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Antibacterial potency and phytochemical screening of the bark of *Terminalia catappa* against some clinical isolates

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Abstract

Context and purpose of the study: To evaluate the antibacterial properties of ethyl acetate and aqueous extracts of the bark of *Terminalia catappa* against some clinical isolates.

Main findings: The antibacterial activity of the *T. catappa* bark extracts was evaluated against five bacterial clinical isolates which are *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using agar-well diffusion method. The minimum inhibitory concentration was done by the broth dilution method. Broad spectrum antibiotics were used as positive control for the antibiotic sensitivity test. Qualitative and quantitative phytochemical screening of *T. catappa* bark were carried out using standard methods. However, ethyl-acetate and aqueous extracts of *Terminalia catappa* bark both showed that *K. pneumoniae* was more sensitive compared with *E. coli, S. typhi, S. aureus* and *P. aeruginosa.* Antibacterial activity of the ethyl acetate and aqueous extracts showed the value range from 10.33 ± 0.58 mg/ml to 9.33 ± 0.60 mg/ml. Ethyl-acetate extract showed minimum inhibitory concentration (MIC) at 80 mg/ml while the aqueous extract shows MIC at the concentration of 100 mg/ml. However the minimum bactericidal concentration (MBC) of ethyl-acetate extract against the clinical isolates was at the concentration of 100 mg/ml while there was no minimum bactericidal concentration (MBC) for the aqueous extract. Phytochemical screening shows the presence of saponins, glycosides, and alkaloids, in larger quantity while flavonoids, tannins, and steroids in smaller quantity.

Brief summary and potential implications: This study indicates that the extracts were efficacious and can be used for the management of diseases caused by the tested organisms. Results obtained support the use of this plant as use in traditional medicine and support that the plant extracts possess compounds with good antimicrobial properties that can be used as antimicrobial agents in the search for new antimicrobial drugs.

Keywords: Ethyl-acetate extract, aqueous extract, *Terminalia catappa*, phytochemicals

Introduction

Common sources of obtaining antimicrobial agents are medicinal plants [1]. Plants are used medicinally in the world as sources of many potent drugs used for various infections and diseases [2]. Phytomedicine is now the order of the day in many parts of the developed and developing countries. Traditional medical practitioners in Nigeria use a variety of herbal concotions to treat different kinds of diseases including microbial infections.

The need for new effective antimicrobial agents has led researchers to screening for bioactive compounds in *Terminalia* species and utilize it for medicinal purposes.. The choice and selection of this plant is based on its uses in traditional medicine both in Africa and in Asia for the treatment of microbial infections. Reports have shown that quite a number of species from Combretaceae family are known to contain antimicrobial constituents. *Terminalia* species growing in Nigeria, West Afica have exhibited substantial antifungal activity [3].

In recent times, there is a provocative flare and interest in traditional medicine. The importance of phytomedicine in solving the health care problems of the world is gaining interesting attention. Due to this emergence of interest, the research on plants of medicinal importance is developing internationally. In fact, most of the developing countries have adopted traditional medical practice as an important part of their customs. There is an increasing demand for more medicines from plant materials such as the bark, leaves, roots and fruits since it is believed that “green medicine” is safer and more reliable than the expensive synthetic drugs, many of which have serious detrimental side effects [4].

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History reveals that majority of medicinal preparations are obtained from different plants, whether in the simple or processed forms of the plant materials and also crude extracts and mixtures respectively [5]. During the development of modern medicine in early times, biologically active compounds from higher plants have played a vital role in providing medicines to combat pain and diseases.

Phytochemicals are chemical compound that occur naturally in plants. They are biologically active but non-nutrient substances; they can be derived from different parts of plants. These chemical compounds are responsible for the organoleptic properties and color of some fruits, such as the deep purple of blueberries and the smell of garlic as reported by [6]. The qualitative and quantitative screenings of the phytochemicals are necessary for drug discovery. *Terminalia catappa* is a large tree commonly found in Africa, Asia and Australia. It is known by the common names such as Bengal almond, country almond, Indian almond, Malabar almond, sea almond and tropical almond as reported by [7]. The seeds are quite buoyant and edible. They are consumed by humans and bats. *T. catappa* is usually grown as an ornamental tree and because of its large green leaves, it provides deep shade. The fruit is edible, fleshy and tastes slightly acidic. The bark is solid and it is been used in different herbal medicines for various purposes. Studies on the medicinal properties of the roots, bark and leaves have been reported. In traditional medicine, various parts of *T. catappa* plant such as the fruits, roots and leaves are used in the treatment of diverse infections such as headaches, dysentery, eye problems, wounds, rheumatism, cough, liver problems and asthma. The fruits have also been exploited for anti-diabetic activity [8]. This work is aimed at utilizing the ethyl-acetate and aqueous extracts of the bark of *T. catappa* to investigate its antimicrobial activity as well as determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against some clinical bacterial isolates such as *Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* Screening for the qualitative and quantitative biologically active compounds in the bark of *T. catappa* such as tannins, alkaloids, steroids, saponins, glycosides and flavonoids were also carried out.

