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Obi Patrick Ebele
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Ujam Nonye Treasure
Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Ugodi Walter Gerald
Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Chukwujindu Chukwunonso Ebere
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Corresponding Author:
Obi Patrick Ebele
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Phytochemical screening and antimicrobial evaluation of ethanol extract and fractions of the leaf of *Terminalia mantaly* H. Perrier (Combretaceae)

Obi Patrick Ebele, Ujam Nonye Treasure, Ugodi Walter Gerald and Chukwujindu Chukwunonso Ebere

Abstract
Medicinal plants contain bioactive chemical substances that are active against external invaders such as pathogenic microorganisms. These bioactive chemicals are primarily manufactured by plant for self defense but have been employed in the treatment of infectious diseases that affect humans due to their therapeutic benefits. The aim of this study is to carry out the phytochemical screen and to evaluate the antimicrobial activity of ethanol extract and fractions of *Terminalia mantaly* leaf. Plant materials were powdered and macerated with ethanol. The crude extract was fractionated with n-hexane, chloroform, ethyl acetate and ethanol successively using column chromatography and silica gel as adsorbent. Qualitative phytochemical screening of basic plant secondary metabolites was carried out using standard procedures. The ethanol plant extract and fractions were reconstituted in dimethylsulphoxide (DMSO) to 200 mg/ml concentration and Agar well Diffusion method was used in the antimicrobial sensitivity screening of clinical strain of enteric microorganisms. Gentamicin and Ketonazole were used as reference standard antibacterial and antifungal drugs respectively. Two-fold dilutions from 200 – 25 mg/ml were done for sensitive extracts and fraction to determine the Minimum Inhibitory Concentration (MIC) and the results obtained were expressed as Mean ± SEM (Standard error of mean). The results of Phytochemical screening revealed the presence of secondary metabolites such as alkaloids, saponins, tannins, flavanoids, steroids and glycosides in the crude extract while the results of the general antimicrobial assay of the extract and fractions showed a comparable potency with gentamicin against *Streptococcus pneumonia, Staphylococcus aureus, Escherichia coli* and ketoconazole against *Aspergillus niger* and *Candida albicans*. The study validated the forkloric uses of this plant as antimicrobial agent scientifically.

Keywords: *Terminalia mantaly*, ketoconazole, Gentamicin, Dimethylsulphoxide, minimum inhibitory concentration (MIC)

Introduction
Medicinal plants application in the cure of ailments has been on the increase in developing countries of the world especially in tropical Africa countries endowed with varieties of plant species. This is as a result of the immense pharmacological properties of African medicinal plants and the remedies that are made from plants which have played important roles in the health of millions of people especially in the rural areas [1]. Also, the level of resistance most pathogenic organisms has developed against most standard anti pathogenic agents has made the search for newer antibiotics a global challenge preoccupying research institutions, pharmaceutical companies and academia [2, 3]. The increasing emergence of resistant strain of pathogenic organisms to newly introduced antibiotics indicates that even new families of antibiotics are expected to have a short life [4]. Hence the importance of search for bioactive molecules from plant source cannot be over emphasized.

Many plants possess antimicrobial activities and are used for the treatment of different diseases [5]. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful [6]. Green plants posses the broadest spectrum of synthetic activity and have been the source of many useful compounds [7]. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine [8].

The generic name of the plant, Terminalia comes from the Latin word ‘terminalis’ meaning ending which refers to the habit of the leaves being crowded at the ends of the shoot [9]. Most members of this genus, Terminalia have been reported with antimicrobial activities.

Terminalia ivorensis trunk barks and roots are used against wounds and as an antipyretic in Côte d'Ivoire [10, 11, 12] and other biological activities associated with *Terminalia ivorensis* include anti-inflammatory and anti-arthritis [13] also antibacterial activity [14].

Terminalia mantaly is drought resistant, deciduous or evergreen tree with erect stem that grows up to 10-20m and clearly visible layered branches. The stem bark is smooth, mottled pale grey while the leaves are smooth, bright green with wavy margin, broadly rounded apex and much tapered base. They are in terminal rosettes of 4-9 unequal leaves on short, thickened stems having length up to7 cm. The flowers are small and greenish in erect spikes to 5 cm long while the fruits are small oval with no obvious wings [9]. It is commonly called umbrella tree and native to Madagascar.

