression equations. Regression equations were used to calculate LC₁₆, LC₅₀ and LC₈₄ values. Confidence intervals (95% CI) were calculated according to a previously reported method (Litchfield and Wilcoxon, 1949). An LC₅₀ value greater than 100 µg/ml was considered to represent an inactive extract (Moshi et al., 2010). The MIC-values are interpreted as follows: 0.05 to 0.5 mg/ml strong activity, 0.6 to 1.5 mg/ml moderate activity and above 1.5 mg/ml weak activity (Mushi et al., 2012).

RESULTS

Antimicrobial activity

Extracts of leaves, stem barks and roots of *C. schumannii* showed varying antibacterial activity against all the tested organisms with MIC-values ranging from 0.313 to 5.0 mg/ml (Table 1). Mycobacteria were highly susceptible to all extracts except for dichloromethane/methanol (1:1) extracts of root and stem bark which had no activity against *M. madagascariense*. Extracts from root showed strong to moderate activity against Gram positive organisms whereas weak activity was observed to most of the Gram negative bacteria except *V. cholerae* which

had moderate activity with MIC-values of 1.25 mg/ml. Leaf and stem bark extracts had similar activity to both Gram positive and Gram negative organisms.

Antioxidant activity

The antioxidant activity of the leaves, fruits and root extracts was measured in terms of radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the results are shown in Figures 1 to 3. Both extracts showed substantial radical scavenging activity in a concentration dependent manner. Generally, activity increases with increase in concentration and the activity of extracts was in the order of 1:1 DCM/MeOH < 100% Acetone < 80% EtOH/H₂O.

Brine shrimp lethality

The LC₅₀ values of different extracts from *C. schumannii* and the reference drug are shown in Table 2. All extracts exhibited LC₅₀ values greater than 100 µg/ml, a value well above the cutoff point for cytotoxic activity. Interestingly, 20% aqueous ethanolic extract of the leaf had no observable activity at the maximum concentration tested (240 µg/ml).

Phytochemical screening results

Results of the phytochemical constituents of the extracts are shown in Table 3. Tannins were detected in all extracts of stem bark and leaves, whereas the other classes of phytochemicals were detected as shown in Table 3.

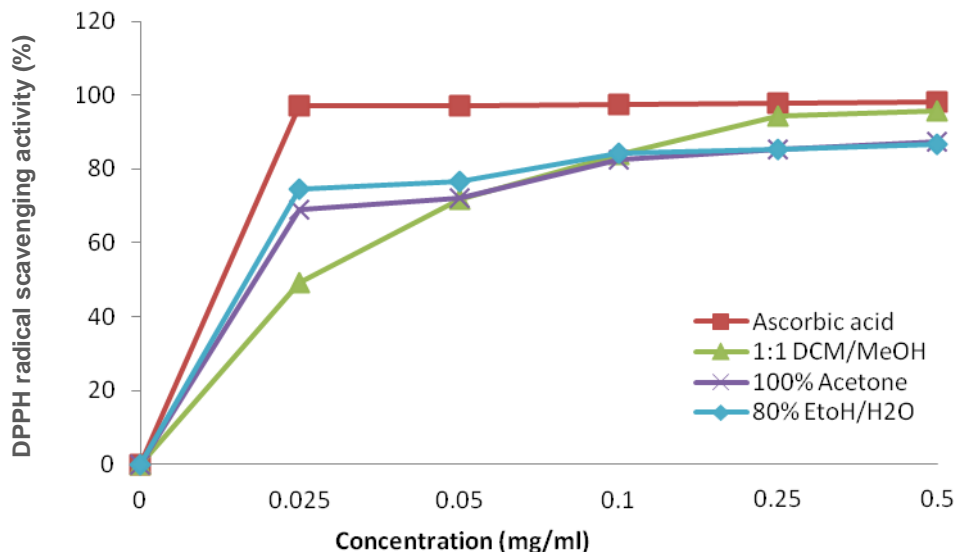


Figure 1. The DPPH radical scavenging activity of extracts from fruits of *C. schummanii* compared with Ascorbic acid (standard antioxidant drug). Each value is expressed as mean \pm SD ($n=3$).