Materials and methods

Collection and identification of plant materials

The bark of *Terminalia catappa* tree was collected from the compound of United Missionary Church of Africa, (UMCA) Offa garage in Ilorin, Kwara State Nigeria. The plant material was taxonomically identified and authenticated with a voucher number of UIH 001/ 975 by the Botany Department, University of Ilorin, Ilorin.

Collection, identification and maintenance of test organisms

Pure isolates of the clinical organisms; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* were obtained at Sobi Specialist Hospital, Alagbado, Ilorin, Kwara State. The isolates were maintained at a temperature of about 4 ^{0}C throughout the period of study. At the commencement of the research, the isolates were sub-cultured into separate McCartney bottles containing nutrient agar slants which were incubated at 37 ^{0}C for 24 hours, to ensure viable growth.

Sterilization of materials

Sterilization of all materials used were followed according to the methods prescribed by [9].

Extract preparation using ethyl acetate and aqueous solution

The bark of T. catappa was rinsed under running tap and later with distilled water, it was air dried at room temperature for two weeks and then grinded into powder with a mechanical grinder to obtain coarse powder. Forty grams of the powder was weighed and dispensed into two conical flasks each, 500 ml of ethyl-acetate and distilled water which was used for extraction of the active ingredients and placed on a shaker for 48 hours. The extracts were then filtered and passed through four layers of muslin cloth. The solvent was subjected to evaporation using hot water bath at 60 0C. Crude extracts obtained from ethyl-acetate and aqueous solvents were placed in the refrigerator at 4 0C prior to use.

Preparation of Extract Concentrations

The Ethyl-acetate and Aqueous extract of the bark of T. catappa concentration was carried out by weighing 1 g of each of the extract into 10 ml of ethyl-acetate and distilled water which was used as the stock culture. Different concentrations used included 100 mg/ml, 80 mg/ml, 60 mg/ml, 40 mg/ml and 20 mg/ml.

Sterility test of plant extracts

The ethyl acetate and aqueous extract were tested for growth or contaminants. This was carried out by inoculating 1ml of each extract on Mueller Hinton Agar and incubated at 37 0C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicated that they were sterile. The different extracts were then accessed for antibacterial activity.



Determination of antimicrobial activity

The aqueous and ethyl acetate extracts of the bark of *T. catappa* were screened for antimicrobial activity by agar well diffusion method. Agar surface was cut with the help of sterile cork borer having a diameter of 5.0 mm size. All bacterial strains were grown in nutrient agar broth (NB) for 18-24 hours at 37 ^{0}C, the turbidity of the broth was adjusted to 0.5 McFarland solution. This gives a suspension containing approximately 1.2 x 10^6 colony forming units (CFU)/ml [10].

Antibacterial sensitivity assay

Agar well diffusion technique as described by [11] was used to determine antimicrobial sensitivity of the extracts. The overnight incubated test organisms were respectively diluted to 0.5 % McFarland solution. Prepared Mueller-Hinton agar, according to manufacturer's instructions was poured into Petri-dishes, 1 ml of the standardized inoculums of each bacterial isolates was dispensed into plates. The seeded plates were allowed to set after a uniform distribution of the inoculums following swirling of the Petri dish. A standard sterile 5 mm cork borer was used to bore five wells on the surface of the agar plates. Five wells on each plate were filled with each of the two crude extracts (1 ml) of various concentrations of 100 mg/ml, 80 mg/ml, 60 mg/ml, 40 mg/ml, and 20 mg/ml with the aid of a sterile dropper pipette. Sterile distilled water and ethyl-acetate was used as controls. All plates were allowed to stay for an hour to ensure proper diffusion of the extract into the agar. The Petri-dishes were then incubated upside down, at 35 ^{0}C for 18- 24 hours [12].

