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Phytochemical Screening and Antimicrobial Activity of *Terminalia catappa* (L.) seed oil obtained from Anyigba, Kogi State, Nigeria, on Some Selected Clinical Isolates

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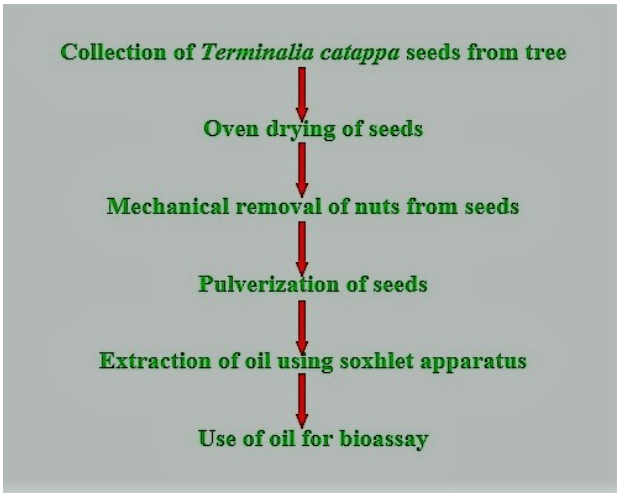
Grafical Abstract



Abstract:

This research was carried out in a bid to seek for alternative ways of dealing with strains of microorganisms that have become resistant to conventional antibiotics. The study was carried out to evaluate the antimicrobial efficacy of *Terminalia catappa* seed oil obtained from Anyigba, Kogi State Nigeria, on some selected clinical isolates of selected microorganisms (*Aspergillus niger*, *Staphylococcos aureus*, *Escherichia coli*). The oil was extracted from the seeds using the soxhlet extraction method with n-hexane as the solvent. The Mueller Hinton (well diffusion method) was used to test the susceptibility of the strains of the microorganisms to the oil, using Ciproflaxin as standard positive controls, alongside Tween and DMSO as negative control. Experiments were carried out in duplicates. The results obtained revealed that *Terminalia catappa* oil was unable to create any inhibition zones on the clinical isolates. From this research, it can be concluded that *Terminalia catappa* oil had no Antibacterial activity.

Keywords: Antimicrobial, *Terminalia catappa*, Essential oils, seed

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.[1] 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.[2] There is therefore a continuous and urgent need to discover new antimicrobial components with diverse chemical structures and novel mechanisms of actions because of the increase in the incidence of new and re-emerging infectious diseases[3] 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)[4] had reported that more than 25% of the prescribed drugs contained at least one active ingredient of plant origin and about 80% of world population relies on traditional medicine for significant part of their primary health care needs.

Belonging to the family Combretaceae, *Terminalia catappa* Linn. is naturally occurring and widespread in the subtropical and tropical zones of the Indian and Pacific Oceans, and is planted extensively in many countries as an ornamental tree.[5] The type of oil that is obtained from *Terminalia catappa* seeds is safe for consumption, in addition Oliveria *et al*., (2000)[6] indicated that the oil content of *Terminalia catappa* (583.0 g/kg dry matter) is comparable to that of other oil seeds such as peanut, rapeseed, and sunflower.
Termianalia catappa is used primarily as an ornamental shade, and salt-tolerant street tree, but the leaves provide food for the Tasar silkworm, and the seeds are edible like almonds with similar oils. On the Malay Peninsula and through the Canary islands this tree is known as the tropical almond. *Termianalia catappa* has been claimed to have therapeutic effects for liver related diseases.[7] It is attributed with cholagogue action. In India, it is used as cardiac stimulant. Its leaves are widely used as a folk medicine in Southeast Asia for the treatment of dermatosis and hepatitis[8]. More and more pharmacological studies have reported that the extract of *Termianalia catappa* leaves and fruits have anticancer by (Fan *et al*., 2004)[9], antioxidant by (Zhai *et al*., 2001)[10], anti-HIV reverse transcriptase, anti-inflammatory, and anti-diabetic effects as stated by (Xu *et al*., 2000)[11] and hepato-protective activities as stated by (Nagappa *et al*., 2003)[12] but the effective components and related mechanisms remain unknown.
Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds[13,14,15,16]. The efforts of scientists in establishing plants with promising antimicrobial property is yielding fruitful results as a number of plants with high antimicrobial property have been elucidated. This work was carried out to determine the phytochemical components as well as evaluate the antimicrobial activity of *Terminalia catappa* seed oil.

