Phytochemicals Screening and Antibacterial activity of Ethanolic leaf extracts of *Terminalia catappa* on some clinical bacterial isolates

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ABSTRACT
This study is aimed at evaluating the antimicrobial activity of ethanolic leaves extract of *Terminalia catappa* on some clinical bacterial isolates. The extracts were tested in vitro on Gram negative bacteria, it was obtained from ethanol using percolation method. The sensitivity of four clinical isolates of *Escherichia coli, Proteus mirabilis, Morganella morganiis, Yersina enterocolitic* was tested. The extract demonstrated strong in vitro antibacterial activity against these organisms at all concentration used (20µg/disc, 40µg/disc, 80µg/disc and 160µg/disc). Of these bacteria screened for antibacterial activity *Morganella morganiis* 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). While the least sensitive organism was observed on *Escherichia coli* 14mm (160µg/disc), 11mm (80µg/disc), 10mm (40µg/disc), 9mm (20µg/disc). Minimum Inhibitory concentration (MIC) of the extract was determined at 25ug/ml for the entire test organism. 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 Bacterial Concentration (MBC) meaning that the extract was only Bacteriostatic at these concentrations.
Keywords: *E. coli, Terminalia catappa, Morganella morganiis, Yersina enterocolitic,* MBC, MIC.

INTRODUCTION

Indian almond: *Terminalia Catappa* is a large tropical tree in the lead wood tree family. The leaves are large (15-25cm) long and (10-14cm) broad, ovoid, glossy, dark-green and leathery. They are dry season decidous, before falling they turn pinkish – reddish or yellow – brown due to pigments such as violaxanthin, lutein and zeaxanthin (Babayi *et al*, 2004). Medicinal plants possess therapeutic properties or exert beneficial pharmacological effects on the animal body (Bukar *et al*, 2009). Plants are the source of about 25% of prescribed drugs in the world (Bashir, Z.A. 2012). In developing countries about 80% people rely on traditional plant based medicines for their primary health care needs (Babayi *et al*, 2004).

There is abundant number of medicinal plants and only small amounts of them were investigated for its biological and pharmacological activities (Babayi *et al*, 2004). The wide range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug possessing a variety of pharmacological activities (Nair *et al*., 2005).

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Discovery of new pharmaceutical agents from medicinal plants can combat the drastic increase in infectious diseases in many countries especially in rural areas and it has been used as an economic reason as well (Bashir, 2012). Nowadays, there is widespread interest of drugs derived from plants which reflect its recognition of the validity of many traditional claims regarding the value of natural products in health care (Nair *et al*., 2005).

Therefore, in order to determine the potential use of medicinal plants, it is essential to intensify the study of medicinal plants that finds place in folklore of microorganisms (Bashir, 2012). In view of the high medicinal potential of *T. Catappa* observed based on report from previous findings, the study was designed to test the antibacterial activities of *T.catappa* leaves against some isolates of bacteria so that its further possible uses in medicine, therapeutics and food preservation could be determined (Bashir, 2012).

Screening of plants materials become necessary to provide scientific bases for validating the traditional utilization of medicinal plants. Several researchers have worked on medicinal plants with activity against different ailments (Bashir, 2012). This could be due to the fact that herbal remedies are individualized (Each person has certain predisposition to disease and is susceptible to environmental factors like genetics, dietary and life style.).

Traditional practitioners are in habit of mixing too many herbs to cure a single ailment which may be very dangerous because every plant has a specific inhibitory activity to a particular organism (sumitra *et al.*, 2011). Demonstration of antimicrobial activity of *T. catappa* provides the scientific basis for the use of this plant in the traditional treatment of diseases and may help to discover new chemical classes of antibiotic and substances that could serve as selective agents for infectious disease, chemotherapy and control (Sumitra *et al*., 2011).

MATERIALS AND METHODS

Study Area

This research was carried out in Microbiology laboratory of Gombe State University. The laboratory is directly opposite with faculty of Medicine and Surgery Library Gombe State University.

Collection and identification of the plant material

The leaves of *T. catappa* used in this study were collected at the nursery site of Gombe State University and were identified by Mallam Aminu Idris at the Herbarium of Department of Biological sciences.

Clinical bacterial isolates

The bacterial isolates used in this research were obtained from the microbiology unit of the laboratory section of Federal Teaching Hospital Gombe. These were grouped into gram positive and gram negatives using standard procedures of Gram staining described by Cheesebrough, (2006) as follows:

Gram staining technique

A wire loop was heated until it was red hot and allowed to cool. A loop full of the isolate was picked and smeared on a slide and air-dried, placed on a staining rack. Some drops of crystal violet stain were added on the smear for 30sec-60sec after which it was washed with clean water. Lugol's iodine was then added for 30secs and rinsed with clean water, it was decolorized with ethanol and rinsed with clean water. Safarannin stain was added for 2mins and raised with clean water. It was finally allowed to air-dry and examined under microscope using 100x objective lens with oil immersion (Cheesebrough, (2002).