Antibiotics sensitivity test

Standard antibiotics disks were used to perform the antibiotic susceptibility test and the tests were carried out according to [13] standards. Each of the isolates were also tested for antibiotic activity with some antibiotics such as Gentamycin, (10µg) Ciprofloxacin, (10µg) Augmentin (30µg) Streptomycin, (30µg) and Erythromycin (10µg). The antibiotic disks were placed aseptically on seeded plates of the isolates with aid of sterile forceps. The plates were incubated upside down at 37 ^{0}C for 16-18 hours (CLSI., 2012).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the extract was done according to the methods of (CLSI., 2012).

Determination of minimum bactericidal concentration (MBC)

The (MBC) of the extracts was determined using the method of (CLSI., 2012).

Qualitative phytochemical screening

Ethyl acetate extract and aqueous extract of T. catappa bark were subjected to some phytochemical analysis using standard techniques for the detection of steroids, glycosides, saponins, tannins, flavonoids and alkaloid. This was determined using chemical methods and by adopting standard protocols to identify the constituents as described by [13,14]. Steroids were determined by the criteria of [15]; flavonoids by [16]; saponins, tannins and alkaloids by the methods of [17,18] while glycosides were determined by the method of [19].

Quantitative phytochemical screening

Quantitative analysis of the extract for total tannins, flavanoids, saponins, alkaloid, glycosides and were carried out using standard procedures suggested by [20,24] respectively.

Statistical analysis

The result obtained where statistically analyzed by ANOVA. The means and standard deviations were computed by one-way ANOVA, using IBM SPSS 20.0 computer software package. The level of significance was determined at $p<0.05$.

Results

Sterility test

The ethyl-acetate and aqueous crude extracts of *T. catappa* bark shows no growth of contaminants

Antibacterial sensitivity test

All the organisms showed high level of sensitivity at 100mg/ ml, and most of the organisms showed some level of resistance at 80mg/ ml, 60mg/ ml and 40mg/ ml of the ethyl-acetate and aqueous extract. However, in this study, *Klebsiella pneumoniae* showed the highest inhibition zone to the ethyl-acetate extract of *T. catappa* bark with (10.33 mm) and aqueous extract with (9.33 mm). This was followed by *E. coli* with (9.67 mm) for the ethyl-acetate extract and (7.70 mm) for the aqueous extract; *P. aeruginosa* (9.33 mm) and *S. typhi* with (8.67 mm). *S. aureus* showed the minimum zone of inhibition to the ethyl-acetate crude extracts with (8.33 mm) zone

of inhibition. The ethyl-acetate extract however shows the highest zone of inhibition on the microbial isolates. The extracting solvent, sterile water and ethyl-acetate where used as controls and showed no zone of inhibition of the isolates. Table 2 and 3 shows the zones of inhibition of the aqueous and ethyl-acetate extracts of *T. catappa* on the bacterial isolates

Antibiotic Sensitivity Testing

Antibiotics sensitivity testing shows that among all the antibiotics, ciprofloxacin shows the highest zone of inhibition against Staphylococcus aureus (20 mm) and Salmonella typhi which shows the lowest zone of inhibition (8 mm). Gentamycin shows low zone of inhibition on the tested organisms, the highest zone for Klebsiella pneumoniae, is 9 mm, while Salmonella typhi is 4 mm, Pseudomonas aeruginosa 3 mm, Escherichia coli and Staphylococcus aureus shows no zone of inhibition. Erythromycin and streptomycin also showed zones of inhibition to the tested organisms. Figure 1. Shows the antibiotic sensitivity range on the microbial isolates.

