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PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL ACTIVITIES OF THE LEAF, STEM BARK AND ROOT EXTRACTS OF *Combretum sokodense*

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ABSTRACT
The phytochemical and antimicrobial properties of the leaf, stem bark and root of Combretum sokodense were examined in this research work. The phytochemical screening of the plant reveals the presence of flavonoids, tannins, saponins, terpenoids, alkaloids, antraquinone, carbohydrates and reducing sugar. The leaf, stem bark and roots extract also show antimicrobial activities.The antimicrobial activities of the plants extracts revealed an inhibitory effect against Staphylococcus aureus, Escherichia coli, Aspergillus niger and Mucorsppat different concentrations. The methanolic extract of the leaf showed the highest level of inhibition on Escherichia coli while Mucor species showed highest level of inhibition on ethanolic extracts. The ethanolic extracts of the stem bark showed the highest level of inhibition on Escherichia coli and Aspergillus niger while the methanolic extract showed less response. Staphylococcus aureus and Aspergillus niger showed highest level of inhibition on the root of Combretum sokodense. The antimicrobial activity against bacterial and fungal isolates may be attributed to the presence of these secondary metabolites. Therefore, Combretum sokodense is found to be a potential source of drugs for the treatment of bacterial and fungal infections.
Keywords: Combretum sokodense, Extract, Phytochemicals, Antimicrobial Activities, Traditional Medicine.

INTRODUCTION

Medicinal plants are known to contain substances that are used for therapeutic purposes and are precursors for the synthesis of useful drugs. In Nigeria, the use of plant parts in the treatment of human disease is as old as the disease itself and herbal medicine was the major form of medicine (Oghenejobo *et al.,* 2014; Yahaya *et al.,* 2012). Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantages of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Barbour *et al.,* 2004; Yasunaka *et al.,* 2005)**.**Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as microbial and viral infections(Koshy *et al.,* 2009).

Combretum, the bush willows or Combretums, make up the type genus of the family *Combretaceae*. The genus comprises about 370 species of trees and shrubs, roughly 300 of which are native tropical and Southern Africa, about 5 to Madagascar, some 25 to tropical Asia and approximately 40 to tropical America. The genus is absent from Australia. Though somewhat reminiscent of willows in their habitus, they are not particularly close relatives of these (Tan *et al.,* 2002)**.**Some of the different species of Combretum plants include; *Combretum sokodense Combretum mossanbicanse, Combretum woodii, Combretum, erythophyllum, Combretum racemosum, Combretum apiculatum* etc (Elegami *et al.,*2007; Eloff *et al.,* 2008). They could be shrubs, trees or climbers (Oghenejobo *et al.,* 2014). In Nigeria, different types of plant are used in the treatment of different types of diseases. The roots, stem, bark and leaf of *Combretum sokodense* are used in the treatment of scrotal elephantiasis, ring worms, typhoid fever, impotence, eye sore and ear ache, stomach pains, snake-bite, leprosy, dysentery, diarrhea, general body swellings, arthritis and other inflammatory conditions and abortion as well as for swelling of the abdomen, sterility and constipation (Fyhrquist *et al.*, 2002; Grønhaug *et al.,* 2008; Nwaeze and Abarikwu, 2006; Ojewole, 2005).

Hutchings *et al.*(1996) reported that some *Combretum* species are used traditionally for the treatment of mumps, syphilis, pneumonia, and colds. Other diseases as stated by traditionalists that could be treated with *Combretum* species are nose bleeds, sore throats, fattening babies, gall stone, hook worm, urinary tract infection, scorpion bite, infertility in women, heart disease, dysmenorrhoea and general debility (McGaw *et al.,* 2001; Oghenejobo *et al.,* 2014; Van Wyk*et al.,* 1997).

These substances are present in different forms. Fresh, dried, capsules, tablets, bottled in liquid forms, tea, bath tincture etc are all good as the quality of the raw plant from which they were made (Winstanly *et al.,* 1997; Yahaya *et al.,* 2012).Phenols, tannins, flavonoids, glycosides, sterols, terpenoids, shikimics acids, and choline are some of the reported phytoconstituents of the herbs (Nascimento *et al.,* 2000). The increasing failure of chemotherapeutic antibiotic resistance showed by many pathogenic infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Plants with possible antimicrobial activity should be tested against appropriate microorganisms to confirm their activity and ascertain other parameters associated with it. Among the different *Combretum* species, no much study work has been done on *Combretum sokodense.* As such, this study is aimed at determining the phytochemicals and antimicrobial potentials of the plant against some selected microbial isolates.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh samples of Leaf, stem bark and roots of *Combretum sokoodense* were collected from Tulu village, of Gumaudistrict in Toro Local Government Area of Bauchi State, Nigeria. The samples were taken to a Botanist in the Department of Plant Biology, Faculty of Science, Bayero University, Kano for taxonomic identification. The samples were identified and given an accession Number of: Bayero University, Kano herbarium Accession Number BUKHAN 0013. This study was carried out between July – September when fresh sample of the plant were readily available.

