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Phytochemical Screening and Antibacterial Activities of Leaf Extracts of *Terminalia catappa* (Umbrella Tree)

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Abstracts: ***The aqueous and organic solvent leaves extracts of Terminalia catappa were screened for antibacterial activity using hole in plate bioassay procedure. The extracts were also screened for their phytochemical constituents. Hexane (Hx) fractions at 30 – 90 mg/ml, showed activity against Pseudomonas aeruginosa, Streptococcus viridians and Staphylococcus aureus.The chloroform (CHL) fractions also showed a significant (P < 0.05) activity on the three (3) Bacteria employed. The petroleum ether (PE), water ethanol extract (W/E) and aqueous water extract (W) all showed significant (P<0.05) activity on Pseudomonas aeruginosa, Streptococcus viridians and Staphylococcus aureus. Tannins, Saponins, Flavonoids, Alkaloids, Anthraquinones, Steroids, saponin glycosides and cardiac glycosides were detectedin the extracts. These results have justified the indigenous use of the plant against the use of synthetic ones.***

Keywords: Herbal plants, *Terminalia catappa*, phytochemicals, antibacterial activity, extracts

1.Introduction

Plants with medicinal potentials and their secondary metabolites have been identified and applicated in dishes from the earliest annals of human habitancy; herbal medicine in ancient systems as well as advanced medicine has created one of the most important science bases for security in various lands of the mankind (1-3). For many of years, herbal plants have been used for distinct goals. Herbal plants are generally defined as one year gramineous herbs with not any strict contexture. Dependent on the type of plants, one part including (flowers, leaves, branch or roots) or whole sectors (aerial parts or roots) could be accomplished in treatment of acute and chronic diseases food products and nutritional improvement (3-5). After entering herbs into digestive system, body starts consuming them and stave off the waste as well as free radicals bounded to the fibres of the herbs and in general purify useful contents into body cells. Because of unending advantages and their considerable benefits, plants with medicinal potentials are anticipated to be applied widely in human nutrition to improve not only the healthiness of human's body cells but also ensure the psychological health (1, 6) A large number of plants are used in traditional medicinal practices and have been so far more than three (3) thousand years such as Chinese traditional medicine. Most of these medicines exert therapeutic effects and would be proven as such if properly evaluated by western standard. Over 2500 plants as having medicinal value and it was been estimated that over 6,000 plants are used in traditional folk and herbal medicine representing about 75% of the medicinal need of the third world, 95% in Africa. Exceptionally, in some countries herbal medicine may contain, by tradition, natural organics or inorganic active ingredients which are not in plant origin. The continued microbial drug resistance has led researchers to find other alternative sources even from medicinal plants. These plants are used locally for the treatment of bacterial diseases without any scientific proofs (7-9).

Terminalia catappa is a species of the family Combretaceaeandis widely distributed in countries with tropical and subtropical climates, especially in coastal regions due to the plant's ability to easily adapt to salinity and winds. In Asian countries, the leaves of this species are commonly used for the treatment of dermatitis, hepatitis, diarrhea and pyresis (10, 11). This plant was also listed in Pharmacopeia vegetables of the Caribbean, where the leaves of this plant are used in a decoction for gastritis and urinary infection .The literature also shows that the polar extract from different parts (leaves, fruits and bark) of Terminalia catappa have shown the following biological activities: antimicrobial, antifungal, antioxidant, antimetastatic, anti-inflammatory, mutagenic, aphrodisiac and antidiabetic. Although the mechanism responsible for this preventive effect remains unknown, thus the use of this specie against gastric bacteria is relevant. Infection caused to *Helicobacter pylori* is considered the most prevalent cause of gastric disease such as ulcers, dyspepsia and stomach cancer The drug therapy available for the treatment of diseases caused by this microorganism has limitation such as the high rate of resistance to conventional drugs and inappropriate patient treatment, as presented to numerous side effects. In this sense, the use of natural products as an alternative to control this bacterium has shown to be satisfactory, what drives the improvement of investigations of medicinal plants as adjuvant or new antimicrobial drugs (11-13).This work, therefore evaluates the antibacterial and phytochemical properties of *Terminalia catappa*. Medicinal plants usually have active ingredient which are used to cure one disease or the other. These ingredients may be in form of alkaloid, saponins, steroids and glycosides, they may be found in a particular part of the plant or all its body.

