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Antibacterial activity of *Tamarindus indica* leaf extract against *Escherichia coli* and *Salmonella sp.*

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Abstract
Plants are naturally endowed with the intrinsic ability to produce many chemical compounds, which defend against bacteria, fungi, insects and larger animals. Humans harness these chemical compounds from various plants and use them as traditional remedies for many infectious and non-infectious diseases. One of such plants is Tamarindus indica (Tamarind), which is a tropical tree that is commonly grown in Africa. The phytochemicals of this plant have been extracted by many studies, and have been demonstrated to have antidiarrheal, antioxidant, anti-inflammatory and antimicrobial activities. In this study, we extracted phytochemicals from the leaves of T. indica and tested their antibacterial activity against Escherichia coli and Salmonella spp. The antibacterial susceptibility test of the extract revealed substantial zones of inhibition around agar wells containing different concentrations of the extract, indicating positive antibacterial activity against the test organisms. The results obtained here could play a vital role in the identification and development of druggable compounds for the treatment of bacterial infections.

Keywords: Antibacterial activity; *Tamarindus indica;* Leaf extract; tamarind

INTRODUCTION
Plants have been has being exploited as herbal medicines for the treatment of several infectious and non-infectious diseases throughout the development of mankind. They possess several phytochemicals as secondary metabolites that acts as plants' defence mechanisms against invasion by many microorganisms. The demonstration of antibacterial activity by extracts of the parts of these plants may help to discover new sources of antibacterial compounds that could aid in the drug development and treatment of diseases caused by these microorganisms.

Antibiotic resistance is a major challenge to the healthcare sector in a large part of the developing world. The emergence and spread of multidrug-resistant (MDR) strains among

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pathogenic bacteria are particularly threatening to the current antibiotic therapy. This continued and rapid, emergence of resistance to newly introduced antibiotics indicates that even new families of antibiotics will have a short life expectancy (Marasini *et al.*, 2015). In this study, antibiotics refer to those antibacterial agents that are originally sourced from microorganisms, which may include their semi-synthetic or synthetic forms.

The development of antibiotic resistance in many developing countries by many pathogenic microorganisms is said to be due to the indiscriminate use of commercial antibiotics commonly used in the treatment of infectious diseases (Abiramasundari *et al.*, 2011). In other to avoid this, these antibiotics should be the drugs of last resort. This has necessitated a search for a new source of antimicrobial substances from plant sources such as tamarind leaf extract, since they produce a variety of bioactive compounds with known therapeutic properties (Boucher *et al.*, 2009).

Tamarindus indica (commonly called Tamarind), is a tropical tree commonly that is found in the fertile areas of the tropical zones of Africa and Asia. It is an ornamental tree that is widely grown for its acidic fruits that used in making drinks. It is also a~~a~~ popular component of many concoctions and decoctions used as health remedies. Its pulps, seeds, leaves and bark have been consumed as herbal medicine and as a traditional food (Kapur and John, 2014). The plant parts have been extensively studied in terms of the pharmacological activity of its major compounds and the results indicate potent antidiarrheal, antioxidant, anti-inflammatory, wound healing and antimicrobial activities (Nikkon, 2003).

In Northern Nigeria, the plant has long been used in traditional medicines, and the fruits are the most valuable part, which is used as a condiment in the preparation of local meal and has also been reported as curative in several ailments. The stem bark and leaves also useful as they are mixed with potash and are taken as a decoction for the treatment of diseases such as stomach disorders, general body pain, jaundice, and yellow fever. It is also used as a blood tonic and skin cleanser (Yusha'u *et al.*, 2014). *T. indica* fruit is popularly employed daily as a flavouring agent in the production of local drinks, and preservation of food. It is also used as a drug conveyor along with other herbs for the treatment of various other diseases such as indigestion, constipation, fever and inflammation, as well as a gargle for sore throat, relieves pains, reduces secondary bacterial infections and promotes healing in traditional medicine (Fabiyi *et al.*, 1993).