Preparation of Plant Materials

The harvested fresh plant samples were rinsed with tap water and air dried under shade for two weeks and reduced to coarse powder using pestle and mortar and then ground to fine powder using a blender. The powder was stored in a plastic container for further use.

Preparation of Extract

Exactly 5g of the powdered samples(leaf, stem bark and roots) were weighed separately in a different beaker and percolated with 50mL of distilled water covered with cotton wool and wrapped with aluminum foil after vigorous shaking. The solution was left to stand at room temperature for 48 h.The mixture was then filtered with Whatman No. 1 Filter paper. The filtrate (extract) was used for the phytochemical screening. The mixture was then filtered using a clean muslin cloth and then Whatman filter paper No. 1 to obtain the extract. The extracts obtained were concentrated using rotary evaporator at 40ºC. The percentage extract yield was estimated as dry weight/dry material weight ×100 (Parekh and Chanda, 2007).

Phytochemical Screening

The extract were subjected to preliminary phytochemical tests using standard methods as described by Yahaya *et al*.(2011; 2012)to detect the presence of antraquinones, tannins, cardiac glycosides, terpenoids, alkaloids, saponin, phenols, glycosides.

Anti-microbial Assay

Preparation of Medium

The clinical isolates used were *Escherichia coli,* a gram-negative bacteria and *Staphylococcus aureus,* a gram positive pathogen. The two fungi used were *Aspergillus niger* and M*ucor* species. All the test organisms were clinical isolates obtained from Aminu Kano Teaching Hospital, Kano and were taken to Microbiology Laboratory, Department of Microbiology, Bayero University, Kano for various biochemical tests for identification. The bacterial isolates were grown in Nutrient Agar (NA) while the fungal isolates were grown in Potato Dextrose Agar (PDA). The media were prepared according to the method of (Wolfgang and Hilda, 1976).

Determination of Antimicrobial Activity

Organic solvent extracts were dissolved in 1mL dimethyl sulphoxide (DMSO) while aqueous extracts were dissolved in 1mL sterile distilled water, i.e. 0.6g of each extract was dissolved in 1mL of the solvent. Half (0.5) mL of the extract was introduced into 50 sterile discs respectively in Bijour bottles to make 60µg/disc concentration. Half mL of DMSO was added into the remaining stock solution making 1mL, 0.5 mL was taken and placed into another bottle containing 50 filter paper discs and labeled 30µg/disc, 0.5 mL of DMSO was added, another 0.5 mL was taken and placed into another 50 filter paper discs and labeled 15µg/disc. With each disc was capable of absorbing 0.01mL of the solution, the procedure was employed to prepare 15, 30 and 60µg/disc concentrations. The same process of serial doubling dilution as explained above was employed in the preparation of organic solvent extract for disc and Minimum Inhibition Concentration (MIC) preparation, and measurement of zones of inhibition as described by National Committee for Clinical Laboratory Standard (NCCLS, 2008).

Preparation of Turbidity Standard

Barium Sulphate (l% w/v) standard suspension was used as turbidity standard. This was prepared by adding 0.6 mL of (l% w/v) Barium Chloride solution with 99.4mL ofH_2SO_4 (l% v/v) solution to yield 1% w/v Barium Sulphate suspension. The turbid solution formed was transferred into the test tube as the standard for comparison(Cheesbourgh, 2005).

Standardization of Inoculum

Using inoculation loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

Preparation of Sensitivity Discs

Whatman No. lfilter paper discs of 6mm in diameter were punched out with the aid of paper punch and placed in Bijour bottles. They were then sterilized by autoclaving at 121°C for 15 minutes. The discs were allowed to cool.