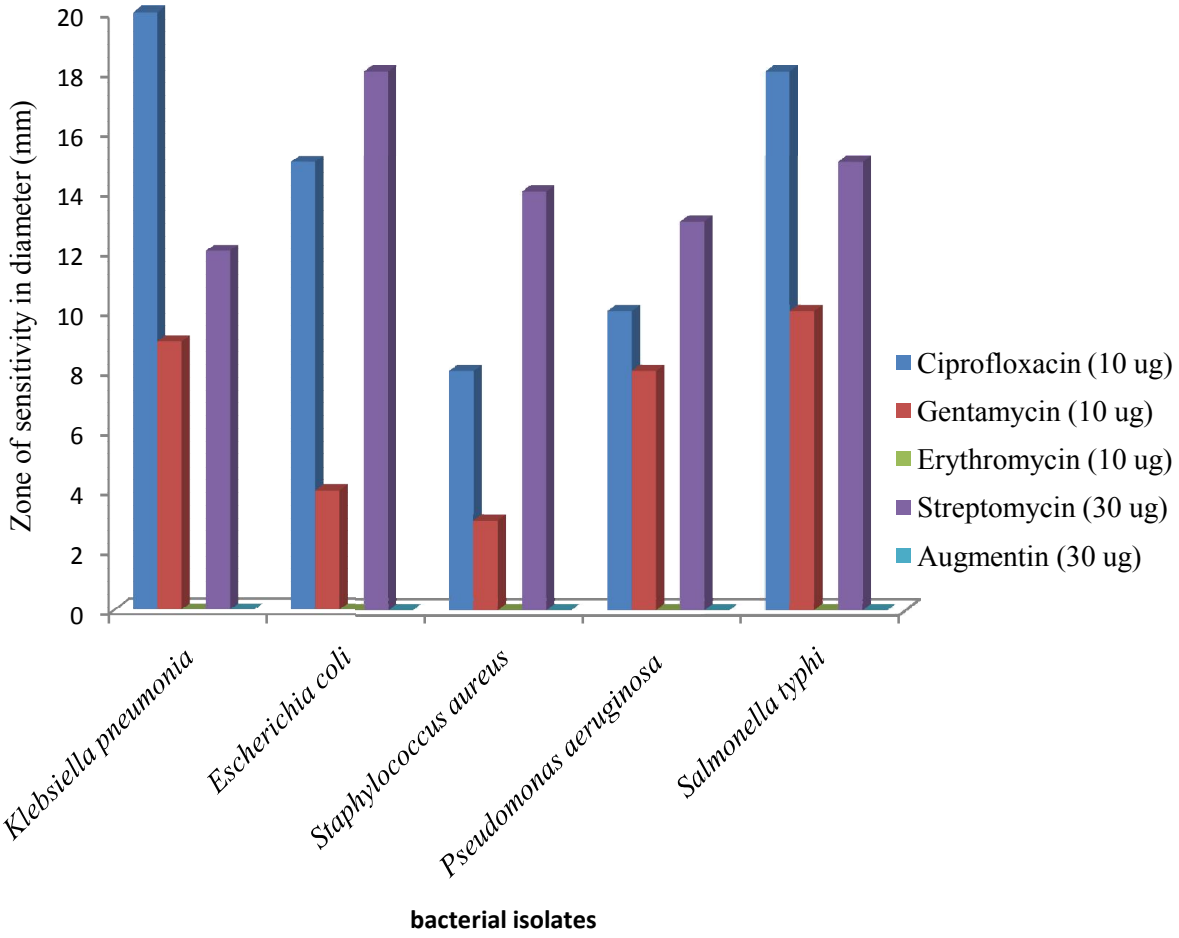


Figure 1: The range of the sensitivity of standard antibiotics on bacterial isolates

Minimum inhibitory concentration (MIC)

Ethyl-acetate extract showed minimum inhibitory concentration at 80 mg/ ml, However aqueous extract shows (MIC) at the concentration of 100 mg/ ml..

Minimum bactericidal concentration (MBC)

Ethyl-acetate extract showed no growth at 100 mg/ml. For the aqueous extract, there was no MBC.

Qualitative and quantitative phytochemical analysis of T. catappa bark extracts

Phytochemical screening of the aqueous, and ethyl-acetate extract of the bark of T. catappa shows the presence of the phytochemicals, saponins, tannins, steroids, flavonoids, glycosides and alkaloids (Table 2). Table 3 shows result of the quantitative phytochemical screening with saponins having the highest quantity and steroids with the lowest.

