Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 3 (3): 424-428

jeteas.scholarlinkresearch.org

Preliminary Phytochemical Screening and Antibacterial Activities of Leaf Extracts of Terminalia Catappa

[1]Opara, F. N.; [1]Anuforo, H. U.; [1]Okechuk Wu, R.I.; [1]Mgbemena, I.C.; [2]Akujobi, C.O. and [1]Adjero, A.

[1]Department of Biology,
Federal University of Technology, Owerri, Imo State, Nigeria.
[2]Department of Industrial Microbiology,
Federal University of Technology, Owerri, Imo State, Nigeria.
Corresponding Author: Opara, F. N

Abstract
Various parts of *Terminalia catappa,* the Indian almond, are claimed useful in trado-medicine for the treatment of bacterial infections and some other ailments. The development of resistance to the antibiotics in current clinical use is a big concern. In view of this, the phytochemical screening and antibacterial activities of the leaf extracts of *Terminalia catappa* were evaluated using ethanol and hot water as solvents to determine the active components, antibacterial potency of the leaf extracts and the minimum effective concentration so as to reduce harm. Cold maceration method was adopted in extracting the active principle, having pulverized the leaves. Phytochemical analysis of the crude extracts indicated the presence of carbohydrates, saponins, tannins, steroids and terpenes. Antibacterial screening with 24hours cultures of clinical isolates of *Salmonella typhi*, and type cultures of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa,* using agar-cup diffusion method indicated that *P. aeruginosa* was the most sensitive while *S. typhi* the least; ethanolic extract was relatively more active than water extract. Minimum bactericidal concentration test showed that ethanolic extract exerted bactericidal effect at 62.5mg/ml on *S.aureus*, but was static on the rest test microorganisms at the said concentration. Water extract exhibited bactericidal activity at 125mg/ml on *S. aureus* and *P. aeruginosa*, but was static on *E. coli* and inactive on *S. typhi*. From the activity obtained, *S.aureus* and *P.aeruginosa* were more sensitive to ethanolic extract than water extract. The concentration should be higher than applied in this study to probably achieve marked activity. The results obtained suggest that *T. catappa* can be used in the treatment of ailments caused by the test microorganisms, and thus lends credence to the application of the plant in traditional medicine as remedy for various infections.

Keywords: Terminalia catappa, antibacterial activity, ethanol and water extract, pathogenic microorganisms, phytochemical

INTRODUCTION

Although many different antibacterial agents are available in the field of medicine, many of these agents are increasingly being incapacitated by the microorganisms through the evolution of different mechanisms that amount to resistance to these drugs (Walsh, 2000).Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also are often with adulterations and side effects (Shariff, 2001).There is therefore a continuous and urgent need to discover new antibacterial components with diverse chemical structures and novel mechanisms of actions because of the increase in the incidence of new and re-emerging infectious diseases (Nair and Chanda ,2008) to replace those that have lost their efficacy. Research has, however, shown that many herbs possess varying degree of antimicrobial activities. Kaufman *et al*., (1999) had reported that more than 25% of the prescribed drugs contained at least one active ingredient of plant origin. About 80% of the world's population relies on traditional medicine for significant part of their primary health care needs (WHO, 2002).Extraction of bioactive compounds from medicinal plants allows the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Ebana *et al., 1991;* Williams, 1996; Pamplona-Roger, 1999; Manna and Abalaka, 2000; Babayi *et al*., 2004). In this study *Terminalia catappa* (Combretaceae) also known as Indian almond, a tall deciduous and erect tree reaching a height of 15-25m,all parts of which(leaves, bark, roots and fruits) are useful in traditional medicine for the treatment of wounds and ulcerations, boils and some bacterial infections (Gao *et al*., 2004; Babayi *et al*., 2004; Nair and Chanda, 2008), with which the test organisms are associated is used. It has been reported that extracts of *T. catappa* leaves show antioxidative, anti-inflammatory and hepatoprotective actions and contains hydrolysable tannins or triterpenoids (Tanaka,*et al.*,1986;Lin, *et*

al.,2001;Gao,*et a*l.,2004;Tang,*et al*.,2004). This study therefore seeks to examine the antibacterial potency of the ethanol and aqueous leaf extracts of *Terminalia catappa*, against some selected clinically important bacteria, the minimum effective concentration and determine the phytochemical components of the medicinal plant.