Disc Diffusion Test

Standard Inocula of the isolate were swabbed onto the surface of prepared and solidified Mueller Hinton agar in separate Petri-dishes. The prepared discs of the extracts and the standard antibiotic discs (Chloramphenicol) were placed onto the surface of the inoculated media at intervals. The plates were incubated at 37°C for 24 h before observation for and measurement of zones of inhibition (NCCLS, 2008).

Minimum Inhibitory Concentration (MIC)

MIC was determined by preparing various concentrations of the extracts by serial doubling dilution (as explained in disc preparation) and incorporated into test tubes containing 2mL nutrient broth. Standardized inocula (0.1mL) of the isolates were introduced and the tubes were incubated at 37^{o}C for 24 h (NCCLS, 2008). The results were taken by considering the zone of growth and inhibition of the organisms by the test fraction (Ridgway, 1989).

RESULTS AND DISCUSSION

The qualitative phytochemical characteristics of the medicinal plant were investigated and summarized in Table 1. Tannins, saponins, flavonoids, terpenoids, carbohydrate and reducing sugars were present in the leaf, stem bark and roots. The quantitative phytochemical analysis of the medicinal plant is shown in Table 2.

Table 1. Qualitative phytochemical analysis of *Combretum sokodense*

Phytochemicals	Leaf	Stem bark	Root
Tannins	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Steroids	-	-	+
Antraquinones	+	+	-
Soluble starch	-	-	+
Terpenoids	+	+	+
Carbohydrates	+	+	+
Reducing Sugar	+	+	+

KEY: +: Present; -: Absent

Table 2. Quantitative phytochemical analysis of *Combretum sokodense*

Test	Leaf	Stem bark	Root
Alkaloids	0.197	0.151	0.102
Tannins	3.596	0.406	0.201
Saponins	12.297	8.028	0.760
Flavonoids	1.090	1.323	0.538

The phytochemical screening of the extract of the plant showed that the leaf, stem bark and roots were rich in some bioactive components as seen in Table 1. The *Combretum sokodense* leaf shows highest concentration of alkaloids, tannins, saponins and flavonoids when compared to other parts of the plant as shown in Table 2.Edeoga*et al.*(2005) reported that alkaloids, tannins, saponins and flavonoids were known to show medicinal activity as well as exhibiting physiological activity and show anti-oxidant, anti-inflammatory and membrane stabilizing property (Perenz *et al.,*1995). The presence of terpenoid has been reported to be useful in herbal medicines (Baker*etal.*, 1983; Hayashi *et al.,*1993). These phytochemicals also have some strong antimicrobial significance against some potential enteric pathogens. Kam and Liew(2002)reported that alkaloids are known to be the largest group of secondary metabolites in plants. Their presence in significant amount is claimed to have powerful effects on humans and hence could be used as pain killer medications, anti-malaria and stimulants (Duke and Ayensu, 1985; Yahaya *et al.,* 2012).The presence of tannin is also important, as it form irreversible complexes with prolin-rich protein, which results in protein synthesis inhibition(Shimada *et al.,* 2006). Parekh*et al.* (2007) also reported that tannins react with proteins to provide tanning effect that helps in the treatment of inflamed ulcerated tissues. Most herbs that contain tannin as a major constituent are claimed to be astringent in nature and useful in the treatment of intestinal disorders like diarrhea and dysentery, wounds, sprains, bruises and arresting bleeding (Oghenejobo *et al.,* 2014; Yahaya *et al.,* 2012). Tanins are known to inhibit tumor growth, and hence could be used for cancer prevention. Thus it can be suggested from the above that *Combretum sokodense* is a source of bioactive compound that could have effect on the treatment and prevention of cancer. These observations could be responsible for the use of *Combretum sokodense* in herbal cure remedies (Li *et al.,* 2006).

The antimicrobial activities of crude extracts of *Combretum sokodense* were estimated by measuring the diameters of the zones of growth inhibition on the tested microbial species and the results were presented in Table 3. The entire test organisms were susceptible to *Combretums okodense* extract though to varying degree. The antimicrobial activities of the plant extracts revealed an inhibitory effect against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Mucor* spp at different concentrations as shown in Table 3. The methanolic extract of the leaf showed the highest level of inhibition on *Escherichia coli* while *Mucor* species showed highest level of inhibition on ethanolic extracts.