Discussion

The antibacterial sensitivity test reveals that ethyl-acetate and aqueous extracts were good solvents for extraction but the ethyl-acetate extract exhibited more potency against the bacterial 3isolates than the aqueous extract (Tables 2 and 3). This could be as a result of higher solubility of bioactive compounds in organic solvents as reported by [25]. This implies that the ethyl-acetate was able to extract the bioactive compounds in the bark of T. catappa and hence the more pronounced activity against the bacterial isolates. The results correspond to those of [26,27] which have respectively shown a greater concentration of the active compounds in the extracts but the ethyl-acetate extract of the bark presented a better activity on most of the tested organisms. Klebsiella pneumoniae was the most sensitive bacteria with the largest zone of inhibition with the extracts while Staphylococcus aureus had the lowest zones of inhibition with the extracts at 20 mg/ml. The activity of the extracts recorded against S. aureus corresponds with the result obtained by [28] which indicated that Staphylococcus aureus were susceptible to methanolic extract of T. catappa leaves. The aqueous extracts of T. catappa shows a low range of zones of inhibition compared to the ethyl-acetate extract. Zones of inhibition by the standard antibiotics used varied from 3mm to 20mm. Antibiotic sensitivity testing on the bacterial isolates was resistant to augumentin (30µg) and erythromycin (10µg) but susceptible to ciprofloxacin, streptomycin and gentamycin (Figure 1). Ciprofloxacin showed high level of effectiveness against all the bacterial isolates especially K. pneumoniae. Streptomycin (30µg) also showed wide range of zone of inhibition against E.coli followed by S. typhi. Gentamycin (10ug) showed low range zones of inhibition for all the bacterial isolates with S. aureus having the lowest zone. In recent times there is over whelming increase in antimicrobial drug resistant strains which necessitate the quest for more potent and effective new antibiotics. Thus there is a need for a continuous search for new effective and affordable antimicrobial drugs. The results of present study signify the potentiality of T. catappa bark as a source of therapeutic agents which may solve the ongoing search for new antimicrobial drugs. The minimum inhibitory concentration (MIC) of both extracts; aqueous and ethyl-acetate reveal that both extracts has antibacterial effect on the test organisms, Table 4 shows the MIC of the extracts against the bacterial isolates. The bacterial isolates showed no growth at the concentration of 80 mg/ml with the ethyl-acetate extract while with the aqueous extract the MIC for the bacterial isolates was at a concentration of 80 mg/ml. However the minimum bactericidal concentration (MBC) of ethyl-acetate extract against the bacterial isolates was at the concentration of 100 mg/ml, while that of aqueous extract will be at a concentration that is greater than 100 mg/ ml because aqueous extract of the bark showed no MBC at the concentration of 100 mg/ml (Table 5). The results have showed also that only ethyl-acetate extract shows bactericidal action at 100mg/ml and this extract was bactericidal for all the tested bacterial isolates.

Studies by [29] revealed that the barks of some plant materials exhibit more potent and stringent activity than the leaves. Marmonier (1990) reported that ethyl-acetate extract is more active than the aqueous extracts of plant materials. Similar results were also shown by [30,33]. It has been documented that Terminalia catappa plant extracts has a remarkable effect on some bacterial isolates as reported by which was also evident in this study. The relatively higher activity achieved in this study by the ethyl acetate extract could imply that it extracted more of the active components in the bark of T. catappa than the aqueous extract. The results obtained from this study revealed that the bark of T. catappa contains bioactive agent that contains antimicrobial properties against some bacterial clinical isolates. Table 6 shows that both the aqueous and ethyl acetate extract of T. catappa contained some phytochemicals. The phytochemical analysis of the extract of T. catappa showed the presence of alkaloids, tannins, saponins, flavonoids, steroid and glycosides. The bark of T. catappa is found to contain more of the phytochemical compound saponin while the least phytochemical compound is steroid (Table 7). Babayi et al. (2004) reported that many traditional healers make use of water to extract the active compounds from plants for medicinal purposes, because water is easily available and very safe for consumption but satisfactory isolation of active compounds from plant material depends to a large extent the nature of solvent used in the extraction process as explained by Masoko et al. (2008). The antibacterial action of the bark of T. catappa plant could be as a result of the presence of the phytochemicals present as documented by Fofana (2004) ; Ackah et al. (2008); Kadam et al. (2011). Therefore it can be used for treatments of bacterial infections.

Table 1: Sterility test for the ethyl-acetate and aqueous extracts of *T. catappa* bark

Extracts	Sterility test
Ethyl-acetate extracts	No growth
Aqueous extracts	No growth

Table 2: Zone of inhibition of the aqueous extracts of *T. catappa* bark on some bacterial isolates

Concentration (mg/ml)/Zone of inhibition (mm)

Bacterial isolates	100	80	60	40	20	WC	EC
Klebsiella pneumoniae	9.33 ± 0.58	8.00 ± 0.00	7.00 ± 0.00	5.33 ± 0.58	3.67 ± 0.58	NI	NI
Salmonella typhi	8.67 ± 1.15	6.67 ± 0.58	6.00 ± 1.00	5.33 ± 1.15	3.67 ± 0.58	NI	NI
Pseudomonas aeruginosa	7.67 ± 0.58	7.00 ± 1.00	5.67 ± 1.53	3.33 ± 1.53	2.00 ± 1.00	NI	NI
Staphylococcus aureus	7.33 ± 0.58	6.33 ± 0.58	5.00 ± 0.00	3.33 ± 0.58	1.67 ± 0.58	NI	NI
Escherichia coli	7.70 ± 0.60	7.00 ± 1.00	5.67 ± 0.58	4.00 ± 0.00	2.66 ± 0.58	NI	NI

Key Notes: WC: Water control EC: Ethyl-acetate control NI: No inhibition. Values shown are the mean and standard deviation of triplicates of the measure of zones of inhibition at $p<0.05$.

