Bacteriostatic Effect of *Terminalia catappa* Leaves Extract on Clinical Isolates of Gram Negative Bacteria

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ABSTRACT— ***To determine the antibacterial activity type, extracts of Terminalia catappa leaves were tested in vitro on Gram negative bacteria. Extract of Terminalia catappa leaves was obtained from ethanol using percolation method. The sensitivity of four clinical isolates of Escherichia coli, Morganella morganii, Proteus mirabilis and Yersinia enterocolitica was tested. The extract demonstrated strong in vitro antibacterial activity against these organisms at all the concentrations used (20µg/disc, 40µg/disc, 80µg/disc and 160µg/disc). Of these bacteria screened for antibacterial activity, M. Morganii appeared to be the most sensitive organism exhibiting growth inhibition zone diameter of 15mm (160µg/disc), 14mm (80µg/disc), 12mm (40µg/disc) and 9mm (20µg/disc). It was followed by P. mirabilis and Y. enterocolitica sharing the same zones of growth inhibition of 14mm (160µg/disc), 13mm (80µg/disc), 10mm (40µg/disc) and 8mm (20µg/disc) while the least sensitive organism was E. coli with 11mm (160µg/disc), 10mm (80µg/disc) and 9mm each for 40µg/disc and 20µg/disc. This suggested that the cell wall composition of the former above mentioned bacteria might be different of that of the latter.Minimum Inhibitory Concentration (MIC) of the extract was determined at 25µg/ml for the entire test organisms. Although the extract revealed a strong inhibitory activity against the test organisms, growth was observed when test-tubes which showed absence of growth at MIC were sub-cultured on solid media to determine Minimum Bactericidal Concentration (MBC) meaning that the extract was only bacteriostatic at these concentrations.***

Keywords— Terminalia catappa, Extract, Biological activity, Clinical isolates, Bacteria

1. INTRODUCTION

Terminalia catappa (tropical almond) is a medium size tropical tree whose branches form layers of canopy and 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 [1]. The fallen leaves are potential in the management of sickle cell disorders [2] and are also used as herb to treat liver diseases [3].

The dried leaves are used for fish pathogen treatment, as an alternative to antibiotics. The leaves have antioxidant as well as anticlastogenic properties [4]. The various extracts of leaves and bark of T. catappa have been reported to be anticancer, anti-HIV reverse transcripts [2] and hepato protective [5] as well as anti-inflammatory [6], hepatitis [7], antidiabetic [8] and aphrodisiac [9]

The moderate consumption of the seed kernel is useful in the treatment of men with sexual dysfunctions, primarily from premature ejaculation [9]. The ethanol extract of the leaves of T. catappa L. (Combretaceae) inhibits osmotically-induced hemolysis of human erythrocytes in a dose dependent manner [7]. Punicalagin and punicalin, from the leaves are used to treat dermatitis and hepatitis as both have strong antioxidative activity [6].

Although many different antibacterial agents are available in the field of medicine, many of these agents are increasingly being incapacitated by the microorganisms through the evolution of different mechanisms that amount to

resistance to these drugs [10]. Indeed, mutations take place in microorganism populations and trigger resistance to antibiotics (X, 190Y). Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also are often with adulterations and side effects [11]. There is therefore a continuous and urgent need to discover new antibacterial components with diverse chemical structures and novel mechanisms of actions because of the increase in the incidence of new and re-emerging infectious diseases [12] to replace those that have lost their efficacy. Research has, however, shown that many herbs possess varying degree of antimicrobial activities [13]. [14] had reported that more than 25% of the prescribed drugs contained at least one active ingredient of plant origin. About 80% of the world's population relies on traditional medicine for significant part of their primary health care needs [15]. Four promising Gram negative bacteria restively to resistance activity against antibiotics were provided to our laboratory by Gombe hospital Specialist. It seems that among them, some would be sensitive to extracts of some leaves. Extraction of bioactive compounds from medicinal plants allows the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity [16], [17], [18]. The present research aimed at determining the antibacterial activity of ethanol extract of Terminalia catappa leaves against four clinical Gram negative isolates of bacterial.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

Fresh leaves of mature *T. catappa* tree were collected at Gombe State University Campus, Gombe State, Nigeria and identified at the department of Biological Sciences of the same University. The leaves were gently cleaned and washed under running tap water to remove dirt after which they were air-dried for two weeks and then ground into powdered form using clean laboratory motor and pestle [19] and the research was carried out within a period of six months (January - July, 2013).

