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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF CRUDE EXTRACTS (AQUEOUS AND ETHANOLIC) OF *Terminalia mollis* LINN (COMBRETACEAE) ROOT

Y. K. E. Ibrahim, *B. A. Tytler and Y. K. Ogunsola
Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Abstract
The bark and wood parts of the root of *Terminalia mollis* was investigated for its phytochemical and antimicrobial properties. Phytochemical screening showed the presence of tannins and resins as the major secondary metabolites. Test for antimicrobial activity of the plant crude extracts using the agar diffusion method showed that both the water and ethanolic extracts of the bark and wood parts of the root exhibited profound inhibitory activities against *Staph. aureus, Bacillus subtilis, E. coli, Ps. aeruginosa* and *C. albicans* and no activity against *A. niger*. MIC values varied from 0.3125%w/w for *S. aureus* and B. *subtilis* to 1.25%w/w for *C. albicans*. MBC/MFC values of the extracts against the test organisms were generally two geometric dilutions higher than their corresponding MIC values. Rate of kill studies indicated that the extracts had microbicidal activities against the susceptible organisms, effecting at least 3 log decimal reduction within one hour of contact with the extracts. Generally, the root bark extracts exhibited slightly greater inhibitory activities against the test organisms compared with the wood parts of the root.

Keywords: *Phytochemical; antimicrobial screenig; crude extract; Terminalia mollis; root*

INTRODUCTION

Medicinal plants have an ancient history of human use. Its usage dates back to late fifth century B. C. when Hippocrates mentioned 300-400 medicinal plants (Schutes, 1978). The Bible recorded about 30 healing plants (Cowan, 1999). Some plants used today appear to have been used in almost the same manner and for the same purposes since 3000 B.C. by the Babylonians e.g. Papyrus Ebers (written in 1550 B.C.). It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). Many of these plants have been used locally with proven efficacy although a substantial proportion has yet to be documented.

During the last two decades, there has been a considerable growth in the study and use of medicinal plants all over the World especially in advanced countries. There was also increase in the international commerce and commercial exploitation of herbal medicines through over the counter labeled products. In some countries, herbal medicines are still a central part of the medical system e.g. India and China. The renewed interest in the use of medicinal plants has been attributed to its abundance, cheapness and ready accessibility by the local populace, beside its serving as raw material base for the elaboration of more complex semi-synthetic chemical compounds and its possible application as taxonomic markers for the discovery of new compounds. The chemical structures derived from plant substances can also be used as models for new synthetic compounds (Akerele, 1992;

* Corresponding author. E-mail: ykeibrahim@yahoo.co.uk; Tel:+234-(0)-803-700-1988
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Sofowora, 1993).

Scientists all over the world have started identifying, isolating and evaluating the active ingredients in herbs and to some extent, they have been able to explain bio-chemically and physiologically, how these herbs work. Farnsworth (1986) reported that 74% of the 119 investigated chemical compounds used as drugs have the same or related use as the plants from which they were derived e.g. andrographolide used for bacillary dysentery was obtained from *Andrographis paniculata* which has been used for dysentery.

Although in Nigeria, there are no available current data, there are indications of high demand for herbal products. Some of the reasons observed to these growths include: desire for self-care using natural products to improve health, increased availability of information on scientific studies documenting on the safety and efficacy of herbal medicine for health care and the escalating cost of health care in modern medicine. Several claims by traditional herbalist in Nigeria have been proven and scientific evidence provided by many natural product scientists to justify the ethnomedical usage of these plants (Sofowora, 1993; Akah *et al.,* 1994; Onwukaeme, 1995).

Terminalia mollis, which belongs to the Family Combretaceae (consisting of 20 genera and 600 species) is a wide spread savannah tree growing mainly in heavy soil (Trease and Evans 1989). It is widely distributed in Africa, growing from Senegal through Guinea on the west coast to Sudan in the east (Irvine, 1961). In Nigeria and Ivory Coast, its powdered bark has been reportedly used as an external antiseptic in wound dressing and in the treatment of pile. The plant is also claimed to have haemostatic action and is employed as a laxative and purgative (Oliver, 1959) for its effect in the relief of cough and stomachache. The Yoruba of southwest Nigeria often use the roots of the plant as chewing-sticks. Traditional herbalists in northwest Nigeria have recently been employing concoctions of the plant root in the treatment of secondary infections of Acquired Immune Deficiency Syndrome (AIDS) patients with no existing scientific evidence.

Although, several genera and species of the Combrateceae family have been investigated, documented studies on *Terminalia mollis* are relatively few. This study was, therefore, designed to provide scientific basis for the local use of the plant by investigating its phytochemical constituents and antimicrobial properties.