Table 3: Zone of inhibition of the ethyl-acetate extract of *T. catappa* bark on some bacterial isolates

Concentration (mg/ml)/Zone of inhibition (mm)

Bacterial isolates	100	80	60	40	20	WC	EC
Klebsiella pneumoniae	10.33±0.58	9.67 ± 0.58	8.00 ± 0.00	6.67 ± 0.58	5.00 ± 0.00	NI	NI
Salmonella typhi	8.67 ± 0.58	7.33 ± 0.58	6.00 ± 0.00	5.33 ± 0.58	3.67 ± 0.58	NI	NI
Pseudomonas aureginosa	9.33 ± 0.58	8.33 ± 0.58	6.76 ± 0.58	5.00 ± 0.00	4.33 ± 0.58	NI	NI
Staphylococcus aureus	8.33 ± 0.58	8.00 ± 0.00	7.33 ± 0.58	5.00 ± 1.00	4.00 ± 0.00	NI	NI
Escherichia coli	9.67 ± 0.58	8.33 ± 0.58	6.67 ± 0.58	5.33 ± 1.53	4.07 ± 0.81	NI	NI

Key Notes: WC: Water control EC: Ethyl-acetate control NI: No inhibition. Values shown are the mean and standard deviation of triplicates of the measure of zones of inhibition $p<0.05$.

Table 4: Minimum inhibitory concentration of the aqueous and ethyl-acetate extract and of the bark of *T. catappa*

Bacterial isolates	Ethyl-acetate 100 mg/ ml	Ethyl-acetate 80 mg/ ml	Aqueous 100 mg/ ml	Aqueous 80 mg/ ml
Klebsiella pneumoniae	-	-	-	+
Salmonella typhi	-	-	-	+
Pseudomonas aeruginosa	-	-	-	+
Staphylococcus aureus	-	-	-	+
Escherichia coli	-	-	-	+

Key (-) No growth (+) Growth

Table 5: Minimum bactericidal concentration of aqueous and ethyl-acetate extract of the bark of *T. catappa*

Bacterial isolates	Ethyl-acetate 100 mg/ ml	Ethyl-acetate 80 mg/ ml	Aqueous 100 mg/ ml	Aqueous 80 mg/ ml
Klebsiella pneumoniae	-	+	+	+
Salmonella typhi	-	+	+	+
Pseudomonas aeruginosa	-	+	+	+
Staphylococcus aureus	-	+	+	+
Escherichia coli	-	+	+	+

Key: - =No growth, + = growth

Table 6: Qualitative phytochemical screening result of the aqueous and ethyl-acetate extracts of *Terminalia catappa* bark

Biochemical constituents	Aqueous extracts	Ethyl-acetate extracts
Alkaloids	+	+
Glycosides	+	+
Tannins	+	+
Steroids	+	+
Flavonoids	+	+
Saponins	+	+

Key: + = Present

Table 7: Quantitative phytochemical screening of the bark of *T. catappa*

Phytochemicals	Quantitative analysis mg/ 100g of dry bark powder
Alkaloids	16.30
Glycosides	16.34
Tannins	8.43
Steroids	2.50
Flavonoids	10.70
Saponins	26.23

Conclusion

The result of this study has revealed that the aqueous and ethyl-acetate of the bark of *T. catappa* has antimicrobial property against bacterial isolates of *S. typhi, S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli.* In addition, the bark extract were shown to possess significant amount of phytochemicals. These findings are significant especially, at this crucial period when there are notable challenges of resistant strains of some microorganisms. Remarkably, *T. catappa* plant is readily available in all parts of African and Asian countries and could be an alternative to curing of bacterial infection at low cost. There ought to be a monitoring strategy for the consumption of the bark of *T. catappa* as many men, women and children do consume the fruit of *T. catappa.*

However, further research should be done to investigate the toxicity effect of *T. catappa* bark as well as monitoring the effective dosage using laboratory animals as well as human clinical trials.

Author's contributions

AE conceived the idea and carried out a full supervision of the study. SK, MR and MA read the manuscript and gave notable contributions to the design and final writing of the manuscript. IB and TA drafted the manuscript and performed the statistical analysis

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