The yield when compared with the results obtained by (Yahaya *et al.,* 2012) when compared with the ethanolic extract of this plant is relatively small. The ethanolic extracts of the stem bark showed the highest level of inhibition on *Escherichia coli* and *Aspergillus niger* while the methanolic extract showed less response. *Staphylococcus aureus* and *Aspergillus niger* showed highest level of inhibition on the root of *Combretum sokodense.*

Table 3. Antimicrobial activity of *Combretum sokodense* showing zone of inhibition diameter (mm) of the Test Organisms

	Leaf						**Stem bark**						**Root**					
	MthE (μg/disc)			**EthE (μg/disc)**			**MthE (μg/disc**			**EthE (μg/disc)**			**MthE (μg/disc)**			**EthE (μg/disc)**		
Organism	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**
S. aureus	8	9	13	8	10	12	11	9	0	12	9	0	8	11	14	12	8	6
E. coli	0	11	14	8	9	10	14	12	8	16	13	10	0	0	0	9	6	0
*Mucor*spp	9	11	13	10	12	14	12	11	9	13	10	8	10	8	0	10	8	0
A. niger	0	10	12	8	10	13	13	10	8	14	12	10	10	14	14	15	13	10

Key: MthE :Methanolic Extract; EthE : Ethanolic Extract

Conclusion

The phytochemistry and antimicrobial activity of *Combretum sokodense* of leaf, stem bark and root extracts shows that the plant contains secondary metabolites and has antimicrobial properties. The antimicrobial activity against bacterial and fungal isolates may be attributed to the presence of these secondary metabolites. Therefore, *Combretum sokodense* is found to be a good source for the treatment of bacterial and fungal infections.

REFERENCES

Baker, C. N., Thomsberg, C., and Hawkinson, R. W. (1983). Inoculum standardization in antimicrobial susceptibility testing: Evaluation of overnight agar cultures and the rapid inoculum standardization system. *J. Clin. Microbiol.*, *17*(3), 450–457.

Barbour, E., Sharif, M. A., Sagherian, V. K., Harbe, A. N., Talkbouk, R. S., and Tasheuk, S. . (2004). Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J. Ethno-Pharmacology*, *93*, 1–7.

Cheesbourgh, M. (2005). District laboratory *practice* in tropical countries. In *part 2. Thatford press* (pp. 64–70).

Duke, J. A., and Ayensu, E. S. (1985). *Medicinal plants of China. Mich. Reference Publcations, Algonae, ISBN: 0917256204.*

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African J. Biotechnol.*,*4*(7), 685–688.

Elegami, A., Osman, S. M., Omer, M. E., and Ishag, K. M. (2007). In- vitro antibacterial activity of some Sudanese Combretum species. *Int. J. Trop. Med.*,*2*, 45– 51.

Eloff, J. N., Katerene, D. R., and Mcgaw, L. J. (2008). The Biological activity and chemistry of the South African Combretaceae. *J. Ethnopharm*, *1119*, 686–699.

Fyhrquist, P., Muwasumbi, L., Haeggotro, C. A., Vuerela, H., Hiltunen, R., and Vuorela, P. (2002). Ethnobotanical and antimicrobial investigation on some species of termindia and Combretum (Combretaceae) growing in Tanzania. *J. Ethnopharm.*, *79*, 169 – 177.

Grønhaug, T. E., Glæserud, S., Skogsrud, M., Ballo, N., Bah, S., Diallo, D., and Paulsen, B. S. (2008). Ethnopharmacological Survey Of Six Medicinal Plants From Mali, West-Africa. *Journal of Ethnobiology and Ethnomedicine*, *4*, 26–36.

Hayashi, T., Okamuka, K., Kawasaki, M., and Morita, N. (1993). 1993. Production of diterpenoids by cultured cells from 2 chemotypes scoparia dulcis. *Phytochemistry*, *53*(2), 353–356.

Hutchings, A., Scott, A. H., Lewis, G., and Cunningham, A. (1996). *Zulu medicinal Plants: An inventory*. *University of Natal Press; Scottsville South Africa*.

Kalemba and Kunicka. (2003). Antibacterial and Antifungal Properties of Essential Oils. *Curr. Med. Chem.*,*10*(10), 813–29.

Kam, P. C., and Liew, A. (2002). Traditional Chinese herbal medicine and anaesthesia. *Anaesthesia*, *57*(11), 1083–1089.

Koshy, P., Sri, N. A. M., and Wirakanain, S. et al. (2009). Antimicrobial activity of some medicinal plants from Malaysia. Am J of Applied Sci. *Am. J. Appl. Sci.*, *6*(8), 1613– 1617.