Methodology

The essential oil used in this study was extracted from nuts of Indian almond. Ripe fresh seeds were collected directly from the tree plant and dried in an oven for 5 days to reduce moisture content and enable easy breaking to get reasonable amount of the nuts. The seeds were cracked open with a nut cracker and the nuts are pounded using mortar and pestle into a smooth powder to increase surface area so that the solvent can permeate properly into it to extract the oil.

Extraction of essential oil by soxhlet method

The extraction of essential oil was done using n-hexane in electro-thermal soxhlet extractor (Gallenkamp, England). 30g of powdered seeds was weighed and put into the thimbles of the soxhlet extractor, the apparatus was mounted and allowed to run for 6 hours, after which the mixture of essential oil and n-hexane was collected in a beaker and evaporated over a hot water bath to collect the oil as residue. The method was repeated thrice to get sufficient amount of oil.[17]

Determination of relative density of oil

Two density bottles were washed and dried in an oven and allowed to cool in a desiccator. Each of the bottles was then weighed empty and the weights recorded. One was filled with water and the other with *Terminalia catappa* seed oil. Both was weighed again and the weight of the water and oil were determined by the difference in weight.[18]

$$Relative\ density = \frac{Weight\ of\ oil}{Weight\ of\ an\ equal\ volume\ of\ water}$$

$$Relative\ density = \frac{(W2 - W1)}{(W3 - W1)}$$

Where:
Weight of empty bottle = W1
Weight of empty bottle + oil = W2
Weight of empty bottle + water = W3

Determination of the phytochemical constituents of oil

Chemical tests is carried out on the seeds oil of *Terminalia catappa* using standard procedures to identify the constituents as described by (sofowora, 1982)[19], (Trease and Evans, 1989)[20] and (Harborne,1973)[21].
Alkaloids: About 0.5ml of the extracts is warmed with 2% of the H_2SO_4 for two minutes. It was filtered and a few drops of Dragondoff reagent is added. Orange red precipitate indicates the presence of alkaloids.
Tannins: Small quantity of extracts is mixed using water and heated on water bath and filtered. Few drops of ferric chloride is added to filtrate. Dark green solutions indicates the presence of tannins.
Glycosides: Extracts is hydrolyzed using HCL and neutralized with NaOH solution. Few drops of Fehling's solution is added. Red precipitate indicates the presence of glycosides.
Saponins: About 0.5ml of the extract is shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.
Flavonoids: Extract of about 0.5ml is dissolved in diluted NaOH and HCL added. A yellow solution that turns colourless indicates the presence of flavonoids.
Total phenol: Extract of about 0.5ml is dissolved in Dennis solution. A red colour precipitate indicates the presence of phenol.

Culture of Clinical Isolates

Clinical isolates (*Aspergillus niger*, *Staphylocuccos aureus*, *Escherichia coli*) were obtained from the Department of Microbiology, Faculty of Natural Sciences, Kogi State

University, Anyigba. Overnight cultures were obtained using 10ml Nutrient agar and used for the study.

Well Diffusion Assays

Antimicrobial activity testing was carried out by using agar diffusion method. Three dilutions (30, 60 and 90%v/v) of *Terminalia catappa* seed oil were made using 0.005%v/v Tween 20 with 0.005%v/v Dimethyl sulfoxide (DMSO) in the ratio 1:1 as a co-diluent. Mueller-Hinton sterile agar plates were flooded with 2ml indicator microbial stains (*Aspergillus niger*, *Staphylocuccos aureus*, *Escherichia coli*), and the excess drained. A cork borer was flamed and used to bore five wells which contained 0.005% tween and 0.005% DMSO (1:1) (negative control); 30%, 60%, and 90% v/v of oil (treatments), and 0.5mg/L Ciprofloxacin (positive control) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured using venier caliper and a meter rule after 18 to 24 hours of the incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The experiments were done in duplicates.

