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Antibacterial profile of extracts of *Combretum micranthum* G. Don against resistant and sensitive nosocomial isolates

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ABSTRACT

Combretum micranthum G. Don (Combretaceae) is reputed in folk medicine for its anti-infective properties. In this study, we screened aqueous and methanol extracts of *Combretum micranthum* for antibacterial activities against nosocomial bacterial isolates. The methanolic and aqueous extracts of the leaves, root-bark and stem-bark were screened against the 25 different nosocomial isolates each of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* isolated from patients presenting with various ailment at the University of Nigeria Teaching Hospital, Enugu using the agar well diffusion technique. The extracts of *Combretum micranthum* exhibited antimicrobial activities against both Gram-negative and the Gram-positive isolates including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Generally, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* showed the highest level of susceptibility to the extracts of *Combretum micranthum*. The hot aqueous, the methanol and the cold aqueous extracts were more effective than other extracts in inhibiting all the isolates tested. The extracts of the plant *C. micranthum* G. Don showed high antimicrobial effectiveness against the different 200 clinical isolates of both Gram positive and Gram negative isolates screened and could therefore be harnessed as a potent antibacterial agents or could possibly provide leads for the synthesis of novel anti-infective agents.

Keywords: Antibacterial, *Combretum micranthum*, nosocomial.

INTRODUCTION

In recent times, we have witnessed a resurgence of interest and patronage of phythomedicinal products, even in developed countries where herbal alternatives are increasingly being promoted. Herbal formulations have also been improved in developing countries, not only to rescue the ancient folk remedies but also as effective alternatives to orthodox therapies. A lot of medical problems, especially in developing countries, have been associated with microbial infections. It is therefore not surprising that so many folk therapies have claims of anti-infective effectiveness. Some of these traditional antimicrobial therapies have for a long time been used successfully against microbial infections in developing countries where there is also unequivocal evidence as to the potency of these therapies. *Combretum micranthum* G. Don (Combretaceae) is believed to possess antibacterial properties and is used traditionally for the treatment of several infections (McGaw *et al.*, 2001). It is native to western Africa, distributed from Senegal, Mauritania, and Niger to Nigeria.

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It is a savannah plant, found on dry sites, sandstone, clay, laterite, crystalline rocks, and skeletal soils. *Combretum micranthum* is a small tree, shrub or liana of 4 m high (attaining up to 10 m under favourable conditions). It may reach a height/length of 20 m by twining around the branches of nearby trees. The bark is grey and fibrous, with orange to brown-red slash, and hairy and scaly red brown stems. The whole plant is densely covered with red scales. Leaves are variable, oblong-elliptic, 5-10 cm long and 2.5-5 cm wide and are alternate, shining light green when young; typically rust coloured when mature, in the dry season (MSBP, 2007).

The leaves are used to make the popular 'quinquelibas drink', a refreshing tea traded as 'kinkéliba'. The seeds are edible and the leaves are used as fodder for small ruminants. Leaves, roots and barks have many medicinal usages (antipyretic, tonic, diuretic, antidiarrhoeal and choleric) (MSBP, 2007). Leaf extracts have been found to exhibit anti-viral and anti-inflammatory properties. It is also commonly used in Burkina Faso and Cote d'Ivoire by native healers for the treatment of *malaria* (MSBP, 2007).

Nosocomial and multi-resistant infections constitute major global challenge and have increased morbidity, mortality and health care cost which are direct results of increased hospital turn-over and longer hospitalization periods (Ponce-de-Leon 1991; Emori and Gaynes, 1993). The interest in screening and harnessing medicinal plants for anti-infective application is therefore justifiable. In this study, we screened *Combretum micranthum* for antibacterial activities and evaluated its potential usage in treating nosocomial infections *vis-a-vis* the claims in folk medicine.

MATERIALS AND METHODS

Preparation of plant material

Various parts of *Combretum micranthum* used in this study were collected from a community, Alluma in Kogi state, Nigeria. The plant parts were authenticated by a taxonomist, Mr. A.O. Ozioko of the Department of Botany University of Nigeria, Nsukka. Excised plant parts (stem-bark, leaf and root) were rinsed thoroughly in running tap water. The leaves were dried at room temperature for one week, while the stem-bark and root were sun dried. The different plant parts were powdered using mechanical grinder.

