

Phytochemical Screening and Antibacterial Activity of *Combretum glutinosum* Extract against Some Human Pathogens

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Abstract: The aim of this study is to determine the antibacterial effects of *Combretum glutinosum* plant extract on some clinically isolated bacteria species. *Combretum glutinosum* powdered plant materials was extracted using methanol and sterile distilled water. The Agar diffused methods was used to determine the antimicrobial activity of the plant against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The methanolic and water both provide yield from different parts of the plant and both showed effectiveness. Phytochemical screening of the crude extract revealed the presence of tannins, saponins, phenols, flavonoids, cardiac glycosides, anthroquinones and alkaloids and the growth of all bacteria were inhibited though to varying degrees thus justifying their use in traditional medicines in treating enteric infection and other diseases across Africa.

Keywords: *Combretum glutinosum*, enteric infections, methanolic extract, phytochemicals

INTRODUCTION

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 in Nigeria. Infectious disease is major cause of death in developing countries and according to WHOM as many as 80% of world population depends on traditional medicines for their primary health care needs today and that 25% of the drugs are based on plants and their derivatives (Azu and Onyeagba, 2007). Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics which increase the likelihood to increase the length of stay in the hospital (Winstanly *et al.*, 1997) and increased use of a particular antibiotics could lead to increased bacterial resistance (Mordi and Erah, 2006). Despite advanced orthodox medicines availability, there has been an increased interest in the complementary and alternative medicines particularly by those who have apprehension concerning toxicity and safety of modern drugs (Cowan, 2005). In Nigeria, different varieties of plant are used in the treatment of different types of diseases. The roots, stem, bark and leaves of *Combretum glutinosum* are used in the treatment of scrotal elephantiasis, dysentery, ring worms, syphilis, typhoid fever, eye sore and ear ache (Nwaeze and Abarikwu, 2006). It was also reported that the stem bark and leaves of *Combretum glutinosum* are used as antipyretic and in the treatment of stomach ache,

gonorrhoea and typhoid fever while the stem bark of *Daniella oliveri* was used in the treating fever, boil and back ache (Tony, 2005).

Combretum glutinosum is a very large genus comprising of about 250 species found in both temperate and tropical regions of the world (Nascimento *et al.*, 2000). Substances derived from medicinal plants remain the basis for a large proportion of commercial medications for the treatment of various diseases like heart diseases, high blood pressure, pains, asthma, malaria, typhoid fever, snake bites, arrow poison and other problems. These substances are available in a variety of forms. Fresh, dried, tablets, capsules, bottled in liquid forms, tea, bath tincture etc are all good as the quality of the raw plant from which there were made (Burkill, 1994; Winstanly *et al.*, 1997). The medicinal usefulness of plant has been the subject of numerous chemical and microbiological studies. Some of the reported phytoconstituents of the herbs included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acids. And some of the other uses include antispasmodic, antiasthmatic, expectorant, anticatarrhal and antisiphilitic (Nascimento *et al.*, 2000). The increasing failure of chemotherapeutics antibiotics resistance exhibited 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 parameter associated with it. The objective of the study is to access the antimicrobial potentials of *Combretum glutinosum* plant in Kogi East, Nigeria on some selected clinical bacteria isolates.

MATERIALS AND METHODS

Collection and identification of plant materials: The fresh sample of Leaves and stem bark of *Combretum glutinosum* was collected from Okenya village in Igalamela/Odolu Local Government Area of Kogi State, Nigeria. The taxonomic identification of the plant was confirmed by a botanist in the Department of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State, Nigeria. This study was carried out in the dry season (December-February) when fresh leaves of this plant were readily available.

Preparation of plant materials: The fresh plant was harvested, rinsed with tap water and air dried under shade for 20 days and reduced to coarse powder using the pestle and mortar and then grinded to fine powder using a blender. The powder was stored in an air tight bottle for further use.

Preparation of extract: Hundred grams of the powdered sample (stem bark and leaves) were weighed separately in different beaker and percolated with 150 mL of methanol and water each and covered with cotton wool and rapped with aluminum foil after vigorous shaken. The mixture was left to stand at room temperature for 72 h. The mixture was then filtered using a clean muslin cloth and then what man paper no. 1 filter paper and the extract obtained. The extract obtained were concentrated using rotary evaporator at 40°C. The percentage extract yield was estimated as dry weight/dry material weight $\times 100$ (Parekh and Chanda, 2007). For the preparation of dilutions of crude extracts for antibacterial assay, the extracts was reconstituted by dissolving in the extracting solvent and water and further diluted to obtain 400, 200, 100, 50, 25, 12.5 mg/mL etc. and maintained at room temperature between 2-8°C.