Statistical Analysis

One way Analysis of Variance (ANOVA) was used to compare the mean inhibition zones of the different treatment groups and Duncan Multiple Range Test (DMRT) was used to separate means where significant. $P < 0.05$ was considered significant.

Results

Results revealed that essential oil of *Termianalia catappa* seed contained tannins, flavonoids and glycosides (Table 1)

Table 1: Phytochemical Components of Oil Extract

Parameter	Test/Reagent	Observation	Inference
Alkaloids	Dragondorff	No colour change	(-)
Tannins	Ferric chloride	Dark green colour	(+)
Saponins	Frothing test	No frothing	(-)
Flavonoids	NaOH-HCL test	Slight yellow colour	(+)
Glycosides	Fehlings A and B test	Slightly red colour	(+)
Phenols	Dennis test	No colour change	(-)

Key: + Present - Absent

Relative Density of the Oil

The relative density of *Terminalia catappa* oil obtained was 0.875 (Table 2)

Table 2: Relative Density of *Terminalia catappa* Oil

Sample	Weight of Empty Density Bottle	Weight of Empty Bottle × Sample	Mass of Substance	Density g/ml
Oil	9.55	18.35	8.80	0.875
Water	9.55	19.61	10.06	

Effect of the Extracts on Isolates

Results showed that the extract (*Termianalia catappa* seed oil), at 30% (A) , 60% (B) , and 90% (C) v/v had no significant effect on the test organisms, the three organisms being *Escherichia coli*, *Staphylococcus aureus* , and *Aspergillus niger* respectively. The negative control (NC), like the extract, had no effect as well on all the test organisms. The positive control (PC) however had effects on *E.coli, S. aureus, A. niger* with inbition zones of 12mm, 17mm and 22.5mm respectively (Table 3; Fig 1 -3).

Table 3: Antimicrobial activity of *Terminalia catappa* seed oil extract on a few selected pathogenic organisms

Test Organism	Inhibition zone(mm)				
	NC	A	B	C	PC
E. coli	-	-	-	-	12.0 ± 2.0
S. aureus	-	-	-	-	17.0 ± 3.0
A. niger	-	-	-	-	22.5 ± 2.5

Pictorial Presentation of the Agar Plates

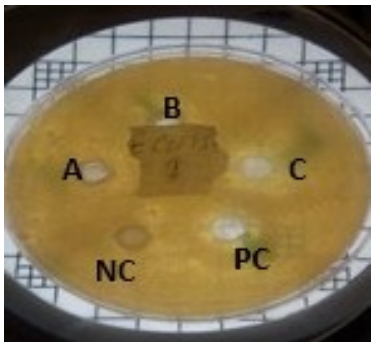


Figure 1: Effect of *Terminalia catappa* seed oil on *Escherichia coli* : NC = Negative Control (0.5% DMSO and 0.5% Tween 20) ; A = 30% oil extract ; B = 60% oil extract ; C = 90% oilextract; PC = Positive Control (0.5mg/L ciprofloxacin)

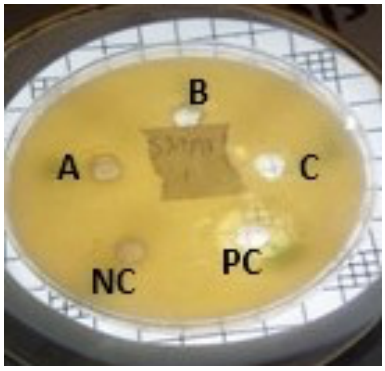


Figure 2: Effect of *Terminalia catappa* seed oil on *Staphylococcus aureus*: NC = Negative Control (0.5% DMSO and 0.5% Tween 20) ; A = 30% oil extract ; B = 60% oil extract; C = 90% oil extract; PC = Positive Control (0.5mg/L ciprofloxacin)