The bark and wood are natural astringent and are used in the treatment of dysentery in Madagascar [15]. Most traditional healers especially in southern region of Côte d'Ivoire have preference for *Terminalia mantaly* bark over *T. catappa* for medicinal purposes [16] and the leaves of *Terminalia mantaly* are used against loss of voice [9]. The aim of this study is to carry out the phytochemical screen and to evaluate the antimicrobial activity of ethanol extract and fractions of *Terminalia mantaly* leaf

Materials and Methods

Collection and Authentication of Plant Material

Fresh leaves of *Terminalia mantaly* were collected from Madonna University, Elele, Rivers state, Nigeria and were authenticated by Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka, Enugu State. A voucher specimen number ESUT/COG/204 was preserved in Department of Pharmacognosy herbarium, Enugu State University of Science and Technology, Enugu, Nigeria.

Preparation of Plant Extract and Fractions

The fresh leaves sample was dried at room temperature and grind with suitable grinding mill into moderately coarse powder. A 700 g of the pulverized leaves was macerated in 2200ml of ethanol for 48 hours and then filtered with Whatman filter paper. The filtrate was concentrated at 40 ^{0}C using rotary evaporator and the concentrated extract was further evaporated to dryness using hot oven at 40 ^{0}C. The weight of the dried extract was gotten using electronic weighing balance and the percentage yield was calculated. The dried ethanol extract was washed with Chloroform, Ethylacetate and 50% ethanol respectively using column chromatography with silica gel of 60-120 mesh size as stationary phase.

Phytochemical Screen

The phytochemical screening was carried out using standard procedures outlined by Evans (2009) and Haborne (1973) to detect the presence of glycosides, tannins, alkaloids, flavonoids, steroids and saponins.

Test for Flavonoids (Shinoda Test): Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids

Test for Saponins: 4ml aqueous solution of extract in a test tube was shaken vigorously. The formation of stable froth indicates the presence of saponins

Test for Alkaloids: 0.5g of extract was dissolved in 10ml of 5% acetic acid, heated for 10 minutes and allowed to get cooled. 2ml of the filtrate was tested with few drops of Dragendorff's reagent (Bismuth potassium iodide solution). The formation of reddish precipitate indicates the presence of alkaloids.

Test for Tannins: 1ml of 2% ferric chloride solution was added 3ml aqueous solution of extract. A blue-green or black coloration indicated the presence of phenols and tannins

Test for Glycosides: 10ml of 1% sulphuric acid was added to 0.5g of extract in a test tube and boiled for 15 minutes on a water bath, then cooled and neutralized with 10% potassium hydroxide solution. 10ml of a mixture of equal volume of fehling's solution I and II was added and boiled for 5 minutes. A brick red precipitate indicates the presence of glycosides.

Test for Steroids (Salkowski Test): 0.5 g of extract was dissolved in 5ml chloroform and equal volume of concentrated sulphuric acid was added to the mixture. The formation of reddish coloration at the chloroform layer indicates the presence of steroids

Preparation of Test Microorganisms and Plant Extract Concentration

The microorganisms used for the evaluation were clinically isolated bacteria (*Streptococcus pneumonia, Staphylococcus aureus, Escherichia coli*) and fungi (*Candida albicans and Aspergillus niger*) obtained from the Madonna University Teaching Hospital, Elele, Rivers State. The bacteria and fungi were purified in nutrient agar and saboraud dextrose agar respectively and individual bacterium and fungus suspensions of 0.5 McFarland turbidity standard were prepared.

2g of each of the extracts were carefully weighed and transferred into different test tubes previously labeled. 10ml Dimethylsulphoxide (DMSO) was transferred into each labeled test tubes containing the weighed extracts to form homogenous stock solution.

Antimicrobial Screening

Antimicrobial sensitivity test for the plant extract and fractions was done using the agar well-diffusion method as outlined by the standard of the United State National Committee for Clinical Laboratory Standards [19]. Three cups 5 mm diameter wells were bored into set nutrient agar and saboraud dextrose agar media using a sterile borer. All plates were inoculated with the test bacterium and fungus by streaking the plates with appropriate bacterium or fungus suspension of 0.5 McFarland turbidity standard. A sterile cotton swab was properly dipped into the appropriate suspension and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum. The entire surface of the agar plate was streaked with the cotton swab containing the inoculum and the plates were allowed to stand for 3 min to dry the excess moisture [20]. Standard solutions of the extract/fractions were prepared in DMSO and 0.5 ml of each transferred to each cup in the inoculated agar. The plates were allowed to stand at room temperature for about 10 minutes and then incubated at 37 ^{0}C for 24 hours (for bacteria) and 48hours (for fungi). After 24 hours and 48 hours for bacteria and fungi cultures respectively the inhibition zone diameters (IZD) were measured [21]. Gentamicin and Ketoconazole were used as standard for the antibacterial and antifungal respectively.