2.2 Extraction

20g of the powder (*T. catappa* leaves) was soaked in 200ml of 95% ethanol in a conical flask for two weeks with regular shaking at room temperature. This was then filtered and the solvent evaporated using Rotary Evaporator and kept at 4^{o}C before sensitivity testing [20]

2.3 Bacterial Isolates

The isolates used in this research were clinical bacterial isolates obtained from Gombe State Specialist Hospital. They were subjected to standard identification procedures described by [21] and confirmed to be the followings: *Escherichia coli*, *Morganella morganii*, *Proteus mirabilis* and *Yersinia enterocolitica.* These were subcultured on nutrient agar slant and preserved at 4^{o}C before use.

2.4 Inocula Preparation

The inoculum was prepared from the stock cultures maintained on nutrient agar slant at 37^{o}C for 24 hours and emulsified into sterile distilled water in a test-tube using sterile were loop and compared with 0.5 Mcfarland standard of Barium sulphate solution (1%v/v) [21]

2.5 Preparation of Sensitivity Disc

Disc of 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 disinfected by autoclaving at 121^{o}C for 15 minutes. The stock solution of the plant extract was used to make the required disc potencies. Disc potencies of 20, 40, 80 and 160µg/disc were prepared [22]

2.6 Antibacterial Susceptibility Test

Disc agar diffusion method of Kirby-Bauer described by [21] was employed for the antibacterial assay. Sterile swab sticks were used to swab the standardized inocula of the test organisms onto the surface of prepared Mueller-Hinton agar plates. Sterile forceps was used to place the different concentrations of the plant material incorporated into sensitivity disc with standard disc of Ciproflaxacin (10µg) at the centre serving as the positive control on the inoculated plates. The plates were inverted and than incubated at 37^{o}C for 24 hours after which the zones of growth inhibition were measured.

2.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration was determined by serial doubling dilution of the plant extract in sterile distilled water and achieved four different concentrations of 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml. 2ml of each concentration was pipette into test-tubes containing 2ml of nutrient broth. 0.1ml of standardized suspension of the test organisms was then introduced into these test-tubes. Two test-tubes labeled as positive and negative controls containing plant extract plus nutrient broth and nutrient broth plus test organism respectively were also set up along side. All the test-tubes were incubated at 37^{o}C for 24hours after which the MIC was determined. MBC was carried out by sub-culturing all test-tubes without an evidence of growth during MIC and incubated at 37^{o}C for 24 hours.

3. RESULTS

The physical property of *Terminalia catappa* leaves extract obtained from ethanol appeared dark-brown in colour, gummy relatively to its texture and weighed 20g and 2.9g before and after extraction respectively (Table 1).

The entire Gram negative organisms employed for this research were sensitive to the leaves extract of *T. catappa* at all concentrations used (Figure 1). *Morganella morganii* was the most sensitive organism followed by *Proteus mirabilis* and *Yersinia enterocolitica* with the same sizes of zones of growth inhibition produced at all concentrations. The least sensitive organism was *Escherichia coli* (Table 2).

Minimum Inhibitory Concentration (MIC) of the extract against these bacteria was determined at 25µg/ml while the Minimum Bactericidal Concentration (MBC) determined after the sub-culture of all tubes with no growth at MIC on solid media revealed microbial growth following an overnight incubation which probably showed that the extract was only bacteriostatic at the concentrations used (Table 3).

Table 1: A Physical Property of the Leaves Extract of *Terminalia catappa*

Property	Extract
Weight of the Plant Material	20g
Weight of the Extract Recovered	2.9g
Colour	Dark Brown
Texture	Gummy

Table 2: Antibacterial Activity of leaves Extract of *T. catappa* against the Gram Negative Bacteria Using Disc Diffusion Method

S/N	Bacteria	Concentrations and Zones size of inhibition (MM)			
		20µg/disc	40µg/disc	80µg/disc	160µg/disc
1	*Escherichia coli*	9	9	10	11
2	*Morganella morganii*	9	12	14	15
3	*Proteus mirabilis*	8	10	13	14
4	*Yersinia enterocolitica*	8	10	13	14

Table 3: Antibacterial Activity of the leaves Extract of *T. catappa* Leaves Using Broth Dilution

S/N	Bacteria	Concentrations (µg/ml)						
		MIC				MBC		
		100	50	25	12.5	100	50	25
1	*E. coli*	-	-	-	+	***	**	**
2	*M. morganii*	-	-	-	+	***	**	**
3	*P. mirabilis*	-	-	-	+	***	**	**
4	*Y. enterocolitica*	-	-	-	+	***	**	**