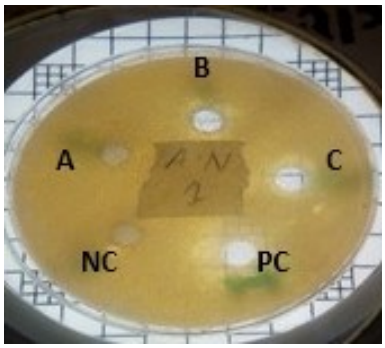


Figure 3: Effect of *Terminalia catappa* seed oil on *Aspergillus niger*: NC = Negative Control (0.5% DMSO and 0.5% Tween 20) ; A = 30% oil extract ; B = 60% oil extract ; C = 90% oil extract; PC = Positive Control (0.5mg/L ciprofloxacin)

Discussion

The seed oil extract of *Terminalia catappa* was found to have tannins, flavonoids and glycosides; but had no inhibitory or antimicrobial activity against the micro-organism isolates (*Escherichia coli*, *Staphylococcus aureus*, and *Apergillus* niger) this was confirmed by its failure to exert any form of inhibition zone around the wells containing the oil. This could be due to the fact that the concentration of the phytochemicals present in the oil was not enough to exert antimicrobial activity against the organisms[22] and the low concentration of the phytochemicals could be due to the soxhlet method used in extracting the oil, which has been reported to be less effective than methods like steam distillation.[23] Miksusanti *et al* (2019)[24] reported that 0.5% *Terminalia catappa* seed oil showed no antibacterial effect on *Streptococcus mutans* (with inhibition zone of 0 mm^2), but on ethanolysis of the oil the resultant product had antimicrobial effect on the same isolate (with inhibition zone of 183 mm^2).The lack of antimicrobial activity of the oil may also be as a result of its inability to diffuse through the medium properly due to its poor solubility.[22,23]
Other parts of *Terminalia catappa* have however shown antimicrobial activities. Studies by Babayi *et al*. (2004)[25] using leaf extracts of *Terminalia catappa* containing saponins, tannins and steroids had antimicrobial activity against *Escherichia coli* and *staphylococcus aureus* which are well known to be involve in cutaneous diseases, wounds, burns and other different ailments. The antimicrobial activity of the leaf extracts of *Terminalia catappa* may be due to the presences of saponins and steroids present which are absent in the seed oil. Studies by Pawar and Pal (2002)[26] on the chloroform extracts of *Terminalia catappa* root also show good antimicrobial activity against Gram-positive and Gram-negative microorganisms.

Conclusion

The phytochemical analysis carried out on the oil extract revealed the presence of tannins, flavonoids and glycosides and the absence of saponins, alkaloids and phenols.
The antimicrobial investigations carried out on *Terminalia catappa* seed oil base on this study prove had no antimicrobial activity against *Escherichia coli*, *Aspergillus niger*, and *Staphylococcus aureus*.

Recommendations

Though *Terminalia catappa* oil had no significant effect on the organisms tested in this research, it may be further tested on other microorganisms at different concentrations.
Adoption of more suitable and effective method of extraction and isolation of active compounds such as stem distillation and column chromatography is suggested.
Other uses of the oil should be investigated; such as the possibility of its use as a cooking oil and in the manufacture of cosmetics.

References

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

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

3. Nair, R. and Sumitra, C. (2008). Antimicrobial activity of Terminalia catappa, Manikara zapota and Piper betel leaf extract. *Indian Journal of Pharmaceutical Science*, 70 (3):390-393.

4. Kaufmann, B. P., Calson, F. T., Dayanandan, P. E., Fisher, B. J., Parks, C and Wells, R. J. (1999).Plants: Their biology and importance. New York : Harper and Row Publishers. 681-700.

5. World Health Organization statistics (2002). Are herbs safe. *Herb quarterly*. pp.37.

6. Oliveria, J.T.A., Vasconcelos, I.M., Bezerra L.C.N.M., Silveira, S.B., Monteiro, ACO. And Moreira, R.A. (2000). Composition and nutritional properties of

seeds from Pachira aquatica Aubl, Sterculia striata St Hil *et* Naud and *Terminalia catappa* Linn. *Food Chemistry*, 70:185-91.