MATERIALS AND METHODS

Plant Collection and Treatment

The plant was collected from Area BZ of Ahmadu Bello University staff quarters and identified by Mal. U.S. Gallah of the Herbarium Unit of the Department of Biological Sciences of the university. Specimen voucher was deposited with the Herbarium. The roots of the plant, after collection, were washed, chopped into small pieces and sun dried. Thereafter, the bark of the root was separated from the wood and the two portions further size reduced in a mortar and pestle.

Extraction

Three extraction processes were employed. One portion of the powdered plant material was subjected to cold-water extraction by the maceration technique. Maceration was done for 24 hours, filtered and the filtrate dried over water-bath thermostated at 40°C. Hot-water extraction was done on another portion of the powdered plant part by boiling the mass in water for 15 minutes. After filtering, the filtrate was evaporated to dryness over a water bath at 100°C. A third portion of the powdered material was subjected to ethanol extraction by percolation in a soxhlet extractor for 3hr. The resultant filtrate was evaporated to dryness at 100°C over a water-bath. All the extracts were

stored in a dessicator for subsequent use.

Phytochemical Screening

Several tests were carried out to detect the presence of secondary metabolites in the plant root, namely:

Test for Resin: This was carried out as described by Trease and Evans (1989), using 10% copper sulphate solution reagent to detect the formation of green precipitate.

Test for Saponins: The method described by Trease and Evans (1989) was also employed, end-point being the formation of honey-comb froth.

Test for Alkaloids: The procedure described by Harbone (1993) and Trease and Evans (1989) was adopted. Three reagents (Mayer, Dragendorff and Wagner) were used to detect the formation of precipitates or turbidity.

Test for Tannins: Detection of tannins was as described by Trease and Evans (1989) using ferric chloride reagent for the development of dark green colour.

Test for Steroids: Salkowskis test for the identification aglycones in steroids was performed as described by Sofowora (1993). Production of reddish-brown colour at the interface on addition of sulphuric acid was taken as indicative of the presence of steroids.

Test for Flavonoids: The sodium hydroxide test described by Trease and Evans (1989) was adopted. Ferric chloride solution was used as reagent for the development of greenish, bluish or violet colour that are indicative of the presence of flavonoids.

Test for Carbohydrates: This was carried out as described by Trease and Evans (1989). Molisch reagent was used for the formation of purple colouration at the interface (indicative of the presence of carbohydrates).

Tests for Anthraquinones: Two tests were carried out to detect the presence of either free or combined anthraquinones. Borntrager's test described by Trease and Evans (1989) was performed to detect the presence of free anthraquinones while in the test for combined anthraquinones, formation of a pink colour was recorded as positive for anthraquinones.

Preparation of Culture Media and Test Organisms

Appropriate quantities of the dehydrated media of Nutrient broth, Nutrient agar (both Lab M, UK) and Sabouraud dextrose Medium (Oxoid, UK) were reconstituted using freshly distilled water, distributed into bottles and sterilized according to manufacturer's instructions. They were stored in refrigerator at 10^{o}C until needed.

The five test organisms used in this study, namely *B. subtilis* (NCTC 8326B76), *Escherichia coli* (ATCC 11775), *Ps. aeruginosa* (ATCC 10145), *S. aureus* (ATCC 021001), *A. niger* and *C. albicans* (both laboratory strains) were first sub-cultured and revalidated by microscopy and biochemical tests. The overnight culture of each test organism was standardized to give an inoculum size of 10^{7} cfu/ml as officially recommended (NCCLS, 1991).

Determination of Antimicrobial activity

Evaluation of the antimicrobial properties of the crude extracts of the plant carried out involved determination of (1) susceptibility of the extract to various test organisms, (2) minimum inhibitory concentrations and (3) rate of kill studies.

Antimicrobial Susceptibility Test

The agar diffusion method was employed (NCCLS, 1991). Cork-borer of 8mm diameter was used to make holes. 100 l each of 1% and 10%w/v concentrations of each extract was placed in the holes. A pre-incubation diffusion time of 2hours was allowed, followed

by incubation in a Gallenkamp Incubator at 37°C for 18-24 hours. Diameter of inhibition zones was measured (mm) and recorded.

MIC/MBC Determination

The minimum inhibitory concentration (MIC) and MBC of the aqueous extracts of the barks and woody part of the root of *Terminalia mollis* were determined as described by Sahm and Washington (1990).

Rate of Kill Studies

The rate of kill study of 10%w/v water extracts of the bark and wood parts of the root against *S. aureus* and *Ps. aeruginosa* were determined as described by Reybrook *et al.,* (1979). Aliquots of extract-organism mixtures were, at intervals of 30 minute withdrawn to determine their viable counts by the pour plate method.