MATERIALS AND METHODS

Collection, Preparation of Plant Materials and Extract

Fresh healthy leaves of mature *Terminalia catappa* trees were collected randomly from Ngor Okpala L.G.A., Imo State, Nigeria, identified and authenticated at the Herbarium unit of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The leaves were gently cleaned and neatly washed under running tap water to remove dust and dirt before they were sun-dried for 5 days to remove moisture. They were then pulverized using clean sterilized pestle and mortar to fine powder and stored in airtight bottles. Using standard methods, sixty grammes (60g) of the processed plant material were weighed out in duplicate into two clean conical flasks of 1000ml each, one flask containing 600ml of 99% ethanol and the other 600ml of hot water. The mixtures were vigorously shaken, the flasks stoppered, the preparation then allowed to stand for 24hours at room temperature. The next day, the mixtures were shaken and separately filtered into sterile conical flasks using suction pump. The filtrates were then concentrated by boiling to dryness using a water bath to obtain the extract. The concentrated extracts were collected using spatula, poured into sterile universal bottles and stored for 48h at room temperature.

Phytochemical Screening of the Crude Extracts

The phytochemical components of the leaf extracts of *Terminalia catappa* were screened for the presence of tannins, saponins, alkaloids, steroids, terpenes, and anthraquinone derivatives using the method of Trease and Evans (1989). Flavonoids and phlobutannins were screened for using the methods of Kumar *et al*., (2007) and Sofowora (1982) respectively.

Collection and Preparation of Test Micro Organisms

The four test microorganisms used in this study were type cultures of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* obtained from the Microbiology/Biotechnology Departments of NIPRD, Idu, Abuja and clinical isolates of *Salmonella typhi* obtained from the Diagnostics Unit of the same Institute. Viability and purification were carried out by streaking each test culture on a sterile plate and incubating at 37^{o}c for 24hours according to Cheesbrough (2000). Minute quantities of each test culture was sub-cultured in sterile broth in bijou bottles and incubating for another 3hours before used (Cowan, 1974).

Antibacterial Potency and Minimum Inhibitory Concentration

The antibacterial potency of the ethanol and aqueous extracts of the plant was determined by the method of agar disk diffusion method alongside the minimum inhibitory concentration (MIC). 8 sterile nutrient agar plates prepared according to manufacturer's specification were used, four for each extract; one in each case streaked with one of the four test microorganisms as labelled. Four 10mm diameter cork borers were used to bore 5 holes on each of the 8 plates. 250 mg/ml of each extract was prepared by separately dissolving 1g of each extract in 4ml sterile distilled water. Using separate sterile syringes with needles, 2ml of the respective 250mg/ml solutions were pipetted and few drops of each used to fill one hole as labelled on the plates. After filling an appropriate hole, in each case, volume of distilled water equal to that of extract remaining in the syringe was pipetted to obtain a double-fold dilution of the extracts until 15-625mg/ml dilution was attained. The plates were prepared in duplicates then incubated at 37^{o}c for 24 hours, after which observations were made and zones of inhibition recorded. Control plates made up of extract without inoculum and inoculums without extract were made in parallel.

Minimum Bactericidal Concentrations (MBC)

The minimum bactericidal concentrations for each extract on the test organisms were determined by gently touching the respective zones of inhibition produced in MIC test with sterile wire loop and streaking accordingly on fresh antibacterial-free agar plates. Zones of inhibition from the first three concentrations ascending from the MIC were tested. The plates were then incubated at 37^{o}c for24hours, and there after examined for growth.