Extraction

The leaf, root and stem-bark powder were extracted with methanol, cold and hot water. A 900 g portion of each powder was macerated in a total of 2 L of 95% methanol for 24 h, with intermittent agitations until exhaustively extracted at room temperature. The methanol extracts were filtered and the filtrates evaporated to dryness yielding solid extracts. For the cold aqueous extraction, 900 g of each of the powdered plant parts were soaked in distilled water and was left at room temperature (27°C) with occasional agitation for about 24 h. Similarly, in the hot aqueous extraction, 900 g of each of the powdered plant material was soaked in 2.7 L of boiling water and kept at 100°C for 1 h. The

extract was filtered and concentrated by evaporation in a steady air current. The extracts were placed under UV rays for 24 h for sterilization and after which checked for sterility by streaking a sample suspension on nutrient agar plate.

Phytochemical tests

A preliminary phytochemical study of the plant parts was carried out and involved testing for the presence or absence of the following secondary metabolites: alkaloids, saponins, glycosides, resins, flavonoids, tannins, steroids and triterpenes, oils carbohydrates and proteins. The methods of Harbourne (1998) and Evans (1998) were adopted in the screening.

Test microorganisms

Strains of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumoniae* isolated from different clinical specimens were obtained from the Medical Microbiology Laboratory, University of Nigeria Teaching Hospital, Enugu State, Nigeria. The isolates were confirmed by standard bacteriological methods (Cheesbrough, 2000; Murray *et al.*, 1995) and purified by three successive sub-culturing on nutrient agar. Purified cultures were stored for a longer period on nutrient agar slants at 4°C. The organisms were activated by successive daily sub-culturing into fresh agar slants for a period of 3 days before use. Overnight (18 – 24 h) cultures in nutrient both were standardized with McFarland 0.5 standard (Cheesbrough, 2000).

Testing susceptibility of microorganisms to Extracts

Susceptibility testing was by the modified agar well diffusion technique (Esimone and Adikwu, 2002). A solution of the methanol extracts (100 mg/ml) was prepared in Dimethyl sulfoxide (DMSO) and a solution (100 mg/ml) of the aqueous extracts was made in sterile distilled water. Molten Mueller Hinton agar (19.9 ml) was seeded with 0.1 ml of the standardized broth cultures of bacteria and was allowed to set. A total of 5 wells (8 mm in diameter) were bored in the agar using a sterile cork-borer. Two drops (about 0.04 ml) of 5 mg/ml concentration of each of the extracts was carefully placed into each of the wells. Two drops of a 2-fold dilution of DMSO was put in the middle well as negative control. The plates were left for 1 hour at room temperature for diffusion of the extracts before incubating at 37 °C for 24 h. Triplicate determinations were made and the average zone of inhibition diameter (IZD) recorded after the incubation. Similar experiments were set up to compare the antibacterial activities of the extract with those of standard antibiotics, gentamicin, ampicillin, and ciprofloxacin.

RESULT

With the exception of the undefatted cold water extracts of the stem bark, all other stem bark and root bark extracts showed high level of inhibition against *Staphylococcus aureus* isolates. *Staphylococcus aureus* isolates were more susceptible to the stem

bark hot water extract (SBHW) with 88% susceptibility rate (Figure 1a). The stem bark hot water extract (SBHW) and the root bark hot water extract (RBHW) inhibited *Streptococcus pneumoniae* the most with 80% of the isolates showing different degree of susceptibility. The RBHW extract also caused a higher level of inhibition against *Streptococcus pyogenes* (Figure 1b).

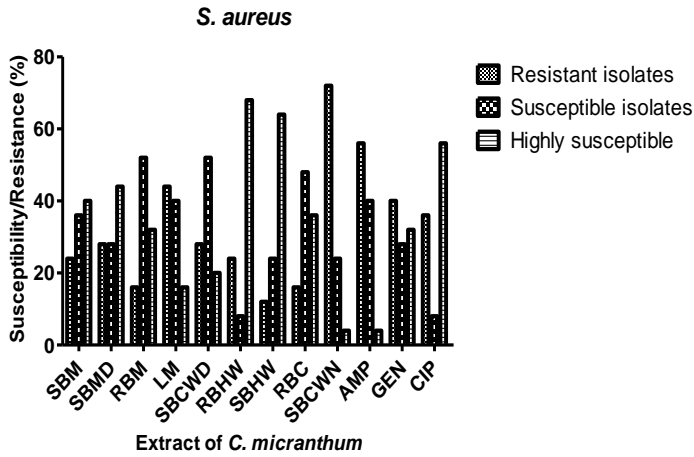


Fig. 1a:

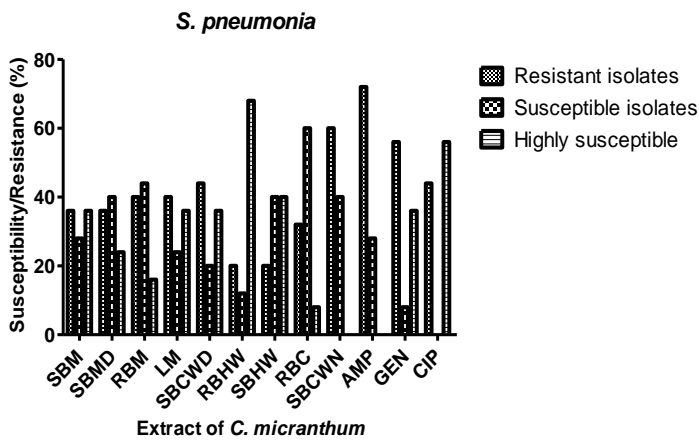


Fig. 1b:

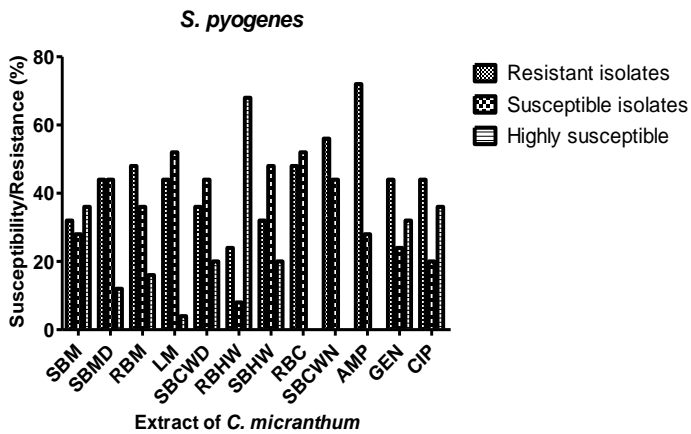


Fig.1(a-c): 1a-1c: Susceptibility of clinical isolates of some Gram positive isolates to different extracts (5 mg/ml) of *C. micranthum*. Inhibition zone diameter ≤ 15 mm (Susceptible isolates); Inhibition diameter > 15 mm (Highly susceptible isolates) SBM-Stem bark methanol extract, SBMD-defatted stem bark methanol extract, RBM-Root bark methanol extract, LM-Leaf methanol extract, SBCWD- defatted stem-bark cold water extract, RBHW-Root bark hot water extract, SBHW-Stem bark hot water extract, RBC- Root bark cold water extract, SBCWN-undefatted stem bark cold water extract, AMP-ampicillin, GEN-gentamycin, CIP-ciprofloxacin

All the bark extracts of *C. micranthum* showed strong inhibition of *Pseudomonas aeruginosa* at a level significantly higher than ampicillin, gentamycin and ciprofloxacin (Figure 2a). All the *Pseudomonas aeruginosa* isolates screened were susceptible to the root bark hot water (RBHW) and stem bark hot water extracts (SBHW) of *C. micranthum*. Apart from the undefatted stem bark cold water extracts, all other extracts tested inhibited isolates of *E. coli* at a degree comparable to that recorded for ciprofloxacin. The stem bark hot water (SBHW) and undefatted stem bark cold water (SBCWN) extracts did not inhibit the *Klebsiella pneumoniae* isolates. Other extracts showed significant inhibition of *Klebsiella pneumoniae* with the root bark methanol extract (RBM) showing the highest degree of susceptibility (88%) as shown in Figure 2c. *Proteus mirabilis* and *Enterococcus faecalis* were also susceptible, but not to the undefatted cold water extract of the stem bark.