Li, Q., Wang, J., Ivanochko, G., and Huang, Y. (2006). Anti-cancer effects of extracts from a North American medicinal plant-wild Sarsaparrilla. *Anti-cancer Res.*, *26*, 2157–2164.

McGaw, L. J., Rabr, T., Sparg, S. G., Jäger, A. K., Eloff, J. N., and Van Staden, J. (2001). An Investigation of the biological activity of Combretum species. *J. Ethnopharm.*, *75*, 45–50.

Nascimento, G. G. F., Locatelli, J., Paulo, C. F., and Silva, G. (2000). The antimicrobial activity of plant extract and phytochemical on antibiotic resistant bacteria. *Brazil J. Microbiol.*,*31*, 247–256.

National Committee for Clinical Laboratory Standards; NCCLS (2008); Performance standards for antimicrobial susceptibility testing; Ninth informational supplement. NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, wayne, PA

Nwaeze, C. U., and Abarikwu, P. O. (2006). Antimicrobial activity of certain medicinal plants used in traditional medicine in Nigeria. *Niger. J. Microbiol.*,*6*(12), 32–40.

Oghenejobo, M., Oghenejobo, B. U. S., Uvieghara, K. E., and Omughele, E. (2014). Phytochemical Screening and Antimicrobial Activities of the Fractionated Leaf Extract of Combretum Racemosum,*Sch. Acad. J. Pharm.,3*(6), 455–462.

Ojewole, J. A. O. (2005). Analgesic and anti-inflammatory effects of mollic acid glucoside, a 1a-hydrozycycloartenoid saponin extractive from Combretum molle. R. Br. ExG. Don (Combretaceae) leaf. *Phyther. Brazilian J. Microbiol.*,*38*, 2 – 6.

Parekh, J., and Chanda, S. (2007). In-vitro antibacterial Activity of Crude Methanolic Extract of Woodfordia Fruticosa Kurz Flower (Lythacease). *Brazilian J. Microbiol.*, *38*, 2–10.

Parekh, J., Darsharna, J., and Chanda, S. (2007). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antimicrobial activity. *Turkish J. Biol.*,*29*, 203–210.

Perenz, R. M., Perenz, S., Zaval, A. M., and Salazar, N. (1995). Anti-inflammatory activity of the bark of Hippocrata excels. *J. Ethnopharmacol., 47*(1), 85–90.

Ridgway, G. L. (1989). Practical medicals microbiology. *J. Clin. Pathol.*, *42*(12), 1315.

Shimada, T., Koumoto, Y., Li, L., Yamazaki, M., Kondo, M., Nishimura, M., and Hara-Nishimura, I. (2006). AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage proteins. *Plant Cell Physiol.*,*47*, 1187–1194.

Tan, D. X., Reiter, R. J., Manchester, L. C., Yan, M. T., El-Sawi, M., Sainz, R. M., … Hardeland, R. (2002). Chemical and physical properties and potential mechanisms: Melatonin as a broad-spectrum antioxidant and free radical scavenger. *Curr. Top Med. Chem.*,*2*, 181–98.

Van Wyk, B. E., Van, O., and Gerick, N. (1997). *Medicinal plants of South Africa. 1st Edition. Briza, Pretoria*.

Winstanly, T. G., Limb, D. L., Egginton, R., and Hancock, F. (1997). A 10m year survey of the antimicrobial susceptibility of urinary tract isolates in the UK. *J. Antimicrob. Chemother.*, *40*, 591 –594.

Wolfgang, K. J., and Hilda, P. W. (1976). *Zinsser microbiology, 18th Edition; Appleton century crofts, New York,*.

Yahaya, O., Tijjani, M. B., Abraham, O. J., Umar, I. O., and Miachi, O. E. (2011). Antimicrobial activity of crude extract of Daniella oliveri against some bacteria associated with enteric infections. *J. Med. Appl. Biol.*,*3*, 26–31.

Yahaya, O., Yabefa, J. A., Usman, B., Polytechnic, F., State, K., and State, B. (2012). Phytochemical Screening and Antibacterial Activity of Combretum glutinosum Extract against Some Human Pathogens. *Br. J. Pharmacol. Toxicol.*,*3*(5), 233–236.

Yasunaka, K., Abe, F., Okabe, H., Mumizi, E. E., Aguilavi, A., and Ryes-Chilps, R. (2005). Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumauries and vanthones. *J. Ethnopharm.*, *97*, 293–299.