RESULTS

Phytochemical screening of the crude leaf extracts of *Terminalia catappa* indicated that the plant had carbohydrates, saponins, tannins, steroids and terpenes while alkaloid, flavonoid, anthraquinones, and phlobutannins were not detected (Table 1).

Table 1.Phytochemical components of the crude extracts of *Terminalia catappa*

Phytochemicals	Results
Alkaloid	-
Carbohydrates	+
Flavonoids	-
Saponins	+
Anthraquinones	-
Tannins	+
Phlobutannins	-
Steroids and Terpenes	+

+: Present *-: Absent*

Antibacterial Activity of the Crude Extracts

The results of the antibacterial activity of the crude ethanol and aqueous extracts of *T. catappa* (Table 2 and Table 3) revealed that both ethanolic and hot water extracts of leaves of *T. catappa* exhibited marked activity against *P.aeruginosa* and *S.aureus* but showed limited activity against *E. coli* and *S. typhi* .Extracts of *T. catappa* inhibited the growth of the test microorganisms. However, the test organisms differed in their level of susceptibility to the extracts. Ethanolic extracts were relatively more potent than aqueous extract. The MIC of crude ethanol extract were15.625mg/ml,250mg/ml and 31.25mg/ml for *P.aeruginosa*, both *E.coli* and *S.typhi* and *S.aureus* respectively while that of aqueous extract were 31.25mg/ml,62.5mg/ml for *P. aeruginosa* and both *E. coli* and *S.aureus*. *S.typhi* exhibited resistance to all the concentrations of the aqueous extract. Results of the minimum bactericidal concentration of ethanol and water extracts are shown in Tables 4 and 5.

Table 2. Antibacterial potency /minimum inhibitory concentration test of the crude ethanol extracts of *T. catappa*

Organisms	Zones of inhibition (mm)				
	250mg/ml	125mg/ml	62.5mg/ml	31.5mg/ml	15.625mg/ml
P. aeruginosa	15	10	8	4	3*
E. coli	2*	-	-	-	-
S.typhi	3*	-	-	-	-
S.aureus	10	8	6	5*	-

* = Minimum inhibitory concentration
- = No activity

Table 3: Antibacterial potency/minimum inhibitory concentration test of the crude water extracts of *T. catap*pa

Organisms	Zones of inhibition (mm)				
	250mg/ml	125mg/ml	62.5mg/ml	31.5mg/ml	15.625mg/ml
P. aeruginosa	15	10	6	1*	-
E. coli	5	3	2*	-	-
S. typhi	-	-	-	-	-
S. aureus	10	8	4*	-	-

* = Minimum inhibitory concentration
- = No activity

Table 4: Minimum bactericidal concentration of ethanol extracts

Organisms	Concentrations mg/ml				
	250	125	62.5	31.25	15.526
P. aeruginosa	NT	NT	+ve	+ve	+ve
E. coli	+ve	+ve	+ve	+ve	+ve
S. typhi	+ve	+ve	+ve	+ve	+ve
S. aureus	-ve	-ve	+ve	+ve	+ve

Key: +ve= Growth, -ve = No growth, *= MBC, NT= Not Tested.

Table 5: Minimum bactericidal concentration of water extract

Test organisms	Concentrations (mg/ml)				
	250	125	62.5	31.25	15.626
P. aeruginosa	-ve	-ve	+ve	+ve	+ve
E. coli	+ve	+ve	+ve	+ve	+ve
S. typhi	+ve	+ve	+ve	+ve	+ve
S. aureus	-ve	-ve	+ve	+ve	+ve