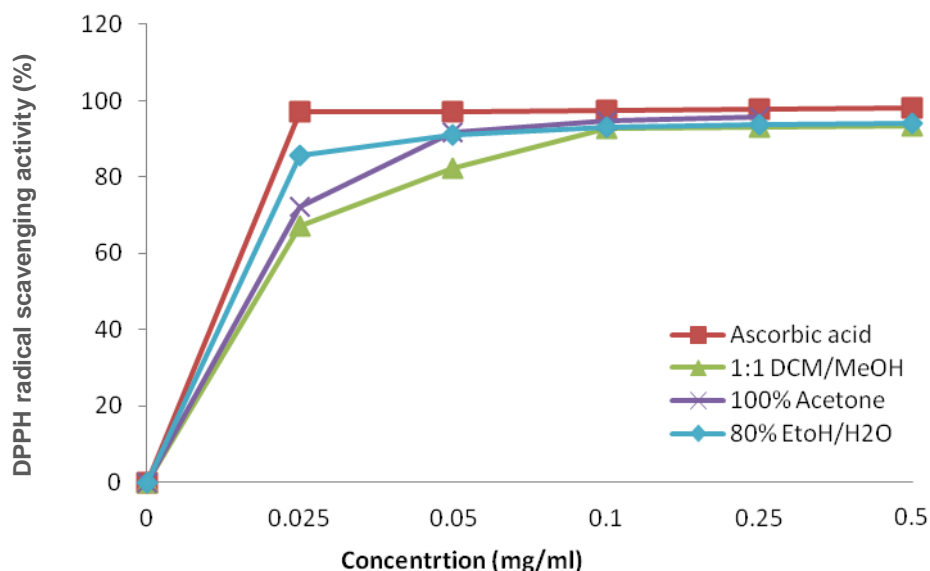


Figure 2. The DPPH radical scavenging activity of extracts from leaves of *C. schummanii* compared with Ascorbic acid (standard antioxidant drug). Each value is expressed as mean \pm SD ($n=3$).

DISCUSSION

Antibacterial test results in Table 1 showed that extracts from stem barks, root and leaves exhibit moderate antimicrobial activity with MIC-values ranging from 0.313 to 5 mg/ml. These results are much weak as compared to the results displayed by standard drugs (Gentamycin) which had MIC values at micro-level. However, these plants should be considered for further studies on

antimicrobial activity as low activity in crude extracts might be a result of the presence of many compounds which sometimes antagonise each other. Mycobacteria were more susceptible to all extracts except for dichloromethane/methanol (1:1) extracts of root and stem bark which could not suppress *M. madagascariense*. Generally, Mycobacteria are mostly more resistant than other bacteria strain and Mycobacterial infection is a public concern. Therefore, these results are encouraging

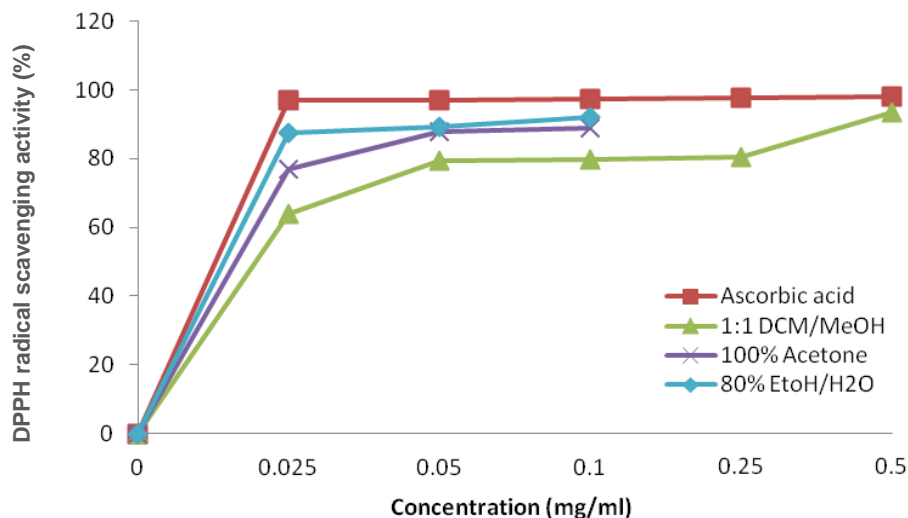


Figure 3. The DPPH radical scavenging activity of extracts from roots of *C. schumannii* compared with Ascorbic acid (standard antioxidant drug). Each value is expressed as mean \pm SD ($n=3$).

