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ANTIMICROBIAL ACTIVITIES OF *Terminalia catappa Linn.* LEAVES EXTRACT ON SOME SELECTED *Salmonella* SPECIES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors LYA and DON designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LYA, JOI and DON managed the analyses of the study. Authors LYA and JOI managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

The aim of this study is to investigate the antimicrobial activity of *Terminalia catappa* leaves extract against *Salmonella* species. Leaves samples were collected randomly from *T. catappa* trees, air dried, pulverized and extracted with distilled water. The extracts were portioned to form three treatments which were boiling, soaking and methanol. The activities of the plant extracts on the microorganism were determined by 96 Well Microtitre Plate method. Ciprofloxacin was used as positive control. The Minimum Inhibitory Concentration (MIC) of *T. catappa* against selected *Salmonella* species ranged between 1.321 mg/ml to 3.124mg/ml. The Minimum Bactericidal Concentration (MBC) of *T. catappa* against selected *Salmonella* species ranged between 100mg/ml to 4000mg/ml. The results obtained from the antibacterial analysis of the treatments of *T. catappa* were statistically analyzed by using one-way Analysis of variance (ANOVA) which revealed that there was no significant difference in the effect of the *T. catappa* (F cal=67.02 at 0.01). The qualitative screening revealed that Alkaloids, Tannins, Steroids, Saponins and Reducing sugar were present in the extract of *T. catappa*. The study revealed that *T. catappa* extracts contains bioactive constituents with high antimicrobial activity against *Salmonella* species and could potentially possess rich medicinal constituents when subjected to further chemical and pharmacological studies.

Keywords: *Terminalia catappa*; *Salmonella typhi*; phytochemicals; extracts.

1. INTRODUCTION

Globally, about 21 million cases and 222,000 deaths per year are caused by *Salmonella typhi* [1]. Humans acquire the disease from other humans through fecal–oral transmission, and also through contaminated water or food. In Nigeria, typhoid fever remains a major burden due to factors such as increased urbanization, inadequate supplies of potable water, regional movement of large numbers of immigrant workers, inadequate facilities for processing human waste, overburdened health-care delivery systems, and overuse use of antibiotics [2].

Microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs [3]. Typhoid *Salmonella* species have increasingly become resistant to conventional antibiotics of choice for the treatment of enteric fever in developing countries [4].

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Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to reports of the World Health Organization, 80% of the world's population relies mainly on traditional therapies, which involve the use of plant extracts or their active substances [1]. The usage of medicinal plants has been reported to have reduced side effects in comparison to some antibiotics [5,6].

Terminalia catappa is a large tropical tree in the leadwood tree family, Combretaceae [7] that predominantly grows in the tropical regions of Asia, Africa, and Australia [8]. It is cultivated in Nigeria solely as a shade tree and for its fruits and seeds which are eaten as fruit as well as for medicinal uses [9].

The leaves are large, 15–25 cm (5.9–9.8 in) long and 10–14 cm (3.9–5.5 in) broad, ovoid, glossy dark green, and leathery. They are dry-season deciduous; before falling, they turn pinkish-reddish or yellow-brown, due to pigments such as violaxanthin, lutein, and zeaxanthin. The falling of these leaves is usually attributed to environmental factors such as humidity, temperature, water availability etc [10].

The leaves of *T. catappa* have been reported to contain several flavonoids, tannins, saponins and phytosterols. Due to this chemical richness, all parts of the tree seemed to be useful ethnomedicinally. The leaves, nuts and the bark are used in different herbal medicines for various purposes. Several pharmacological studies have reported that *T. catappa* leaves and fruits have anti-cancer, anti-inflammatory, wound healing effects, anti-diabetic effects hepato-protective activities [11-15]. It has also been reported to possess antimicrobial activities [16-19].

In several areas of Karu LGA, the locals have been observed to boil and consume *T. catappa* leaves for the treatment of typhoid fever. Therefore, this study was designed to determine the efficacy of *T. catappa* leaves on some selected *Salmonella* species.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Department of Biological Sciences, Bingham University. The university is located in Auta Balefi town, Nasarawa state and has a tropical climate with two seasons; raining and dry harmattan season. The University covers a land mass of 200 square meters and its geographically located at latitude 8° 50°N and longitude 7° 52° E. it found 26km away from Abuja (FCT) the Federal Capital Tertiary of Nigeria [20].