Key: +ve = Growth, -ve =No growth, * =MBC

DISCUSSION

The results of this study revealed that the crude leaf extracts of *T.catappa* contain saponins, tannins, steroids and terpenes and carbohydrates. Babayi *et a*l.,(2004) reported the presence of these components and in addition phenols and saponin glycosides in *T.catappa*. The leaves have been reported to be maturant and emollient,used in the treatment of wound and ulcerations, the bark rich in tannins, the fruits rich in ascorbic acid and seeds contain oil, (Nair and Chanda, 2008). The inhibitory effects of the leaf extract on the test microorganisms may be due to the presence of these components. Though the leaf extracts showed limited activity against all the organisms, marked activity was exhibited against *P. aeruginosa* and *S. aureus*. The low activity recorded

against *E. coli* and *S. typhi* corresponds with the result obtained by Babayi *et al.(* 2004) which indicated that *Bacillus subtilis* and *Staphylococcus aureus* were susceptible to methanolic extract of *T. catappa* . However our result is in contrast to their report of *P. aeruginosa, S. typhi, Candida albicans* and *E. coli* being resistant to extracts of *T.catappa* leaves. The relatively higher activity achieved in this study could imply that ethanol and water extracted more of the active principle than methanol used by Babayi,*et al.(*2004).The extraction of more active principle for antibacterial properties with ethanol, especially when alkaloid and essential oils are the active principles has been reported(Obi and Onuoha, 2000), hence more parity in activity against susceptible microorganisms (Okorondu, *et al.*,2006). The test organisms differed however in their level of susceptibility to the extracts as shown by the results of the minimum inhibitory concentration (MIC) which for aqueous extract was obtained at 31.25mg/ml, 62.5mg/ml and 62.5mg/ml for *P. aeruginosa, E.coli* and *S. aureus* respectively. That of ethanol extract was 15.625mg/ml for *P.aeruginosa*, 250mg/ml for *E.coli* and *S.typhi* and 31.25mg/ml for *S.aureus*. Generally, *P.aeruginosa* was the most susceptible of all the test organisms and suggests that lower doses of antimicrobial agents will be required in treating infections caused by *P.aeruginosa*. Furthermore, in view of the proximity of the activities of both ethanol and water extracts in this study, it is suspected that the active principle is nearly as extractible with water as with ethanol and that *T. catappa* can be used in the treatment of ailments caused by these microorganisms. Having established relative activity of the crude extracts of *T. catappa* against the test organisms, further studies to identify, isolate and purify the active principles responsible for the antibacterial property should be undertaken. Also further studies on the toxicity of the extract is recommended in order to ascertain the safety of the extract for therapeutic use.

CONCLUSION

Terminalia catappa, the Indian almond, was found to contain saponins, tannins, steroids and terpenes and carbohydrates.The leaf extracts of the plant were active against the test microorganisms, *P.aeruginosa, E.coli, S.typhi* and *S.aureus* which are known to be involved in cutaneous diseases, wounds, burns and other ailments. The leaves of the plant therefore can be used in the treatment of these infections thereby giving credence to the trado-medicinal application of the plant. *Pseudomonas aeruginosa* and *S.aureus* were more sensitive to ethanol extract than aqueous extract which also was not effective against *S.typhi*. From this study, ethanolic extracts were more potent than aqueous extracts and *P. aeruginosa* most susceptible of all the test organisms. Further studies to identify and purify the specific active principle responsible for the antibacterial property should be undertaken, and adoption of more suitable and effective method of isolation such as column chromatography suggested. Studies on the toxicity of the extract to ascertain its safety for therapeutic use is also recommended.

ACKNOWLEDGMENT

We the authors wish to express our profound gratitude to the entire staff of the Herbarium Unit and Pharmaceutical Microbiology Division of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria for their assistance and contribution to the success of this study.

REFERENCES

Babayi, H., Kolo, I. Okogun, J. I., Ijah, U.J. J. (2004). The antimicrobial activities of methanolic extracts of Eucalyptus camaldulensis and Terminalia catappa. against some pathogenic microorganisms. Biokemistri. 16 (2):106-111

Cheesbrough, M. (2000). District Laborator y Practrices in Tropical Countries. Cambridge University Press, Cambridge, London. 2nd ed. 110-181.