Phytochemical screening: The extract were subjected to preliminary phytochemical tests using standard techniques to detect the presence of tannins, flavonoids, saponins, alkaloids, steroids, phenols and glycosides, cardiac glycosides as described by Yahaya *et al.* (2011).

Determination of antimicrobial activity: Clinical isolates of *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, were isolated from the Department of Science laboratory technology, Federal Polytechnic, Idah, Kogi State, Nigeria. (1.0 mL of 18 h) culture of each bacterium was adjusted to 1.0×10^8 cfu/mL and spread into a sterile plate so as to achieve a confluent growth using a sterile bent glass rod. Thereafter, holes of 6 mm in diameter and 2.5 mm in deep were bored on the surfaces of the agar medium using a sterile cork borer. (0.5 mL) of the reconstituted extract at concentration of 100 mg/mL was placed into one of the holes and 0.5 mL of sterile distilled water was pipette into a hole as negative control. The plates were allowed to stand for 1 h for proper diffusion of the extract and then incubated at 37°C for 24 h and the zones of inhibitions were measured to the nearest mm. the mean of the plates were taken (Shagal *et al.*, 2011).

Scoring and reading: 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 percentage yield is higher for the methanolic extract of the leaves (4.25%) while water has more yield from the stem bark (1.2%) Table 1. The phytochemical characters of the medicinal plant investigated are summarized in Table 2. Alkaloids, tannins, flavonoids and terpenoids were present in both the leaves and the stem bark. The antimicrobial activity of crude extract of *Combretum glutinosum* were evaluated by measuring the diameters of the zones of growth inhibition on the tested bacteria species and the results are presented on Table 3. The entire test organisms were susceptible to *Combretum glutinosum* extract though to varying degree. The methanolic extract of the stem bark showed the highest level of inhibition on *Salmonella typhi* and *E. coli* while the aqueous extract showed less response. The yield when compared with the results obtained by Owololabi *et al.* (2007) 10.74% for water extract of 52% methanolic when compared with the 4.25% ethanolic extract of this plant is relatively small. Factors like age of the plant and the polarity of the solvent used may have affected the yield. Water and methanol seems to be a good solvent for this plant which supports the use of water

Table 1: Percentage yield of the crude extracts of *Combretum glutinosum*

Plant parts	Extraction solvent	Raw plants powder (g)	Extracted plant powder (g)	Percentage yield (%)
Leaves	Methanol	120	1.4	1.20
	Water	120	2.5	2.10
Stem bark	Methanol	120	5.1	4.25
	Water	120	1.6	1.33

Table 2: Photochemical constituents of *Combretum glutinosum*

Phytochemicals	Stem bark		Leaves	
	Methanol	Aqueous	Methanol	Aqueous
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Phenolics	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	-	-	-	-
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Anthroquinones	+	+	+	+

+: Positive; -: Negative

Table 3: Antibacterial activity of *Combretum glutinosum*

Organism	Zone of inhibition diameter (mm)			
	Stem bark		Leaves	
	Methanol	Aqueous	Methanol	Aqueous
<i>Salmonella typhi</i>	14.0	8.00	10.0	10.0
<i>Pseudomonas aeruginosa</i>	10.0	12.0	-	7.00
<i>Staphylococcus aureus</i>	14.0	8.00	10.0	10.0
<i>Escherichia coli</i>	18.0	20.0	-	8.00

and alcohol as traditional solvent. The phytochemical screening of the crude extract of the plant showed that the leaves and stem bark were rich in some bioactive components as seen in Table 2. Alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Edeoga *et al.*, 2005) and exhibit anti-inflammatory, anti-oxidant and membrane stabilizing property (Perenz *et al.*, 1995). The presence of terpenoid has been reported to be useful in herbal medicines (Baker *et al.*, 1983; Hayashi *et al.*, 1993). These phytochemicals also have some strong antimicrobial significance against some potential enteric pathogens; the presence of Alkaloids in significant quantities may be used as antimalaria, analgesics and stimulants (Duke and Ayensu, 1985). The other phytochemicals present in the plants are known to inhibit tumor growth, treatment of intestinal disorder like dyarrhoea and dysentery, tennin are used in treating wounds, sprains, bruises and arresting bleeding (Akinpelu and Onakoya, 2006).