Results and Discussion

The results of phytochemical screening of the plant extracts as shown in table1 revealed the presence of secondary metabolites, some of whose members are recorded in official monographs to exhibit antimicrobial activities. The ethanol extract and ethylacetate fraction indicate the presence of saponins, tannins, flavonoids, steroids, alkaloids and glycosides while the ethanol fraction indicates all but steroids and chloroform fraction indicates the presence of only steroids and flavonoids. Flavonoids are hydroxylated polyphenolic compounds that have the ability to form complexes with extracellular proteins and bacterial cell walls [22] and are believed to be produced by plants in response to harmful external invaders such as microorganisms that can cause microbial infections [23]. Saponins have surface active property which alters the permeability of microorganisms cell wall by binding with the lipoprotein to cause microporations that trigger free entry of exterior toxic materials or leakage of cellular constituents from the cell there by inducing cell lyses [24, 25]. Tannins are polyphenolic compounds that can bind to protein and inhibit protein synthesis [26, 27, 28] thereby destroying bacterial cells [29, 30]. The results of the antimicrobial Screenings as showed in table 2 revealed variations in antimicrobial activities of the extract and individual fraction which is as a result of variation in distribution of active secondary metabolites in the extract and fraction based on polarities. The ethanol extract though effective against all the tested organisms but less effective than ethylacetate fraction due to lower Minimum Inhibitory Concentration (MIC) values of the ethylacetate fraction against tested organisms as shown in table 3. The ethylacetate fraction was not sensitive against *Staphylococcus aureus* as shown in table 2. The chloroform fraction was sensitive only against *Aspergillus niger* and ethanol fraction was sensitive against *Escherichia coli* and *Candida albicans* only. The general results of the Minimum Inhibitory Concentration (MIC) of the extract and fractions to susceptible microorganisms are comparable to gentamicin against *Streptococcus pneumonia, Staphylococcus aureus, Escherichia coli* and ketoconazole against *Aspergillus niger* and *Candida albicans*

Table 1: Phytochemical analysis of *Terminalia mantaly* leaf

S/N	Secondary metabolites	Observations			
		Ethanol extract	Chloroform fraction	Ethylacetate fraction	Ethanol fraction
1	Saponins	+	-	+	+
2	Tannins	+	-	+	+
3	Flavonoids	+	+	+	+
4	Steroids	+	+	+	-
7	Alkaloids	+	-	+	+
8	Glycosides	+	-	+	+

Key: + = Present - = Absent

Table 2: Antimicrobial sensitivity studies using Agar well diffusion (borer diameter = 9mm)

Clinical isolates	Plant extract/fractions (200mg/ml)				Standards (100 µg/ml)	
	Ethanol extract	Chloroform Fraction	Ethylacetate Fraction	Ethanol Fraction	Gentamicin	Ketoconazole
Escherichia coli	+	-	+	+	+	-
Staphylococcus aureus	+	-	-	-	+	-
Streptococcus pneumoniae	+	-	+	-	+	-
Candida albicans	+	-	+	+	-	+
Aspergillus niger	+	+	+	-	-	+

Key: + = Sensitive; - = Not Sensitive

Table 3: Results of Minimum Inhibitory Concentration (MIC)

Clinical isolates/Micro-organisms	Ethanol extract	Chloroform fraction	Ethylacetate fraction	Ethanol fraction	Gentamicin	Ketoconazole
Escherichia coli	22.39±1.08	-	19.50±0.87	23.44±1.75	16.22±0.34	-
Staphylococcus aureus	42.66±1.14	-	-	-	20.42±0.71	-
Streptococcus pneumoniae	47.86±0.99	-	11.75±0.19	-	11.22±0.34	-
Candida albicans	31.62±2.22	-	9.77±0.36	33.88±1.95	-	11.75±0.27
Aspergillus niger	19.95±0.42	43.65±2.49	16.98±0.23	-	-	11.22±0.38

N=3; results expressed in Mean ± SEM Key: – = Not Sensitive

Conclusion

The effectiveness of the ethylacetate fraction is attributed to the accumulation of polyphenolic compounds such as tannins and flavonoids that are reported in several research publications to exhibit antimicrobial properties. Suffredin *et al*. (2006) stated that antimicrobial activities of medicinal plants with minimum inhibitory concentration of 200 mg/ml or below are considered significant hence, all reported antimicrobial activities in this research are significant and the folkloric uses of *Terminalia mantaly* as antimicrobial agent in some quarters have been validated.

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