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COMPARATIVE PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF LEAVES OF *Terminalia catappa* LINN. (COMBRETACEAE)

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Abstract
Terminalia catappa Linn (Combretaceae), a tree commonly found in the tropics is used in Hausa ethnomedicine in the treatment of liver diseases, diabetes, asthma, diarrhoea and typhoid fever. In the latter case however, only the dried fallen leaves are used. Comparative preliminary phytochemical screening of the fresh and dried fallen leaves extracts revealed the presence of saponins, steroids and triterpenes, fatty acids, tannins and glycosides in both types of the leaves. Alkaloids and flavonoids were found only in the dried fallen leaves. The antibacterial assay of both the fresh and dried fallen leaves extracts indicated higher activity against *Salmonella typhii* (clinical isolates) and *Pseudomonas aeruginosa* (ATCC 10145) by extracts of dried fallen leaves.

Keywords: *Terminalia catappa*; *extract*; *phytochemical*; *antimicrobial*

INTRODUCTION

Terminalia catappa, a big tree found commonly in Asia, India and the tropics is used in ethnomedicine in the treatment of several ailments. The leaves of this plant are reported to be use as anti diarrhoea (Coee and Anderson, 1996), astringent (Esposito-Avella *et al.,* 1996), antidiabetes (Gurib-Fakim *et al.,* 1996), mild-laxative (Hordsworth, 1991), antiasthmatic and also used in treating certain liver diseases (Weninger *et al.,* 1986). In Northern Nigeria folklore use of the dried fallen leaves of *Terminalia catappa* is in the treatment of typhoid fever. Surprisingly the tradition specifies that the leaves used must be the ones that dry and fall by themselves. This prompted the current research into the comparative phytochemical content and also the comparative microbiological activity of both the fresh and the dried fallen leaves of the plant.

Fresh leaves of *Terminalia catappa* were found to be active against *Vibrio cholerea* (Aynechi, 1998), weak molluscidal activity (Pinheiro, 1974), antisickling activity (Mgbemene and Ohiri, 1999), anticlastogenic activity (Liu *et al.,* 1997), anti hepatoxic activity (Lin *et al.,* 1997) and are also reported to inhibit human immunodeficiency virus type 1 (HIV-1) and act by inhibiting the reverse transcriptase (Tan *et al.,* 1991).

Phytochemical components isolated from the fresh leaves of *T. catappa* include eugenin, 1-degalloyl and chebulagic acid (Tanaka *et al.,* 1986), aeraniin (Cen *et al.,* 1991), gentisic acid (Griffith, 1959), terflavin A and B, tergallagin and punicallin. The only steroid isolated from the leaves of this plant is danconsterol (Tanaka *et al.,* 1986).

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Tannins are generally present in all the members of the genera *Terminalia* and some of the biological activities like the inhibition of human immune deficiency virus reverse transcriptase and the hepatotoxic activity are attributed to the presence of gallotannin and formic acids respectively (Cen *et al.,* 1991). Apart from tannins and amino acids, a triterpene-arjunolic acid was also reported to have been isolated. Resin, ascorbic acid, coumarin and anthraquinone are also found to be present in some species of *Terminalia* (Cen, 1991)
There is no reported phytochemical work on the dried fallen leaves.

MATERIALS AND METHOD

Phytochemical screening

The fresh and dried fallen leaves of *Terminalia catappa* growing as ornamental plants in Ahmadu Bello University, Zaria, Nigeria were collected and authenticated at the herbarium, Department of Biological Sciences, Ahmadu Bello University Zaria and Voucher Specimen (number 1556) was deposited.

The plant materials were air dried and powdered. 1kg of this was weighed, continuously extracted with petroleum ether in a soxhlet extractor and subsequently methanol. The petroleum ether extract of the freshly collected leaves was coded PEF while that of dried fallen leaves coded PED. The methanolic extracts of the freshly collected leaves is coded MEF while that of the dried fallen leaves was coded MED.

The various extracts were subjected to preliminary phytochemical screening to identify the presence or absence of the basic phytochemical groups using standard methods (Brain and Turner, 1975; Trease and Evans, 1986).

Antibacterial testing

Preparation of the test bacteria

Clinical isolates of *Salmonella typhii* and *Pseudomonas aeruginosa* (ATCC 10145) were used. *Ps. aeruginosa* was obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and purified by growing on cetrimide agar at 37°C for 24 hours.
For *S. typhii,* blood sample was inoculated into selenite F broth, which was inoculated for 24 hours at 37°C. Subculture of the broth culture was made onto slant of triple sugar iron (TSI) agar by stabbing and incubated for 24 hours at 37°C. TSI agar slants with black colouration at the slant were identified as *S. typhii.*

Determination of Minimum Inhibitory Concentration (MIC)

Two-fold serial dilution of 20%w/v concentration of the extracts in 4% dimethyl sulfoxide (DMSO) was made using nutrient broth. Each dilution was then inoculated with 0.1 ml suspension of the 1:5000 dilution of the test bacteria (inoculum size is approximately 10^6 cfu/ml). These were incubated for 24 hours at 37°C. After incubation, the mixtures were subcultured on nutrient agar plates and incubated for 24 hours at 37°C. After incubation the least concentration of the extracts that did not show any detectable growth was considered as the MIC.

Susceptibility Testing

Molten nutrient agar (19 ml at 45°C) was inoculated with 1ml of suspension of the bacterial species, shaken gently and poured into petri-dish. These were allowed to solidify and wells were bored into each seeded nutrient agar plate with sterile cup borer
(8 mm diameter). The wells were filled with varying concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078 %) of the extracts and one with sterile distilled water as control. These were left for 1 hour at room temperature for the extracts to diffuse into the agar medium. The plates were incubated at 37°C for 24 hours. After incubation, zones of inhibition produced by the extracts were measured to the nearest millimetres.