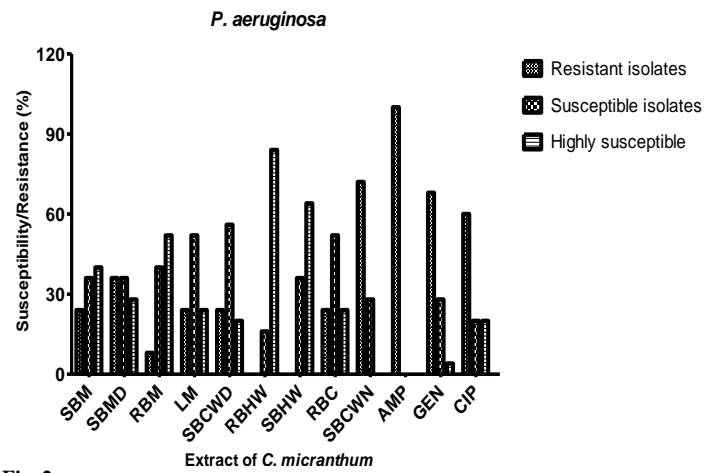


Fig. 2a:

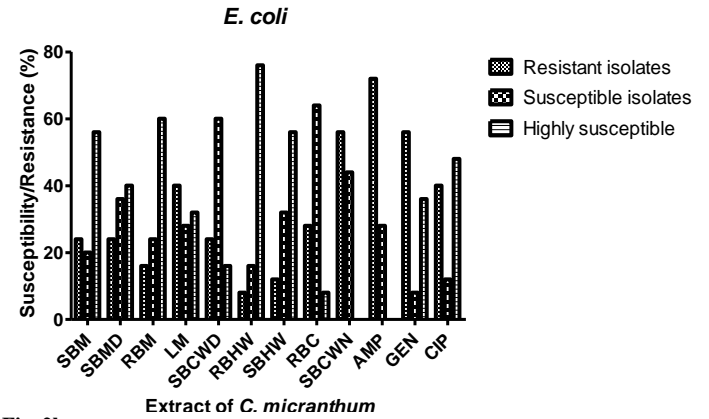


Fig. 2b:

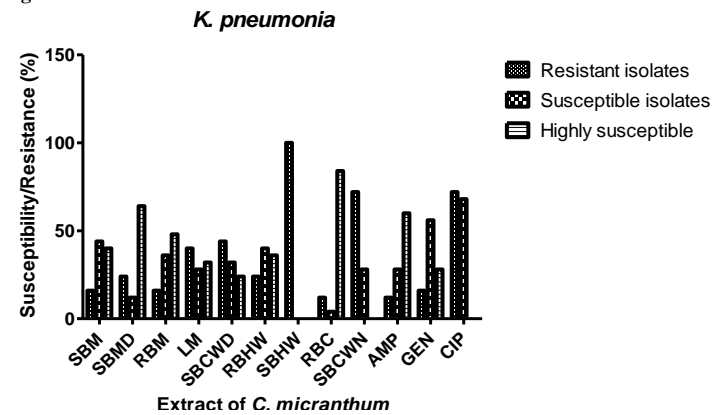


Fig 2c:

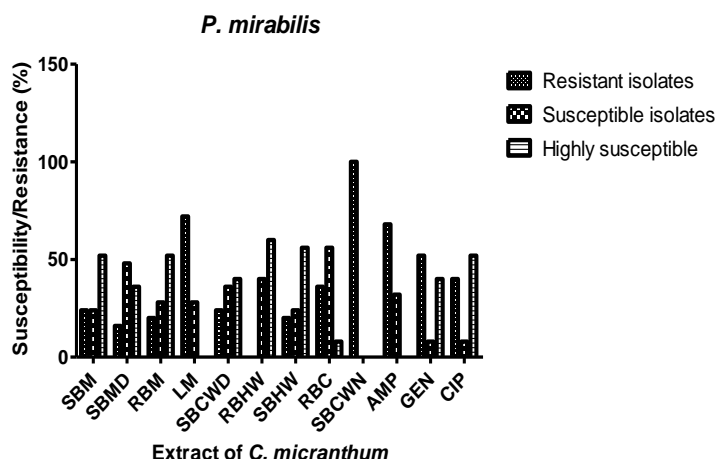


Fig. 2d:

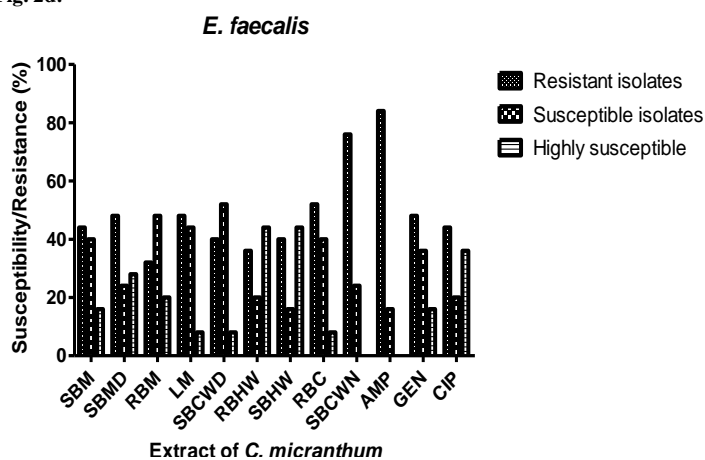


Fig. 2 (a-e): Susceptibility of clinical isolates of some Gram negative isolates to different extracts (5 mg/ml) of *C. micranthum*. Inhibition zone diameter ≤ 15 mm (Susceptible isolates); Inhibition diameter > 15 mm (Highly susceptible isolates) SBM-Stem bark methanol extract, SBMD-defatted stem bark methanol extract, RBM-Root bark methanol extract, LM-Leaf methanol extract, SBCWD- defatted stem-bark cold water extract, RBHW-Root bark hot water extract, SBHW-Stem bark hot water extract, RBC- Root bark cold water extract, SBCWN-undefatted stem bark cold water extract, AMP-ampicillin, GEN-gentamycin, CIP-ciprofloxacin

DISCUSSION

The screening of the aqueous and methanol extracts of *Combretum micranthum* root-bark, leaf and stem-bark extracts for antibacterial properties revealed broad spectrum and potent antibacterial activity against various bacterial isolates. The different extracts of this plant differed significantly in their antibacterial activities against the clinical bacterial isolates ($P < 0.05$). Other species of *Combretum* have also been shown to show similar antibacterial activities in earlier studies (Ferrea *et al.*, 1993; Eloff and Martini, 1998; Rogers and Verotta, 1995). The hot aqueous root-bark extract, followed by the hot aqueous stem-bark extract showed the highest antibacterial activities. The least activity was shown by the cold aqueous stem-bark extract. The test bacterial isolates also differed significantly in their susceptibility to the extracts. Although the Gram-positive and Gram-negative bacteria were both susceptible to the extracts of *Combretum micranthum*, the extracts appear to have better activity against Gram negative isolates. Remarkably, the extracts of *C. micranthum* also showed good anti-pseudomonal and anti-staphylococcal

activities which make the extracts suitable in combating nosocomial infections (Mackie JT and McCartney, 1996).

The root-bark and the stem-bark extracts exhibited greater antibacterial activity than the leaf extracts. Preliminary phytochemical test on *Combretum micranthum* revealed the presence of secondary metabolites such as saponins, tannins, resins, glycosides, flavonoids and alkaloids. These classes of secondary metabolites have frequently been found in several other *Combretum* species (Rogers and Verotta, 1995) and have also been associated with antimicrobial activities. The higher abundance of these metabolites in root and stem bark extracts than the leaf extracts may partly explain the higher activities of the bark extracts.

The abundant presence of bioactive tannins and flavonoids in *C. micranthum* could be responsible for the observed antimicrobial activities. Tannins form complexes with proteins through covalent bonds formation and through the so-called non-specific forces, such as hydrogen bonding and hydrophobic effects (Stern *et al.*, 1996; Haslam, 1996). The antibacterial mode of action is considered similar to that of the quinones, and may be related to their ability to inactivate microbial adhesins, enzymes, and cell envelopes and transport proteins. They are also able to form complexes with polysaccharides (Ya *et al.*, 1988) Our initial screening data encourages further purification and use of *Combretum micranthum* extracts as antimicrobial agents in primary health-care, especially for multi-antibiotic resistant strains.

CONCLUSION

The extracts of the plant *C. micranthum* G. Don showed high antimicrobial effectiveness against the different 200 clinical isolates of both Gram positive and Gram negative isolates screened and could therefore be harnessed as a potent antibacterial agents or could possibly provide leads for the synthesis of novel anti-infective agents. The extracts could also be applied in formulating antiseptic/ disinfectant solutions where the broad antimicrobial spectrum will be an advantage.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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