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Phytochemical Screening and Antimicrobial Activities of *Terminalia catappa*, Leaf Extracts.

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ABSTRACT: The powdered leaf of *Terminalia catappa* was extracted using ethanol and partitioned into n-hexane, chloroform, ethyl acetate and aqueous methanol fractions. Test for the presence of secondary metabolites showed the presence of alkaloids, reducing sugars, saponins, tannins, resins and steroids in ethanol soluble fraction. The antimicrobial assay of n-hexane, chloroform and ethyl acetate fractions indicated a positive activity against the bacterial isolates tested. Chloroform fraction showed activity against Gram-negative *Escherichia coli and Salmonella typhi* at 500µg/disc, while ethanol and aqueous methanol fractions were active only on *Salmonella typhi at* concentrations of 300 – 500µg/disc. The present study revealed that the plant extracts contain phytochemicals responsible for the activity against Salmonella typhi

Key words: *Terminalia catappa*, extraction, secondary metabolite, antimicrobial assay.

Introduction

The use of crude herbal remedies in the form of water-based extracts, tinctures, and concoctions (Donald *et al.*, 1999) is highest in individuals afflicted with chronic diseases like cancer, human immunodeficiency virus (HIV), diabetes and arthritis (Dolin *et al.*, 1994). This trend is aggravated by the poor social-economic situation, ignorance, and exorbitant cost of most western medicine. The search for new drugs has turned researchers to plant sources for the active molecules (Guzdek and Nizankowska, 1996).

Terminalia catappa (tropical almond) is a medium size tropical tree whose branches form layers of canopy. All parts of the plant are used in traditional medicine. The leaves have been shown to protect against acute liver injury produced by some hepato-toxicants. In Taiwan fallen leaves are used as herb to treat liver diseases (Wee, 1992) and a potential in the management of sickle cell disorders (Tan *et al.*, 1991).

The dried leaves are used for fish pathogen treatment, as an alternative to antibiotics. The leaves have antioxidant as well as anticlastogenic properties (Masuda *et al.*, 1999). The various extracts of leaves and bark of *T catappa* have been reported to be anticancer, anti-HIV reverse transcripts (Tan *et al.*, 1991) and hepato-protective (Lin *et al.*, 1997) as well as anti-inflammatory (Lin *et al.*, 1999), hepatitis (Chen *et al.*, 2000) antidiabetic (Nagappa *et al*., 2003) and aphrodisiac (Ratnasooriya and Darmasuri, 2000).

The moderate consumption of the seed kernel is useful in the treatment of men with sexual dysfunctions, primarily from premature ejaculation (Ratnasooriya and Darmasuri, 2000). The ethanol extract of the leaves of

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Terminalia catappa L. (*Combretaceae*) inhibits osmotically-induced hemolysis of human erythrocytes in a dose-dependent manner (Chen *et al.*, 2000). Punicalagin and punicalin, from the leaves are used to treat dermatitis and hepatitis as both have strong antioxidative activity (Lin *et al.,* 1999). In view of this the present study was set up with the objective of assessing the activity of the plant extracts against some selected bacterial pathogens.

Materials and Methods

Extraction and fractionation of plant material

The leaves of *Terminalia catappa* were collected from Zangon Dakata Quarters , Ungogo Local government area, Kano State. The sample was air dried, grounded and soaked in 95% absolute ethanol at room temperature for 2 weeks. This was filtered and the solvent evaporated. The ethanol extract was partitioned into n-hexane, chloroform, ethyl acetate and aqueous methanol soluble fractions. All the solvents used were evaporated using Rotary Evaporator.

Chemical Analysis

Plant extracts were phytochemically screened using standard techniques for the qualitative detection of alkaloid, flavanoids, resins, steroids, sugars, tannins and saponins (Sofowora, 1984).

Sources of Microorganisms

Pure cultures of *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae and Salmonella typhi* were obtained from Microbiology Laboratory, Department of Biological Sciences, Bayero University Kano, Nigeria. These bacterial cultures were maintained in nutrient agar slant at 4^{o}C before use.

Preparation of Inocula

The inoculum was prepared from the stock cultures which were maintained on nutrient agar slant at 37^{o}C overnight and subcultured in nutrient broth using a sterilized wire loop and incubated at 37^{o}C for 24 hours. The density of suspension to be inoculated was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (1% v/v).

Preparation of Sensitivity Disc

Discs of about 6mm diameter were punched from whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 110^{o}C for 24hours .The stock solution of 50mg/ml of the plant extract was prepared by dissolving 0.1g of each fraction in 2ml Dimethylsulphoxide (DMSO). Concentration of 30mg/ml and 10mg/ml were prepared by dissolving 0.6ml and 0.2ml of the stock solution into 0.4ml and 0.8ml of DMSO respectively. One milliliter (1ml) of the extract from 50mg/ml, 30mg/ml and 10mg/ml concentrations were each transferred into separate bottles containing 100 discs. Since each disc can absorb 0.01 ml, the three bottles yielded discs of 500µg/disc, 300µg/disc and 100µg/disc respectively (British Pharmacopoeia, 1998).