RESULTS

Phytochemical

These are presented in tables 1 and 2

Table 1: Showing weight of Extracts

	Weight of extracts (grams)	
Plant sample	Pet. Ether extract	Methanol extract
Dried fallen leaves (1.0 kg)	63	147
Fresh leaves (1.0 kg)	56	125

Table 2: Phytochemical Constituents of the Various Extracts of the fresh and dried fallen leaf

Compound/group		Fresh Leaves		Dried fallen Leaves	
		Pet. Ether extract	Methanol extract	Pet ether extract	Methanol extract
Steroid/triterpenes		+	+	+	+
Saponin	Lieberman – Buchard	NT	+	NT	+
	Haemolytic	NT	+	NT	+
	Frothing	NT	+	NT	+
Tannins		NT	+	NT	+
Flavones		-	-	+	+
Alkaloids		-	-	+	+
Volatile oils		+	-	+	-
Coumarins		+	-	-	+
Free carboxylic acid		-	+	-	+
Free anthraquinone		-	-	-	-
Combined anthraquinone		-	-	-	-
Carbohydrate		NT	+	NT	+
Cardiac glycoside		NT	-	NT	-

+ = Present, - = Absent, NT = Not Tested.

Microbiological

The MIC of the methanolic extract of the fresh leaves against *S. typhi* was 2.5% while the pet. ether extract of fresh leaves was 5.0%. For the dried leaves, the MIC of methanolic extract of dried leaves was 0.3125% while that of petroleum ether extract of dried leaves was 1.25% against the same organism (Table 3).

Table 3: Minimum inhibitory concentrations of the dried and fresh leaf extracts of *T. catappa* on *Salmonella typhi* and *Ps. Aeruginosa*

Conc (%)	Fresh leaves				Dried leaves			
	Pet. ether extract		Methanol extract		Pet. ether extract		Methanol extract	
	S. typhi	*Ps. aeruginosa*	*S. typhi*	*Ps. aeruginosa*	*S. typhi*	*Ps. aeruginosa*	*S. typhi*	*Ps. aeruginosa*
0.0780	+	+	+	+	+	+	+	+
0.1568	+	+	+	+	+	+	+	+
0.3125	+	+	+	+	+	+	-	+
0.625	+	+	+	+	+	+	-	-
1.25	+	+	+	+	+	+	-	-
2.5	+	+	-	-	-	-	-	-
5.0	-	-	-	-	-	-	-	-
10.0	-	-	-	-	-	-	-	-

+: Growth indicated
-: No growth

Table 4: Susceptibility of *S. typhi* and *Ps. aeruginosa* to fresh and dried leaf extracts of *T. Catappa*.

Concentration	Average zone of inhibition (mm) of extracts							
	Pet. ether extract of dried leaves		Methanol extract of dried leaves		Pet. ether extract fresh leaves		Methanol extract of fresh leaves	
	S. typhi	*Ps. aeruginosa*	*S. typhi*	*Ps. aeruginosa*	*S. typhi*	*Ps. aeruginosa*	*S. typhi*	*Ps. aeruginosa*
10%	19	17	24	21	13	12	14	13
5%	16	14	21	19	11	11.5	12	11
2.5%	12	12	17	16	8	8	11	10
1.25%	11	11	14	12	8	8	8	8

DISCUSSION AND CONCLUSION

After successive extraction of respective crude plant materials, higher yield of extract was obtained with methanol. This could be due to the fact that methanol penetrate and extract both intracellular and extracellular components of the plant material more than petroleum ether (Cannell, 1998).

Results of phytochemical screening (Table 2) of the various extracts of the dried fallen leaves and fresh leaves showed the presence of tannins, saponins, steroids/terpenoids in both, while alkaloid and flavonoids were found only in the dried fallen leaves and not the fresh leaves.

From the results of the tests, it is obvious that *T. catappa* leaves have antibacterial activity. The activity is best with MED (Methanol extract of the dried leaves) against the test bacterial species where MIC of 0.3125 and 0.625% were recorded against *S. typhi* and *Ps. aeruginosa* respectively (Table 3). Similar trend was observed in Table 4. Extract MED showed the highest zones of inhibition in all the concentrations of the extracts tested against the two bacterial species. In all these, methanol proved to be a better solvent than petroleum ether. Again, the activity of the leaves, appear to be more in the fallen dried leaves than in the fresh leaves.

MEF has very low activities against the two bacterial species. The difference in the activities of MED and MEF might be related to the chemical constituents of fresh and fallen dried leaves of the plant. The dried leaves contain flavonoids among other phytochemical constituents (Table 2) and flavonoids are known to have antibacterial properties (Sato *et al.,* 1995; Linuma *et al.,* 1994). The flavonoids may have contributed markedly in the activity of the dried leaves. The activities of the extracts are concentration dependent for both bacteria. PED has no activity on the two test organisms at both 2.5% and 1.25% concentrations (Table 4). Also at 1.25% concentration MEF did not inhibit the test bacteria. The MED with lowest MIC 0.625% appear to be more promising extract for further work on isolation and identification of active compound. It should be born in mind that these MIC are only indicative of the activity of crude extracts and that the MIC of pure active compounds might be significantly lower. In summary this study provides new lead for ongoing research for novel antimicrobial agents. The result of this work lends support to the traditional use of the dried leaves for treating enteric fever.

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