2.2 Sample Collection and Processing

Red dried leaves of *T. catappa* were collected and temporarily kept in a sterile polythene bag. These leaves were obtained from Bingham University and its environs. The plant was authenticated by a plant expert in the Department of Biological Sciences, Bingham University Karu, Nasarawa state, Nigeria. The dried leaves of *T. catappa* was further air dried at room temperature for three days, crushed with a mortar and pestle and further blended into fine powder using the USHA mixer grinder MG2053N and stored in a sterile glass container until it was required for analysis.

2.3 Source of Bacterial Isolates

The bacterial isolates of *Salmonella typhi, Salmonella typhimurum* and *Salmonella gallinarum* were obtained from the bacteriology division of the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The pure culture of each isolate was obtained by sub-culturing the isolate onto nutrient agar and biochemical tests were performed to confirm the *Salmonella* species.

2.4 Preparation of Extracts

2.4.1 Boiling (Hot water extract)

Two hundred grams of the crushed leaves was measured and placed in a container containing 1500 ml of distilled water. The mixture was boiled for 60 minutes with constant agitation, allowed to cool and filtered using laboratory test sieve.

2.4.2 Soaking (Cold water extracts)

Cold water extracts were prepared by soaking 200 g of the crushed leaves into 1500 ml of distilled water. The mixture was kept for 48hours at 4^0C and filtered.

2.4.3 Methanol extraction

Two hundred grams (200 g) of the crushed leaves was measured and extracted by cold maceration in 1500 ml of methanol and shaken intermittently for 72 hours followed by sieving with a muslin cloth and filtered with a Whatman no.1 filter paper. The filtrate was concentrated on the rotary evaporator and lyophilized to get the dry solid residue.

2.4.4 Preparation of diluents, mcfarland turbidity standard and control

Eight grams of each of the extract was dissolved in 8 ml of sterile Dimethylsulfoxide (DMSO) and double

dilution was carried out to obtain various concentration of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. McFarland turbidity standard was prepared according to standard chemical procedures. The antibiotic used was 200 mg of ciprofloxacin. The drug was dissolved in 10 ml of distilled water.

2.5 Qualitative and Quantitative Phytochemical Screening

2.5.1 Determination of alkaloids

One gram of the plant extract was macerated with 20 mls of ethanol and 20% H_2SO_4 at the ratio of 1:1 was centrifuged for 5 minutes. Then, 1ml of the supernatant was transferred into triplicate tubes. In the tubes, 5 mls of 60% H_2SO_4 was added and stirred. After 5 minutes, 5 ml of 0.5% formaldehyde was added into 60% H_2SO_4 and mixed. The mixture was allowed to stand for 3 hours. The absorbance was read at 565 nm [21].

2.5.2 Determination of flavonoid

The extract (1 g) was weighed and macerated with 20mls of ethylacetate for 5 minutes and filtered. To the filtrate (5 mls), 5 mls of dilute ammonia was added and stirred for 5 minutes. The upper layer was collected and the absorbance was read at 565 nm [21].

2.5.3 Determination of tannin

The extract (1g) was macerated with 50mls of methanol for 10 minutes and centrifuged for 5 minutes. To the filtrate (5 mls), 0.3 ml of 0.1M ferric chloride in 0.1M HCl was added and stirred. Then 0.3mls of 0.0008M of potassium ferricyanide was added and allowed to stand for 5 minutes. The absorbance was read at 720 nm [21].

2.5.4 Determination of saponins

The extract (1g) was macerated with 10 mls of petroleum ether and decanted into a beaker. Another 10mls of the petroleum ether was added into the beaker and the filtrate evaporated into dryness. The residue was dissolved in 6 mls of ethanol. The solution (2 mls) was put in a test tube and 2 mls of chromagen solution added into it. It was allowed to stand for 30 minutes and the absorbance was read at 550 nm [21].

2.5.5 Determination of steroids

One gram of the plant extract was weighed and macerated with 20 mls of ethanol and filtered. To the filtrate (2 mls), 2mls of chromagen solution was added and the solution was left to stand for 30 minutes. The absorbance was read at 550 nm [22].