Key: **MIC=Minimum Inhibitory Concentration**
MBC=Minimum Bactericidal Concentration
****= Growth observed**
*****= MBC above 100g/ml**
- =Not turbid
+ = Turbid

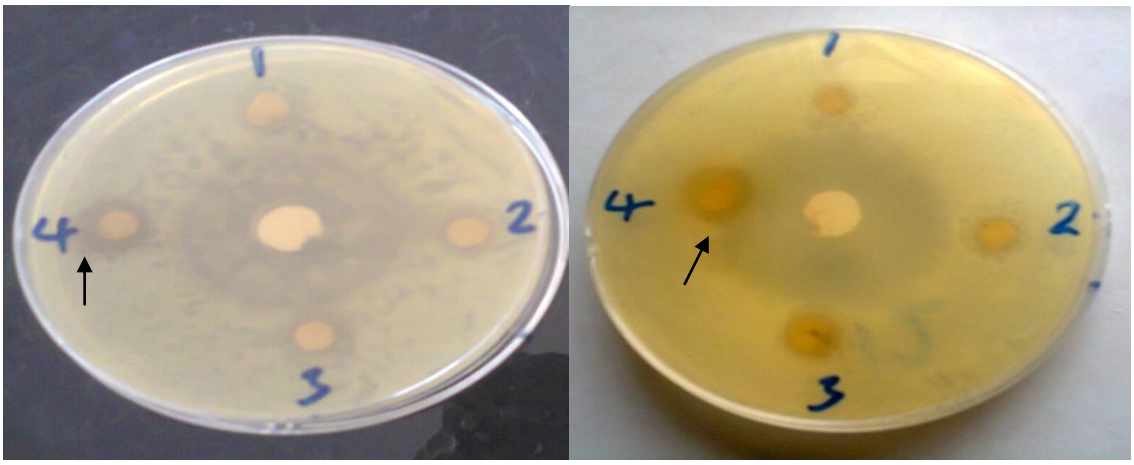

Figure 1: Result of Antibacterial Activity of *T. catappa* Leaves:
Plates showing the results of antibacterial activity of leaves extract of *Terminalia catappa* leaves (shown by arrows) against the test Gram negative bacterial Isolates (*Morganella morganii*, left and *Escherichia coli*, right) on Mueller-Hinton Agar plates using Agar disc diffusion method with Ciproflaxacin disc (10µg) at the centre which served as a positive control.

4. DISCUSSION

Four clinical isolates of Gram negative bacteria were screened for the antibacterial activity of ethanol extract of *Terminalia catappa* leaves using agar disc diffusion method. The Gram negative bacteria tested included, *Escherichia coli, Morganella morganii, proteus mirabilis and Yersinia enterocolitica.*

The dark-brown colour and gummy texture with weight of 2.9g are Physical characteristics of leaves extract (Table 1).

The ethanol extract of *T. catappa* leaves demonstrated a strong *in vitro* antibacterial activity against all the Gram negative bacteria tested (Figure 1). This activity could be as a result of some active components of *T. catappa* leaves believed to have antibacterial activity reported by several researchers among which are [1]'[16]. Of these Gram negative bacteria screened for the antibacterial activity of *T. catappa* leaves, *M. morganii* appeared to be the most sensitive organism followed by *P. mirabilis* and *Y. enterocolitica* shearing the same zones of Growth inhibition at all concentrations used where as the least sensitivity was observed with *E. coli* (Figure 1;Table 2). [23] Reported the antimicrobial activity of *T. catappa* leaf extract against both bacteria and fungal strains and the result showed that Gram positive bacteria were more susceptible than negative ones. In the same vein the activity was more pronounced against bacteria than fungal strains. Our research confound to the findings of [16] which documented the antibacterial activity of ethanol and hot water extracts of *T. catappa* leaves against the clinical isolates of *Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* It also agrees with the work of [1] which reported an antibacterial activity of *T. catappa* leaves extract using different solvents against the bacterial isolates of *E. coli* and *S. typhi.*

Minimum Inhibitory Concentration (MIC) of the extract was determined at 25µg/ml for all the test organisms. However, the test organisms were able to grow when all tubes which showed no evidence of growth at MIC were sub-cultured on solid media for Minimum Bactericidal Concentration (MBC Table 3). This indicates that the extract was only bacteriostatic at the concentrations.

5. CONCLUSION

Our work postulated the assumption according to which out of four Gram negative bacteria, some would be more sensitive than others. Effectively, Morganella morganii was found to be more sensitive. It can be concluded based on this research that the ethanol extract of *T. catappa* leaves demonstrated *in vitro* bacteriostatic.

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