RESULTS AND DISCUSSION

The extracts obtained were of varying shades of brown colour with different percentage yield values (Table 1). The ethanolic soxhlet extraction method gave the highest percentage yield. This might not be unconnected to the property of ethanol as a universal solvent: able to extract water soluble substances as well as insoluble organics coupled with the use of soxhlet extractor that is generally associated with ability to exhaust materials of its extractive constituents. Yield was generally higher with the bark extract than with the wood part of the root. This may be attributable to the presence of more extractable soluble substances in the bark compared with the wood that consists largely of insoluble fibrous structures.

The phytochemical tests revealed the presence of only resins and tannins as the secondary metabolites present in the root of *Terminalia mollis* (Table 2). The presence of tannins and resins in this plant is not unexpected since they have been reported in other members of the species of *Terminalia*, for example, *Terminalia macroptera* (Bhatia and Balat, 1979).

As shown in Table 3, the lower concentration of the extracts only exerted activity against *S. aureus*. The higher concentration of 10%w/v exhibited pronounced inhibitory activities against four of the five test organisms (*B. subtilis, S. aureus, E. coli. Ps. aeruginosa and C. albicans*). No inhibitory activity was observed against *A. niger*. Diameters of the zones of inhibition showed that the bark extracts were more active against the organisms. The cold-water extracts also exerted higher antimicrobial activities against the test organisms with S. aureus being the most susceptible. The difference observed in susceptibility among the test organisms may be attributable to differences in the nature of their cell wall structures; Gram positive organisms possessing thick cell-walls made largely of mureins compared with the cell-walls of Gram negative organism composed of molecules of polysaccharides, lipids and relatively low amount of peptidoglycan strands that confer more resistance to chemical penetration and subsequent cell inactivation (Baird-Parker & Holbrook, 1971). Although *C. albicans* and *A. niger* are both fungi, the former is a unicellular organism as against the latter that is a spore former. Spores are generally associated with high resistance to chemical agents due to the presence of spore coat that contains high amount of dipicolinic acid and very little amount of water (Balassa *et al*. 1979).

The MIC values of the two extracts against the test organisms were generally similar: lowest for the two *S. aureus* and *B. subtilis* (Gram positive) and lowest for *C. albicans* (Table 4). The MBC/FBC values were very much higher, generally two geometric dilutions greater than their corresponding MIC values, which indicates that these extracts exert

largely microbicidal activities against the four susceptible test organisms.

Result of the rate of kill studies as depicted in Fig 1, clearly shows that extracts from both roots parts exerted rapid bactericidal activities against the two test organisms, inhibitory activity greater on S. *aureus* than *Ps. aeruginosa*. This is more aptly demonstrated in Table 5, which show the decimal reduction factors for the extracts on the two test organisms at various contact times. As depicted in this table, the reduction factors for both test organisms were similar with the bark extract but with the woody part, more cells of *S. aureus* were killed at a time compared with *Ps. aeruginosa* at contact times up to 90 minutes. The bark extracts also caused slightly higher reduction in cell population. The slight differences in the level of inhibitory activities between the bark and woody parts of the root of *Terminalia mollis* as seen in the MIC/MBC data and percentage kill/decimal log reduction values, might be due to possible differences in the chemical composition of the extracts as could be seen even in the percentage yields.

The observed antimicrobial activities of these extracts (bark and woody parts of the root of *Terminalia mollis*) might be due to the secondary metabolites: tannins and resins. Tannins are polyphenolic in nature and at high concentration have been reported to coagulate cell wall proteins resulting in microbial death, while at low concentrations are bacteriostatic (Oliver, 1959).

Conclusion

The result of this study shows that *Terminalia mollis* root possesses inhibitory activity against *B. subtilis, E. coli, Ps. aeruginosa, Staph. aureus* and *C. Albicans,* some of which (S. *aureus, Ps. aeroginosa and C. albicans*) have been involved in secondary infections in HIV patients such as superficial wound and burn infection, oral candidiasis, thus providing scientific justification for its local use.