Cowan, S. T. (1974). Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge University Press, Cambridge, London 2nd ed.

Ebana R.U.B., Madunagu, B.E., Ekpe, E.D. and Otung, I. N. (1991). Microbiological exploitation of cardiac glycoside and alkaloids from Garcinia kola, Borreria ocymoides, Kola nitida and Citrus aurantifolia. Journal of Applied Biotechnology 71:398-401.

Gao, J.,Tang, X., Dou, H., Fan, Y., Zhao, X., Xu,Q. (2004). Hepatoprotective activity of Terminalia catappa L. leaves and its two triterpenoids. Journal of Pharmacy and Pharmacology.11: 1449-1455.

Kaufmann, B. P., Calson, F. T., Dayanandan, P. Evan, Fisher, B. J., Parks, C. Wells, R. J. (1989). Plants: their biology and importance. Harper and Row Publishers, New York. 681-700.

Kumar, G.S., Jayaveera, K.N., Kumar, C.K.A., Umachigi, P. S., Vrushabendra, B M. S., Kumar, D. V.K. (2007). Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Tropical Journal of Pharmaceutical Research. 6(2):717-723.

Lin, C.C., Hsu, Y. F., Lin, T. C., (2001). Antioxidant and free radical scavenging effects of the tannins of Terminalia catappa L. Anticancer Res 21:237-2.

Manna, A. and Abalaka, M. E. (2000). Preliminary screening of the various extracts of Physalis angulala (L.) for antimicrobial activities. Spectrum Journal 7(2):119-125.

Nair, R. and Sumitra, C. (2008). Antimicrobial activity of Terminalia catappa, Manikara zapota and Piper betel leaf extract. Indian J. Pharm Sci., V.70 (3): pp. 390-393.

Obi, V. I., Onuoha, C. (2000). Extraction and characterization method of plants and plant products. In: Biological and Agricultural Techniques. Ogbulie,

J.N. and Ojiako, O. J.(eds). Webmedia Publications, Owerri. pp. 271-286.

Okorondu, S.I., Braide, W., Ogbulie, T.E. and Akujobi, C.O. (2006). Antimicrobial and phytochemical property of some traditional spices. Nigeria Journal of Microbiology. 20(3): pp. 301-304.

Pamplona-Roger, G.D., (1999) Encyclopedia of Medicinal Plants. Vol. 1 and2, (2nd Edn).Education and Health Library, The European Union, U.K. pp. 128-1

Shariff, Z.U. (2001). Modern Herbal Therapy for Common Ailments .Nature Pharmacy Series Vol. 1, Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, pp. 9-84.

Sofowora, A. (1982).Medicinal plants and traditional medicine in Africa. !st Ed. John Wiley and Sons, Chichester, New York, pp. 256.

Tanaka, T., Nonaka, G.I., Nishioka, I.(1986). Tannins and related compounds.XL11. Isolation and characterization of four new hydrolysable tannins, terflavins A and B, tergallagin and tercatan from the leaves of Terminalia catappa L. Chem. Pharm. Bull. 34: pp. 1039-1049.

Tang, X.H., Gao, J., Fan, Y.M., Xu, L.Z., Zhao, X.N., Xu, Q. (2004). Mechanisms of hepatoprotection of Terminalia catappa L. extract on D-galactosamine-induced liver damage. Am. J. Clin. Med.,2: pp. 509-519.

Trease, G.E., Evans, W.C. (1989). Pharmacognosy. 13th ed. English Language Book Society, Bailliere Tindal, London, pp. 378-480.

Walsh, C. (2000). Molecular mechanisms that confer bacteria drug resistance. Nature. Vol. 406(84):pp. 235-289.

Williams, V. L. (1996). The Witwater Strand Multitrade. Veld and Flora 82:pp. 12-14.

World Health Organization statistics (2002).” Are Herbs Safe”. Herb *quarterly*, pp.37.