Biochemical test; Further biochemical tests were carried out on the gram-negative organism using standard procedures as follows:

This process was repeated until a drop of the solvent from the siphon tube was colourless.

Citrate utilization test; Simmon's citrate agar was prepared according to manufacturer's instruction and poured into bijou bottles.The bijou bottles were slanted and left undisturbed to solidify.The subculture organism was streaked on the slope of the medium as well as stabbing the butt and incubated at 37^{o}c for 24hrs (Cheesbrough, 2006).

Urease test

Urease agar was prepared according to the manufacturers instruction and poured into bijou bottles, the bijou bottles were slanted and left undisturbed to solidify. The test organism was streaked both on the butt and slope of the medium in the bijou bottles and incubated for 24hrs at 37^{o}C (Cheesbrough, 2006).

KIA test

Kligler iron agar was prepared according to the manufacturers instruction and poured into test-tubes. The test-tubes were slanted a little bit and left undisturbed to solidify. The butt of the media was stabbed with the test organism and the slope was streaked and incubated at 37^{o}c for 24hrs (Cheesbrough, 2006).

Indole test

Peptone water was prepared according to the manufacturers instruction and poured into bijou bottles. The test organisms were inoculated in the pretone water and incubated at 37^{0}C for 24hrs. Following the incubation, about 0.5ml of Kovack's reagent was added to each bijou bottle and observed within 10 minutes (Cheesbrough, 2006)

Preparation of *T. catappa* leaves extracts

The leaves of *T. catappa* were collected and washed thoroughly with tap-water and air-dried for one week under shade. The leaves were crushed to fine powder, using clean laboratory motar and pestle and kept for further analysis (Dawoud *et al.,* 2013).

Extraction using Soxhlet method

25g of the plant material (powder) was placed on Whatman no. 1 filter paper size (6mm) and folded. It was then placed on the soxhlet apparatus chamber. The extracting solvent (ethanol) in the flask was heated and it vapours condensed in a condenser. The condensed extractant dripped into a thimble containing the plant material and extracted it by contact. When the level of the liquid in the chamber rises to the drop of siphon tube, the liquid contents in the chamber siphon into flask.

Rotary evaporator was used to obtain the crude extract which was kept at 40^{0} C for disc

preparation. (Enyi *et al*, 2012). And phytochemicals screening was done as described by (Dawoud *et al.,* 2013).

Bioassay

Disc preparation; Sensitivity disc of the extract was prepared by serial doubling dilution in dimythyl sulfoxide(DMSO) followed by placing sterile whatman no 1 filter paper discs of 6mm in diameter to obtain the disc potencies of 40,80,160,320yg/disc (Cheesebrough, 2006).

Inoculum Standardization

Few colonies of the bacterial isolates were emulsified in normal saline of about 2-3mls in test-tubes to match the 0.5 Mcfarland standard for sensitivity test as described by (Cheesbrough, 2006). The Mcfarland standard was prepared by mixing 0.6ml of 1 % (w/v) dihydrate barium chloride solution with 99.4ml of 1 %(v/v) sulphuric acid solution (Chessebrought, 2006)

Preliminary antimicrobial testing

The standard inocula of the bacterial isolates were swabbed on to the surface of Mueler-Hinton agar media in Petri dishes, followed by placing the prepared discs and the control disc of ciprofloxacin (CPX) 10mg. The plates were incubated at 37^{O}c for 24hrs before the zones of inhibition formed were observed and measured according to the procedure of Kirby- Bauer described by (Cheesbrough, 2006)

Determination of minimum inhibitory concentrations (MIC)

Different concentrations of *T. catappa* leaves extract were prepared by serial doubling dilution of 100yg/ml in distilled water to obtain 50µg/ml, 25µg/ml and12.5µg/ml. Standardized inocula of the test organism were introduced into test-tubes containing 2ml of nutrient broth and 2ml of plant extract and incubated at 37^{0}c for 24hrs.Test-tubes containing broth without plant extract was inoculated with the test organism and incubated at 37^{0}c for 24hrs to serve as negative control, and un-inoculated test tubes containing broth and plant extract was incubated to serve as positive control. The tubes were observed after incubation and the MIC was determined (Mukhtar and Okafor, 2000)

Minimum bactericidal concentration (MBC)

It was determined after selecting the tubes that showed no growth at MIC. A loopful was picked using a sterile wire loop from each of these tubes and sub-cultured onto the surface of nutrient agar contained in Petri dishes and incubated for 24hrs at 37oc.After incubation; they were observed for the presence of growth or otherwise of microbial (Babayi *et al*, 2004).

RESULTS

The result of the antibacterial activity of the ethanolic extract of *T. catappa* revealed that the leaves extract of *T.catappa* exhibited marked activity against *P. Mirabilis and M. Morganii* but showed limited activity against *E. Coli* and *Y. enterolitical*. The physical properties of *T.catappa* leaves extract, (**Table 1**) shows the weight of plant material used, weight of extract recovered and the extract color and texture. **Table 2:** Shows the Classification of the bacterial isolates based on Gram staining protocol. The biochemical reaction of the observed microorganisms is shown in (**Table 3**).