2.6 Determination of Minimal Inhibitory Concentration (MIC) Using 96 Well Microtitre Plate Method

The Minimum Inhibitory Concentration (MIC) for plant extract was evaluated according to method described by Satyajit et al. [23] employing 96-well micro plates. The plates were prepared in triplicates and for each plate 100μl of Mueller Hinton Broth (MHB) was placed to each well followed by 100 μl of plant extract (which contains 400 mg/ml of plant extract) added to the first column of the microplates. This made each well of the first column have a total volume of 200 μl. Starting from the first column serial dilution was conducted up to 10^{th} column with double folding; the final volume (3.125 μl) of the plant extract and the broth were drawn from the 10^{th} column. The 50 μl of MHB was mixed with 100 μl of bacterial suspension from which 50 μl was filled into the wells up to 10^{th} column aseptically. Subsequently, the plates were incubated for 24hrs at 37^0C incubator. After incubation, a 30 μl (2 mg/ml) of 0.02% resazurin dye was added and incubated at37^0C for one hour. Resazurin dye was used as an indicator for bacteria growth; bacteria metabolize it and changed into pink color McNicholl et al. [24]. The color change was then assessed visually. Any color change from purple to pink or colorless were recorded as positive.

The lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC value for the test material and *Salmonella* species.

2.7 Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration was recorded as the lowest concentration of the extract that prevented the appearance of visible growth of the organism after one hour of incubation.

This was determined by aseptically subculturing the contents of the wells with the lowest inhibition from the MIC results on Mueller Hinton agar (MHA) plates. The plates were incubated for twenty-four (24) hours, after which it was observed for growth [25].

2.8 Data Analysis

The plates were prepared in triplicates hence, the Analysis of Variance (ANOVA) was used to analyze the data obtained.

3. RESULTS

Table 1 shows results of the phytochemical analysis of *T. catappa* extract. The phytochemicals alkaloids, tannins, steroids, saponins and reducing sugar were present while resins were not detected in the plant. In term of degree of occurrences of phytochemicals tannins was the most abundant phytochemical present in the *T. catappa* and saponins was moderately presence while Alkaloids, Steroids and reducing sugar were present in normal amounts.

Table 2 shows the quantitative analysis for *T. catappa* plant extracts. It revealed the amount of the various phytochemicals present. The highest absorbance (1.337) was observed in tannins while the least absorbance (0.139) was observed in saponins. The higher absorbance is an indication of the quantity of the phytochemical present in the plant extract.

Table 3 shows the results of the Minimum inhibitory concentration (MIC) of *T. catappa* against *S. typhi*, *S. typhimurium and S. gallinarum* from aqueous and methanol extract. The aqueous extract was the most effective with a minimum inhibitory concentration of 3.125 mg/ml for *S. typhi*. For *S. typhimurium* it was observed that both the aqueous and methanol extract was very effective with the minimum inhibitory concentration of 3.125mg/ml, while the aqueous extract had a minimum inhibitory concentration of 200 mg/ml. The aqueous and methanol extract were all effective on *S. gallinarum* with the minimum inhibitory concentration of 3.125 mg/ml.

Table 4 shows the Minimum Bactericidal Concentration of the three plant extracts for *S. typhi S. gallinarum* and *S. typhimurium* with the values of 400 mg/ml for all the extract except for *S. typhimurium* which was taken at 100 ul for aqueous extracts and 400ul for aqueous and methanolic extracts. When plated on Mueller Hinton Agar (MHA), growth was observed. They were no statistically significant relationship (P>0.05) between the various plants extracts on the *Salmonella* species.

Table 1. Qualitative analysis of *T. catappa* extract

Phytochemical constituents	Degree of occurrence
Alkaloids	+
Tannins	+++
Steroids	+
Resins	-
Saponins	++
Reducing sugar	+

Keys: +++ = Strongly present; ++ = moderately present; + =Present; -= absent

4. DISCUSSION

This study was conducted to examine the antimicrobial effect of *T. catappa* leaves against some *Salmonella* species. The qualitative and quantitative analysis of the phytochemical constituents of the extract of *T. catappa* revealed that tannin and saponins occurred in higher amounts than the other phytochemicals. Tannin as an antimicrobial agent in large amounts in *T. catappa* as characteristic of this

Table 2. Quantitative phytochemical analysis of *T. catappa* extract

	Wavelength	Transmittance	Absorbance
Saponins	550	72.583	0.139
Alkaloids	565	10.416	0.982
Tannins	720	4.603	1.337

Table 3. Minimum Inhibitory Concentration (MIC) of *T. catappa* against selected *Salmonella* species