7. Chiu N. Y. and Chang, K. H. (1986). *The Illustrated Medicinal Plants of Taiwan* (Vol.1). Taipei: SMC Publishing, Inc. p. 129.

8. Lin, C.C., Chen Y.L, Lin, J.M. and Ujiie, T. (1997). Evaluation of the antioxidant and hepatoprotective activity of *Terminalia catappa*. *American Journal of Chinese Medicine*, 25:153-61.

9. Fan, Y.M., XU, L.Z., Gao .J., Wang, Y., Tang, S.H., Zhoa X.N. and Zhang Z.X. (2004) Phytochemical and anti-inflammatory studies on *Terminalia catappa*. *Fitoterapia*, 75:253-60.

10. Zhai, Y. F, Yao, J., Fan, Y. M., Xu, L. Z., Gao, J. and Zhao, X. N. (2001). Inhibitory effects of LR-9 on Proliferation of hepatocarcinoma cells. *Journal of Nanjing University of Natural Science*, 37 (2001): 213-217.

11. Xu, L. Z., Gao, J., Zhu, L., Xu, M., Lu, S. Y., Zhao, X. N. and Zhang, Z. X. Protective effects of LR-98 on hepato-toxicity induced by carbon tetrachloride and D-Galac-tosamine in mice. *Journal of Nanjing University of Natural Science,* 36 (2000):197-201.

12. Nagappa, A.N, Thakurdesai P.A., Venkat Rao .N., and Singh .J. (2003); Anti-diabetic activity of *Terminalia catappa* fruits. *Journal on Ethno Pharmacological Studies*. 88:45-50.

13. Parekh, J., Karathia, N. and Chanda, S. (2006). Screening of some traditionally used medicinal plants for potential antibacterial activity. *Indian Journal of Pharmaceutical Science*, 68 (2006):832-834.

14. Parekh, J. and Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, 10 (2007):175-181.

15. Hediat, M. H. S. and Marraiki, N. (2010). Antimicrobial activity and phytochemical analyses of Polygonum aviculare L. (Polygonaceae), naturally growing in Egypt. *Saudi Journal of Biological Sciences*,17 (1): 57-63.

16. Pearson, D. (1991). The Chemical Analysis of Foods (7th ed.). Church Living Stone: Longman Group Limited. pp. 493-494

17. Meyer, E.W. (1966). Soya bean protein concentrate and isolate. Proceedings of International Conference on Soy bean Protein Foods. USDA/ARS-71:36-38

18. Sofowora, A. (1982). Medicinal plants and traditional medicine in Africa. Chichester, New York John: Wiley and Sons. p. 256.

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

20. Harborne, J.B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. London: Chapman and Hall limited. pp. 279.

21. Knobloch, K., Weigand, H., Weis, N., Schwarm, H.M. and Vigenschow, H. (1986) Action of terpenoids on energy metabolism. *In Progress in Essential Oil Research*; Brunke, E.J., (Ed.) (Berlin, Germany: Walter de Gruyter). pp. 429-445.

22. Samie, A., Nefefe, T., Gundidza, M., Mmbengwa, V., Magwa, M. and Mtshali M. S.(2012). Antimicrobial activities and time kill profiles of five essential oils from Southern Africa against selected bacterial and fungal organisms. *African Journal of Pharmacy and Pharmacology*. 6(44):3086-3095.

23. Soković, M., Glamočlija, J., Marin, P.D., Brkić, D. and van Griensven, L.J. L. D.(2010) Antibacterial Effects of the Essential Oils of Commonly Consumed. *Medicinal Herbs Using an In Vitro Model*. Mol., 15, 7532-754

24. Miksusanti, Herlina, Fithri A.N., Ferlinahayati (2019). Properties of ethanolysis product from Ketapang seed oil (Terminalia catappa Linn.) incorporated in mucoadhesive path film. IOP Conference Series: Earth and Environmental Science 347 (012034): 1 – 9

25. 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.

26. Pawar, S.P and Pal S.C. Antimicrobial activity of extracts of *Terminalia catappa* root. *Indian Journal of Medicinal Science*. 2002; 56(6): 276-278.