The sensitivity of clinical isolates to the leaves extract of *T. catappa* using disc diffusion method are shown in (**Table 4**). And **Table 5.** Shows Sensitivity of clinical isolate to the extract of T. catappa leaves using broth dilution method

Table 1: Physical properties of *T. catappa* leaves extract

Physical properties	Ethanolic extract of *T. catappa*
Weight of plant material	25g
Weight of extract recovered	2.9g
Plant extract colour	Dark brown
Plant extract texture	Soft

The table above showed the weight of the plant material used, the weight of the extract recovered, and the color and texture of the plant extract used.

Table 2: Classification of the bacterial isolates based on Gram staining protocol

Gram reaction	No. Collected	Positive No.
Gram +ve	50	10
Gram –ve	50	40
	Total	**50**

The table above shows the total number of the isolates collected from the specialist hospital Gombe.

Table 3: Biochemical reactions of the observed microorganism

S/N		Urea	Cit	Ind	KIA			
					Slope	Butt	H2S	gas
1	*Escherichia coli*	-	-	+	Y	Y	-	+
2	*Proteus mirabilis*	+	+	-	R	Y	+	+
3	*Morganella morganiis*	+	-	+	R	Y	_	D
4	*Yersina enterocolitica*	+	-	D	R	Y	-	-

Key: Urea: Urease test , **Cit:** citrate test, **Ind**: Indole test, **H_2S:**hydrogen sulphide, **R:** Red Pink (alkaline reaction,**Y:** YellowA(cid reaction) , **d:** different strains give different result. The table above shows the biochemical reactions of the observed Gram-ve bacterial isolates used in this research.

Table 4: Sensitivity of clinical isolates to the leaves extract of T. catappa using disc diffusion method (mm)

	Isolates	20µg/disc	40µg/disc	80µg/disc	160µg/disc	STD
1	*E. coli*	9	10	11	14	28
2	*P. mirabilis*	8	10	13	16	29
3	*M. morganii*	9	12	14	15	27
4	*Y. enterocolitica*	8	10	13	14	28

STD: standard antibiotics (ciproxicilin 10 µg).

The table above shows the zones of inhibitions measured in millimeters of the standard disc (ciproxicilin) and that of prepared disc (susceptibility test disc) against the tested Gram-ve bacteria.

Table 5: Sensitivity of clinical isolate to the extract of T. catappa leaves using broth dilution method

Isolates	Ethanolic extract						
	MIC				MBC		
	100	50	25	12.5	100	50	25
E. coli	-ve	-ve	-ve	+	***	**	**
P. mirabilis	-ve	-ve	-ve	+	***	**	**
M. morganii	-ve	-ve	-ve	+	***	**	**
Y. enterocolitica	-ve	-ve	-ve	+	***	**	**

Key: **MIC**:Minimum inhibitory concentration , **MBC**:Minimum bactericidal concentration

+ve:Turbid, **-ve:**Not turbid, ****:**Growth observed , *******:MBC above 100µg/ml

The table above shows the result of MIC and MBC with different concentration of hundred 100, 50, 25 and 12.5 in MIC. And concentration of 100, 50 and 25 in MBC respectively.

DISCUSSION

The ethanolic extract of *Terminalia catappa* leaves were screened for its antibacterial activity against *Escherichia coli, Morganella morganii, proteus mirabilis and Yersinia enterocolitica* using agar disc diffusion and broth dilution methods. The dark-brown colour and gummy texture of the extract weighted 2.9gwas recovered after extraction, the ethanol extract of *T. catappa* leaves demonstrated a strong *in vitro* antibacterial activity against all the Gram negative bacteria tested. 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 (Adwan and Mhanna, 2008) and (Dawoud *et al.,* 2013). 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* sharing the same zones of Growth inhibition at all concentrations used whereas the least sensitivity was observed with *E. coli* (Dawoud *et al.,* 2013) who reported the antimicrobial activity of *T. catappa* leaf extract against both bacterial and fungal strains and the result showed that Gram positive bacteria were more susceptible than the negative ones. In the same vein the activity was more pronounced against bacteria than fungal strains. Our research confounds to the findings of (Mansir *et al.,* 2015) 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 (Lawal *et al.,* 2014) which reported the 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 which is in accordance of the work done by (Dawoud *et al.,* 2013) who obtained same results. This indicates that the extract was only bacteriostatic at the concentrations.

CONCLUSION

This 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 ethanolic extract of *T. catappa* leaves demonstrated *in vitro* bacteriostatic.

Recommendations

The antimicrobial activity of the leaves extract of *T. catappa* should be carried out against some resistant bacteria.

The antimicrobial activity of *T. catappa* should be conducted on other group of microorganisms apart from bacteria.

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