Studies carried out by Ijeh and Agbo (2006) indicated the possibility that the use of plant extract in high doses could lead to toxic injury to kidney which may interfere with renal tubular functioning and could induce acute renal failure. This study shows that some plants show much promise in the development of in the development of phytomedicines with great antimicrobial properties as seen presently with the traditional medical practitioners. It can be concluded that this plants showed much antimicrobial potential against the selected test microorganisms and has greater promise in the development of phytomedicines.

REFERENCES

- Akinpelu, D.A. and T.M. Onakoya, 2006. Antimicrobial activities of medicinal plants used in folklore remedies in south western Nigeria. *Afr. J. Biotechnol.*, 6(22): 2502-2505.
- Azu, N.C. and R.A. Onyeagba, 2007. Antimicrobial properties of extracts of allium cepa (onions) and zingiber officinale (ginger) on escherichia coli, salmonella typhi and bacillus subtilis. *Inter. J. Trop. Med.*, 3(2): 246-249, DOI: 10.5580/1f5e.
- Baker, C.N., C. Thomsberg and R.W. Hawkinson, 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, PMID: PMC272664.
- Burkill, H.M., 1994. The useful plants of West Africa families. MFT, Royal Botanic Gardon Kew, 4: 605.
- Cowan, M.M., 2005. Plant products as antimicrobial agents. *Clin. Microb.*, 126: 564-582.
- Duke, J.A. and E.S. Ayensu, 1985. Medicinal plants of China. Mich. Reference Publications, Algonae, pp: 705, ISBN: 0917256204.
- Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian Medicinal plants. *Afr. J. Biotechnol.*, 4(7): 685-688.
- Hayashi, T., K. Okamuka, M. Kawasaki and N. Morita, 1993. Production of diterpenoids by cultured cells from 2 chemotypes scoparia dulcis. *Phytochemistry*, 53(2): 353-356.
- Ijeh, H. and C.A. Agbo, 2006. Body Organ weight Administration of aqueous extract of *F. exasperata*. *Anim. Veter. Adv.*, 5(1): 277-279.
- Mordi, R.M. and P.O. Erah, 2006. Susceptibility of common urinary tract isolates to the commonly used antibiotics in a tertiary hospital in Nigeria. *Am. J. Biotechnol.*, 5(11): 1067-1071.
- Nascimento, G.G.F., J. Locatelli, C.F. Paulo and G. Silva, 2000. The antimicrobial activity of plant extract and phytochemical on antibiotic resistant bacteria. *Brazil J. Microbiol.*, 31: 247-256.
- Nwaeze, C.U. and P.O. Abarikwu, 2006. Antimicrobial activity of certain medicinal plants used in traditional medicine in Nigeria. *Nigeria J. Microbiol.*, 6(12): 32-40.
- Owololabi, O.J., E.K.I. Omogbai and O. Obasuyi, 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extracts of *Kigelia Africana* (Bignoniaceae) stem bark. *Afr. J. Biotechnol.*, 6(14): 1677-1680.
- Parekh, J. and S. Chanda, 2007. *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. *Afr. J. Biotechnol.*, 6(16): 1905-1909.

- Perenz, R.M., S. Perenz, A.M. Zavala and N. Salazar, 1995. Anti-inflammatory activity of the bark of *Hippocrata excels.* *J. Ethol. Pharmacol.*, 47(1): 85-90.
- Ridgway, G.L., 1989. Practical medical microbiology. *J. Clin. Pathol.*, 42(12): 1315, PMID: PMC502096.
- Shagal, M.H., D. Kubmarawa and F. Hassan, 2011. Phytochemical screening and antimicrobial efficacy of extract from ficus exasperate against human pathogenic bacteria. *J. Med. Appl. Biosci.*, 3: 11-18.
- Tony, F., 2005. Nutritional and Diet Resource for Cancer prevention and Survival. 3rd Edn., Replika Press Ltd., Washington DC, pp: 51-60.
- Winstanly, T.G., D.L. Limb, R. Egginton and F. Hancock, 1997. A 10 m year survey of the antimicrobial susceptibility of urinary tract isolates in the UK. *J. Antimicrob. Chemother.*, 40: 591-594.
- Yahaya, O., M.B. Tijjani, O.J. Abraham, I.O. Umar, O.E. Miachi, *et al.*, 2011. Antimicrobial activity of crude extract of *Daniella oliveri* against some bacteria associated with enteric infections. *J. Med. Appl. Biosci.*, 3: 26-31.