Table 1.: Percentage Yields of the extracts of the root of *Terminalaia mollis* using various solvents

Extract	Percentage Yield (%)
Ethanolic:	
Bark	26.27
Wood	5.30
Aqueous (Hot):	
Bark	10.90
Wood	3.20
Aqueous (Cold):	
Bark	10.13
Wood	1.20

Table 2: Secondary metabolites present in the different extracts of the root of *Terminalia moliis*

Secondary Metabolites	Extract					
	I	II	III	IV	V	VI
Alkaloids	-	-	-	-	-	-
Resins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Carbohydrates	-	-	-	-	-	-
Saponins	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Steroids	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

- = absence of substance + = presence of substance
I = root wood (cold extract) II = root wood (hot water)
III = root bark (cold water extract) IV = root bark (hot water extract)
V = root wood (ethanol extract) VI = root bark (ethanol extract)

Table 3: Diameters of Zones of Inhibition of Extracts of the root of *Terminalia mollis* against the test organisms

Test Organisms	Conc. Of extract	Extract					
		I	II	III	IV	V	VI
A. niger	1%w/v	0.0	0.0	0.0	0.0	0.0	0.0
	10%w/v	0.0	0.0	0.0	0.0	0.0	0.0
B. subtilis	1%w/v	0.0	0.0	13.0 ± 1	0.0	0.0	0.0
	10%w/v	20.0 ± 0.5	16.0 ± 2	23.0 ± 2	21.0 ± 1	21.0 ± 1	21.0 ± 1
C. albicans	1%w/v	0.0	0.0	0.0	0.0	0.0	0.0
	10%w/v	15.5 ± 0.5	16.0 ± 1	25.5 ±0.5	27.5 ±2.5	17.5 ±2.5	25.0 ±0.5
E. coli	1%w/v	0.0	0.0	0.0	0.0	0.0	0.0
	10%w/v	20.5 ± 0.5	15.5 ±0.5	25.0 ± 1	20.0 ±0.5	17.5 ±0.5	20.0 ±0.5
Ps. aeruginosa	1%w/v	0.0	0.0	0.0	0.0	0.0	0.0
	10%w/v	20.0 ± 0.5	16.5 ±0.5	22.0 ±0.5	20.0 ±0.5	18.0 ±0.5	20.5 ±0.5
S. aureus	1%w/v	20.0 ± 0.5	20.0 ±0.5	26.0 ± 2	20.0 ±0.7	28.0 ± 2	20.0 ±0.5
	10%w/v	28.0 ± 2.0	21.0 ± 3	30.0 ±0.5	27.0 ± 3	29.0 ± 1	29.0 ± 1

Diameter of cork borer = 8.0 mm
I = root wood (cold extract) II = root wood (hot water)
III = root bark (cold water extract) IV = root bark (hot water extract)
V = root wood (ethanol extract) VI = root bark (ethanol extract)

Table 4: Minimum Inhibitory and Minimum Bactericidal/Fungicidal Concentrations of the Aqueous Extracts of the Root *Terminalia mollis* against the Test Organisms

Test Organisms	MIC		MBC/FBC	
	Root bark (%w/v)	Root wood (%w/v)	Root bark (%w/v)	Root wood (%w/v)
S. aureus	0.31	0.31	1.25	5.00
B. subtilis	0.31	0.63	10.00	>10.0
C. albicans	2.50	5.00	5.00	10.00
E. coli	2.50	2.50	5.00	10.00
Ps. aeruginosa	1.25	1.25	1.25	5.00

MIC = minimum inhibitory concentration
MBC/MFC = minimum bactericidal/fungicidal concentration

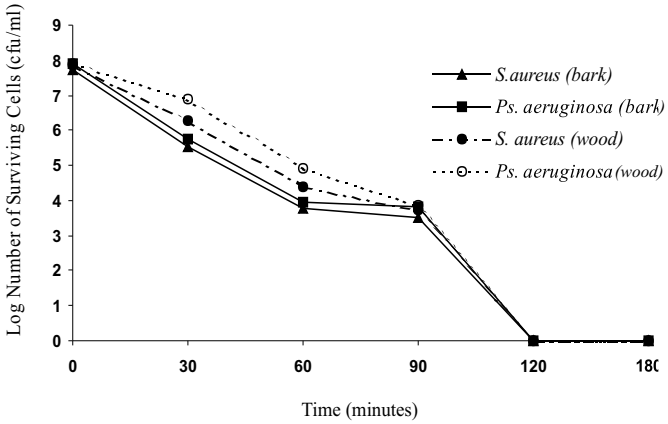

Fig 1: Effect of 10%w/v Aqueous Bark and Wood Extract of *Terminalia mollis* on *S. aureus* and *Ps. Aeruginosa*

Table 5: Decimal Reduction Values Effected by 10%w/v aqueous bark and wood extracts of the root of *Terminalia mollis* on *S.aureus* and *Ps. Aeruginosa*

Time (Min.)	Decimal Reduction Factor			
	10% Aqueous Bark Extract		10% Aqueous wood Extract	
	S. aureus	*Ps. aeruginosa*	*S. aureus*	*Ps. aeruginosa*
30	2.211	2.171	1.538	1.017
60	3.924	3.939	3.441	2.978
90	4.219	4.090	4.106	4.047
120	7.716	7.903	7.839	7.892

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