2.Material and Methods

All chemicals used were of both analytical and general purpose reagent. The leaves of *Terminalia catappa* (umbrella tree) were collected from Runji Sambo Area, Sokoto State, Nigeria. It was packaged in a big envelop and

sealed. The leaves were washed with clean tap water room dried and pulverized into powdered forms and the powder was subjected to aqueous and organic solvent extraction. The species of bacterial organism were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus viridians.* The cultures were maintained on nutrient agar slant at 4^{o}C, re-identified and sub-cultured by biochemical test. Fractionation of the extract was done by activity guided fractionation using ethanol. Water (1:1) and different (hexane, petroleum either, chloroform) organic solvents. The powdered extracts of the leaf (200g) were extracted with ethanol-water (1:1, 500ml), separately at room temperature for 24 hours. The extract was filtered and partitioned in hexane separately (250ml) and clarified by further filtration. Evaporation of hexane fraction to dryness in an oven at 50C yielded residues of 3.75%. The aqueous filtrate (ethanol - water) of the extract fraction was further partitioned (or obtain fraction of different polarities) with petroleum ether (250ml) and chloroform separately remaining water ethanol filtrate. A separate portion of powdered leaf (200g) was extracted with 500ml distilled water at room temperature for 24 hours and then filtrate was evaporated to dryness. The dried extracts obtained from the activity guided fractionation of hexane, petroleum ether, chloroform, and the last remaining water ethanol were formulated into a concentration ranges of 30, 60,and 90mg/ml respectively. 30, 60, and 90mg (0.9g) of each of the extract were weighed using four separate (4) test tubes for each extract. The resultant mixtures (solutions) were in 30, 60, and 90mg/ml respectively, were the chosen concentration. The same procedure was used for the aqueous extract with four separate test-tube at the same interval of concentration range. Ciprofloxacin is an antibiotic that was used as a positive control for the antibacterial activity using a concentration of 90mg (0.09g) of this drug in 1ml 0f distilled water. All media used in this work was prepared according to the manufacturer's instructions. The media used was nutrient agar. 28g of powdered nutrient agar was weighed and dissolved in 1000ml (1litre) of distilled water in a conical flask. The mixture was heated using an autoclave until all the contents were fully dissolved. Out of which was sterilized by the autoclave for 1 hour 30 minutes and then allowed to cool to a lower temperature before it was dispensed into sterile Petri dishes and then allowed to stand overnight in order for it to solidify properly.

3.Results

Table 1.Presents the antibacterial activity of aqueous (WE), Hx, PE and CHL extracts of terminalia catappa. The plant extract showed significant (P<0.05) activity on the entire micro organism used. Table 2.Presents the phytochemical screening of leaf extracts of *Terminalia catappa*.

Table 1: Antibacterial activity of aqueous and organic solvents leaf extracts of *terminalia catappa*

Fractions	*Concentration (mg/ml)*	*Pseudo.*	*Staph.*	*Strep.*
HX	30	10.71±1.60^{a}	15.30±1.21^{b}	14.00±0.82^{a}
	60	15.18±1.23^{a}	15.82±1.21^{b}	15.30±1.43^{b}
	90	15.00±0.8^{a}	14.38±1.89^{a}	16.60±1.61^{a}
PE	30	19.67±0.55^{d}	18.50±1.04^{c}	16.00±2.16^{b}
	60	18.21±1.35^{c}	19.00±2.00^{c}	13.04±2.41^{a}
	90	19.32±1.38^{c}	17.05±1.64^{b}	17.52±2.52^{b}
CHL	30	20.10±1.55^{d}	15.70±1.94^{b}	14.42±1.53^{a}
	60	15.00±1.63^{a}	16.12±2.47^{b}	16.42±0.74^{b}
	90	19.11±2.42^{c}	18.05±1.64^{b}	17.17±2.73^{b}
WE	30	14.03±3.47^{b}	17.51±1.91^{c}	16.28±1.61^{b}
	60	16.16±2.46^{b}	15.83±2.38^{b}	17.08±1.59^{c}
	90	20.00±3.27^{c}	17.58±2.07^{b}	17.83±2.04^{b}
W	30	17.99±2.93^{c}	13.33±1.92^{a}	14.67±0.94^{a}
	60	18.23±1.45^{c}	14.21±0.89^{a}	17.00±2.93^{c}
	90	17.33±1.51^{b}	14.33±0.27^{a}	18.09±2.64^{c}
Ciproflox acine (control)	90	17.00±1.00^{c}	15.33±1.15^{a}	16.33±1.53^{a}

HX = Hexane, PE= Petroleum ether, CHL = chloroform, WE =water ethanol (last remaining) extract & W =water (aqueous) fractions- = No activity; values greater than 6mm indicates some activity. Zone of inhibition is in 'mm', values are mean ± standard error of triplicates. All values are significantly different from the control using analysis of variance. (ANOVA).