Antibacterial Susceptibility Test

Disc agar diffusion technique described by Bauer and Kirby (1966) was employed for antibacterial assay. Three concentrations for each fraction of the plant extract were prepared namely, 500µg/disc, 300µg/disc and 100µg/disc. These concentrations of the plant extract were subjected to antimicrobial susceptibility test against the selected organisms. Sterile wire loop loaded with the standard culture was used in streaking agar plates evenly and aseptically in an inoculation chamber. The prepared discs, and disc containing only DMSO (as negative control) were aseptically pressed firmly using sterile forceps unto the inoculated plates. The set up was incubated at 37^{o}C for 18 hours. The zone diameter of inhibition was measured to the nearest whole number using meter rule.

Result and Discussion

Terminalia catappa extracts were found to contain some secondary metabolites (Table1). In this work, all the fractions obtained indicate presence of resins. Ethanol extract, petroleum ether, chloroform and ethyl acetate responded positively to a test on the presence of steroids. Alkaloids were detected in ethanol extract only. The distribution of tannins and reducing sugars were detected in ethanol and aqueous methanol extracts, while petroleum ether fraction showed presence of saponins. Some of these metabolites particularly some flavonoids (that are absent) were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Yusha'u *et al*., 2008). In addition to some alkaloids and tannins that are well documented for antimicrobial activity (Sign and Bhat, 2003)

Table 1: Weight of various fractions and Phytochemical Constituents **of *Terminalia catappa* leaf extracts**

Fractions	**Weight (g)**	**Secondary metabolites Present**
TC1	10.00	Alkaloid, Reducing sugar, Resins, Steroids, Tannins, Saponins
TC1-01	5.20	Resins, Saponins, steroids
TC1-02	2.63	Resins, steroids
TC1-03	0.68	Resins, Steroids
TC1-04	1.03	Resins, Reducing sugar, Tannins, Saponins

Key: TC1=crude 95% ethanol extract, TC1-01= n-hexane soluble fraction, TC1-02= chloroform soluble fraction, TC1-03= ethylacetate soluble fraction and TC1-04= aqueous methanol soluble fraction.

The antibacterial tests carried out on all the fractions obtained were shown in the table 2. Herper et al., (1945) reported that, susceptibilityof bacterial culture to extract was determined by measurement in the following ranges: 0-7 mm indicates inactivity; 8-12 mm indicates weak activity and 12mm-above indicates strong activity.
From the result obtained in this work, the activity obtained ranges between weak and strong activity against S*almonella typhi*. Ethanol extract has 7mm and11mm (300, 500 µg/disc); chloroform extracts showed 16mm (500 µg/disc) and aqueous methanol extract with 10mm, 14mm and 17mm (at 100, 300, 500 µg/disc respectively). Other organisms tested were found to have resistant to the plant extracts, which may be attributed to the absence of flavonoids in all the extracts as claimed by Yusha'u *et al*., (2008) as well as Sign and Bhat, (2003).

Conclusion and Recommendation

The results have provided baseline-data on the candidacy of chloroform, ethanol and aqueous methanol fractions as anti-typhoid drugs. Further works should be carried out to substantiate this finding and also isolate and characterize the compounds responsible for the bioactivity.

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Table 2: Antibacterial Susceptibility of Clinical isolates to ***Terminalia catappa,*** Extracts

Fraction	Concentration (µg/disc)	Test organisms with Zone of inhibition in (mm)				
		K.P	S.P	S.A	E.C	S.T
TC1	100	00	00	00	00	00
	300	00	00	00	00	07
	500	00	00	00	00	11
TC1-01	100	00	00	00	00	00
	300	00	00	00	00	00
	500	00	08	00	00	00
TC1-02	100	00	00	00	00	00
	300	00	00	00	00	00
	500	00	00	00	08	16
TC1-03	100	00	00	00	00	00
	300	00	00	00	00	00
	500	00	07	00	00	00
TC1-04	100	00	00	00	00	10
	300	00	00	00	00	14
	500	00	00	00	00	17

Note: Zone of inhibition for disc = 6mm.
Key: K.P = *Klebsiella pneumoniae*, S.P = *Streptococcus pneumoniae*, S.A = *Staphylococcus aureus*, E.C = *Escherichia coli*, S.T = *Salmonella typi*.
TC1=crude 95% ethanol extract, TC1-01= n-hexane soluble fraction, TC1-02= chloroform soluble fraction, TC1-03= ethylacetate soluble fraction and TC1-04= aqueous methanol soluble fraction.

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