Table 2. Brine shrimp lethality results of *Combretum schumannii*.

Plant part	Extractant	LC ₅₀ (μ g/ml)	95% Confidence interval	Regression equations
Leaves	1:1 DCM/MeOH	3097.111	1582.5382 - 6061.3559	Y=31.626logx - 60.405
Leaves	100% Acetone	3839.8713	1367.9102 - 10778.9027	Y=33.22logx - 69.071
Leaves	20% Aqueous ethanol	*	*	*
Stem bark	1:1 DCM/MeOH	280.803	210.1856 - 375.1472	Y=96.663logx - 186.67
Stem bark	100% Acetone	3097.111	1277.9306 - 7505.8485	Y=31.62logx - 60.405
Stem bark	20% Aqueous ethanol	3097.111	1277.9310 - 7505.8485	Y=31.626logx - 60.405
Root	1:1 DCM/MeOH	1244.951	646.8848 - 2395.9082	Y=42.763logx - 82.358
Root	100% Acetone	678.711	405.1038 - 1137.1124	Y=66.441logx - 138.14
Root	20% Aqueous ethanol	253.3044	176.0621 - 364.4290	Y=66.653logx - 110.21
Cylophosphamide (Standard drug)		16.37	12.01 - 22.31	Y= 69.968logx - 34.936

*No activity observed at the maximal concentration (240 μ g/ml).

and more effort possible *in vivo* study should be conducted on this plant for Mycobacterium infections. Extracts from root showed strong to moderate activity against Gram positive bacteria. These results support the findings that mostly plant extracts exhibit strong antimicrobial activity against Gram positive bacteria than Gram negative bacteria (Palombo and Semple, 2001). Fresh leaves which are used for treatment of pneumonia showed strong activity against *K. pneumoniae* with MIC-value of 0.313 mg/ml; hence, supporting their use by traditional healers. Although, these results are not as strong when compared to gentamycin, an *in vivo* should be conducted as some drugs need metabolic activation to be more active. Phytochemical screening indicated that the active extracts were mostly rich in tannins and saponins followed by terpenoids and flavonoids while alkaloids were detected from the stem bark only (Table 3). Similar studies have been carried out on a number of

species from Combretaceae family and showed significant antimicrobial activity (De Morais Lima et al., 2012; Eloff et al., 2008). Extracts of fruits, leaves and roots showed antioxidant activity in concentration dependent manner (Figures 1 to 3). Literature indicates that plants which are rich in antioxidants play protective role against chronic and degenerative diseases (Szeto et al., 2002), thus the present findings support the traditional uses of *C. schumannii* against such diseases as well as HIV-related diseases. Several species from Combretaceae family have also been reported to possess antioxidant activities in previous studies (Masoko and Eloff, 2007).

In the present study, all extracts did not display potential cytotoxic activity as their LC₅₀ values were far greater than 100 μ g/ml, a value well above the cutoff point for cytotoxic activity (Table 2). Interestingly, 20% aqueous ethanolic extract of leaf had no observable toxicity at the maximum concentration tested (240 μ g/ml).

Table 3. Preliminary phytochemical constituents of *C. schumannii* extracts.

Plant part	Extract of plant part	Alkaloids	Terpenoids	Flavonoids	Tannins	Saponins
Stem bark	1:1 Dcm/MeoH	-	+	+	+	-
	100% Acetone	+	+	-	+	-
	20% Aqueous EtOH	+	-	-	+	-
Leaves	1:1 Dcm/MeoH	-	+	+	+	-
	100% Acetone	-	-	-	+	-
	20% Aqueous EtOH	-	-	-	+	+
Fruits	1:1 Dcm/MeoH	-	+	-	+	-
	100% Acetone	-	+	-	-	+
	20% Aqueous EtOH	-	-	+	+	+
Root	1:1 Dcm/MeoH	-	-	+	-	-
	100% Acetone	-	-	-	-	+
	20% Aqueous EtOH	-	-	-	-	+

+ = Present; - = absent

Conclusion

Based on results from this study, it can be concluded that extracts from the leaf, stem bark and roots of *C. schumannii* are safe for human use and that, this plant may be a good source of antimicrobial compounds worth further development. Further study especially *in vivo* studies should be conducted to confirm their relevance in living organism.

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