Table 2: Qualitative analysis of the phytochemical screening of the leaves extracts of *terminalia catappa*.

Fractions	*WE*	*HX*	*CLH*	*PE*
Alkaloids (Meyers)	+++	++	++	++
(Wagner's)	+++	++	++	++
Flavonoids	+++	+++	+++	++ +
Tannins	+++	+++	+++	+++
Saponnins	+++	-	+	+
Glycosides	-	-	-	-
Anthraquinones	+	-	-	-
Steroids	+++	++	++	++
Saponnin glycoside	+++	-	-	++
Cardiac glycoside	+++	++	++	++
Volatile oil	-	-	-	-

HX= Hexane, PE = Petroleum ether, CHL – chloroform, WE = water ethanol (lasting remaining) extract & W = water (aqueous) fractions, + trace amount, ++ = moderate amount, +++ = high amount, - = Not detected.

4.Discussion

In recent times, naturally occurring bioactive compounds from medicinal plants have been used as chemopreventive agents to treat bacteria diseases without risk assessment (14, 15). The finding of antibacterial activity presents an easy in vitro system that can be used for assessing the anti- bacterial activity of plants. In this study, the application of HX, PE, CHL, WE and aqueous extract of leaves of *Terminalia catappa* showed significant (P<0.05) inhibitory activities on isolates of *pseudomonas aeruginosa, staphylococcus aureus* and *streptococcus viridians* used at 30 – 90 mg/ml (Table 1). The in vitro activities of crude plants extracts provided evidence to support the use of such plants (1, 4).The WE extract was the most acute at concentration 30-90mg/dl.

There by, showing that different extracts of some plants may have different pharmacological properties. The results obtained, showed that water which was commonly used as a solvent by traditional healers to extract the pharmacologically active compounds due to its easy availability, and is not the best and most effective solvent for extracting the bioactive components of *Terminalia catappa (13)*.The antibacterial potential of the leaves extract of Terminalia catappa has been elucidated by the result of this study, which may explain its traditional usage for treating bacterial diseases (11).

The results obtained, at 90mg/ml concentration (Table 1) on isolate *Pseudomonas aeruginosa*. The aqueous extract fraction is said to have the same antibacterial effect with the control used, simply because there is no significant difference between them. Unlike that of the Hexane, Water-ethanol, petroleum ether and chloroform extracts fractions which were found to be significantly difference from the control. Therefore there antibacterial activity was found to be effective more than the control used. From the results obtained, at 90mg/ml concentration (Table 1) on isolates of *Staphylococcus aureus*. The aqueous extract fraction was found to have the same antibacterial effect, simply because there is no significant difference between them. Unlike that of the hexane, water-ethanol, petroleum ether and chloroform extracts fractions which were found to be significantly different from the control used. From the result obtained at 90mg/ml concentration, (Table 1) on isolates of *Streptococcus viridians*. Hexane fraction was found to have the same antibacterial effect, simply because there was no significant difference between them. Unlike that of the aqueous, water-ethanol, chloroform and petroleum ether extracts fraction which was found to be significantly different from the control used.

The presence of Tannins, cardiac glycosides, saponins, flavonoid, alkaloids, steroids, saponins glycoside and anthraquinone, in the extracts has been associated with the antimicrobial activity (13, 16). It is probably that the antibacterial agents in the extract of the *Terminalia catappa* act by inhibition of nucleic acid, protein, cell wall and membrane phospholipids biosynthesis

The phytochemical screenings of the leaves extract of *terminalia catappa* were found to contain several flavonoids, several tannins, saponins and alkaloids among others. Due to the chemical richness, the leaves of *terminalia catappa (*umbrella tree) are used in different traditional medicines for various purposes which include the treatment of liver diseases, dysentery and diarrhoea. The antibacterial activity of the plant may be due to its phytochemical constituents since these compounds were reported with anti-bacterial activity (17).

5.Conclusion

In this study the aqueous and organic solvent leaves extracts of *Terminalia catappa* were screened for antibacterial activity using hole in plate bioassay procedure. The extracts were also screened for their phytochemical constituents. The leaves extract of *Terminalia catappa* were found to contain several flavonoids, several tannins, saponins and alkaloids among others. It could be that due to the chemical richness, the leaves of *Terminalia catappa (*umbrella tree) possess these antibacterial activities and therefore it safe to recommend this plant to be used as antibacterial agent.

6.Acknowledgements

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