Test organisms	Plant extracts	MIC [mg/ml]
Salmonella typhi	Cold extract of *Terminalia catappa*	1.321
	Hot extract of *Terminalia catappa*	3.125
	Methanol extract of *Terminalia catappa*	3.125
	Ciprofloxacin	3.125
Salmonella typhimurium	Cold extract of *Terminalia catappa*	1.321
	Hot extract of *Terminalia catappa*	3.125
	Methanol extract of *Terminalia catappa*	3.125
	Ciprofloxacin	3.125
Salmonella gallinarum	Cold extract of *Terminalia catappa*	1.321
	Hot extract of *Terminalia catappa*	3.125
	Methanol extract of *Terminalia catappa*	3.125
	Ciprofloxacin	3.125

Table 4. Minimum Bactericidal Concentration (MBC) of *T. catappa* against selected *Salmonella* species

Test organisms	Plant extracts	MIC [mg/ml]
Salmonella typhi	Cold extract of *Terminalia catappa*	400
	Hot extract of *Terminalia catappa*	400
	Methanol extract of *Terminalia catappa*	400
	Ciprofloxacin	400
Salmonella typhimurium	Cold extract of *Terminalia catappa*	100
	Hot extract of *Terminalia catappa*	400
	Methanol extract of *Terminalia catappa*	400
	Ciprofloxacin	400
Salmonella gallinarum	Cold extract of *Terminalia catappa*	400
	Hot extract of *Terminalia catappa*	400
	Methanol extract of *Terminalia catappa*	400
	Ciprofloxacin	400

study prevented the activity of various microbes in agreement with the findings of Adeshina et al. [26] and Ahmadu et al. [27]. Saponins are also known to have penetrative ability into bacterial cell walls resulting in their degradation and subsequent death. The reported phytochemicals are secondary metabolites which have been reported in the past to be the causative factor behind the antimicrobial effects of several plant materials used in microbiological research [26,28]. These phytochemicals according to Deris et al. [29] are the chemical compounds synthesized by plants to ensure therapeutic effects. This is a possible reason for the antimicrobial effect of *T. catappa* leaves considered in this study. Similarly, it was reported by Song et al. [30] that the antimicrobial activity of *T. catappa* could be either through the inhibition of cell wall, nucleic acid and enzymatic synthesis which may help in protection against chronic diseases such as *S. typhi* in agreement with this study.

Different extraction methods were considered in this study for *T. catappa* leaves. However, there was no significant differences observed in the reactions of the bacterial populations examined with respect to method of extraction used for both minimum inhibitory concentrations and minimum bactericidal concentrations. Nevertheless, methanol extract was the most potent for *S. typhimurium* and *S. gallinarum* while the hot water extract was most potent for *S. typhi.* The effectiveness of methanol can be attributed to its polarity and ability to recover polyphenols or bioactive compounds in plants [31]. Similarly, the effectiveness of hot water extracts may be attributed to the solvent used which is water. Water is the most polar solvent and is used in the extraction of a wide range of polar compounds [32,33]. It could also be due to the application of heat which increase the solubility and diffusion of the plant material in the solvent thereby resulting in the release of secondary metabolites.

The minimum inhibitory concentration (MIC) values of the *T. catappa* leaves in this study showed the plant is a potent antimicrobial agent against some *Salmonella* species because it inhibited the growth of *S. typhi*, *S. typhimurium* and *S gallinarum.* A minimum inhibitory concentration of 3.125 mg/ml for all extraction method of *T. catappa* as observed in this study is lower than that of Adeshina et al. [26]. The MIC of 3.125 mg/ml as observed in this study is also lower than the value reported by [11] who reported a minimum inhibitory concentration of *T. catappa* ranging from 6.7-13.5mg/ml for pathogenic bacteria. The difference in the minimum inhibitory concentrations between this study and previous studies may be due to variation in the extraction methods used.

The Minimum bactericidal concentration (MBC), values ranged from 100-400 mg/ml. This value is higher than the report of [10] with values ranging from 31.3-250.0mg/ml. It is also higher than an MBC ranging from 10.5-30.5 mg/ml for pathogenic resistant and sensitive strains tested by Saleh et al. [34]. Differences between the MBC values of this study and those of previous studies may be attributed to the bacterial variations used in separate studies.

The drug ciprofloxacin was used as a control and it was observed to be effective against the *Salmonella* species. Ciprofloxacin was chosen as a control drug due to its broad-spectrum activities against gram negative and gram-positive bacteria.

5. CONCLUSION

The results of this study have shown that extracts of *T. catappa* leaves possess active components that are effective against *Salmonella* species. This work has corroborated the use of the plants in traditional medicine for the treatment of some *Salmonella* infection. However, further studies should be carried

out to determine the toxicity and lethal dosage of the plant for effective administration which can reduce unwarranted side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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