The choice of such plant extracts for drug development against infectious microorganisms may reduce the burden or the indiscriminate use of antibiotics, which will slow down the emergence of antibiotic resistance. For these reasons, this study was designed to investigate the antimicrobial activity of tamarind, which is widely available against the clinical isolates of *Salmonella sp.* and *E. coli.*

Findings from this study has shown that the plant *Tamarindus indica* commonly available in Kaduna has the potential to be used as or part of a plant based concoction against the organisms especially because it has been used for centuries without any reported case of toxicity.

MATERIALS AND METHODS

Collection of Samples

Samples of *Tamarindus indica* leaves together with a portion of its stem were collected from Unguwan Mu'azu Kaduna North Local Government area and taken to the Biological Science Department of Kaduna State University for authentication.

Clinical isolates of the test organisms (*Salmonella sp* and *E. coli*) were collected from the pathology laboratory of 44 Nigerian Army reference Hospital Kaduna and transported to the Department of microbiology laboratory, Kaduna state university, kaduna. Both the isolates were re-inoculated on Salmonella Shigella agar (SSA) and eosin methylene blue (EMB) agar which are selective for the organisms. The isolates were reconfirmed using the phenotypic characterisation technique such as Gram reaction and biochemical reactions.

Extraction procedure

The leaves of Tamarind tree collected were rinsed with distilled water and allowed to dry at room temperature in the laboratory for 10 days. The dried leaves were pulverized using a clean mortar and pestle to pass through a size 30 mesh screen. A 150g of the powdered leaves were extracted in 1.5L (1500ml) of distilled water and 95% ethanol for 72 hours using the cold maceration technique at room temperature. The extracts were filtered through Whatman No. 1 filter paper and evaporated in water bath at 40^{o}C and 80^{o}C to remove excess ethanol and water respectively.

Phytochemical screening

Both the aqueous and ethanolic leaf extracts were tested for the presence of phytoconstituents such as saponins, glycosides, terpenoids, alkaloids, flavonoids, steroids, tannins, and phlobatannins using the standard procedure as described by Saxena and Arora (2018).

Antibacterial activity

To determine the antibacterial activity, ten grams (10 g) of each extract was reconstituted in 100ml of 2% dimethyl sulfoxide (DMSO) to produce a stock solution of 100mg/ml. This is diluted to 75mg/ml, 50mg/ml, and 25mg/ml by transferring 0.75ml of the stock solution to 0.25ml of 2% DMSO, 0.50ml of the stock solution to 0.50ml of 2% DMSO and 25ml of the stock solution to 0.75ml of 2% DMSO respectively. The sensitivity of the aqueous and ethanolic extract was determined using the agar well diffusion method as described by Ahmad and Beg (2001). For a test organism's suspension, a loop full of the bacterial colony was transferred to a tube of sterile distilled water. The turbidity was adjusted to that of 0.5 Mac-Falland solution to make a population of 1.5 x 10^{6} cfu/ml. A 0.1ml was evenly spread into a sterile plate of solidified Mueller-Hinton agar. Four wells of 6mm each were bored using a sterile cork borer. The wells were carefully filled up with 0.1ml of the extract concentrations of 25, 50, 75 and 100mg/ml. Ciprofloxacin was used as a control for the experiment. The inoculated plates were allowed to stand for 1 hour to ensure proper diffusion of the extract into the medium and then incubated at 37°C for 24 hours, to observe for appearance of zones of growth inhibition.

Determination of minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration, five test tubes were prepared with each tube containing 2ml of Mueller-Hinton broth and 1ml of the four different concentrations (100, 75, 50 and 25mg/ml) of the extract while the last one with no extract to serve as control. A 0.1ml of the standardised bacterial suspension was inoculated and incubated at 37^{o}C for 24 hours. After incubation, the test tubes were examined for turbidity (Ahmad and Beg, 2001).

Determination of Minimum Bactericidal Concentration (MBC)
The minimum bactericidal concentration of the extract was determined by taking a loop full of samples from the tubes in the MIC that does not show any sign of tubidity and inoculated on sterile nutrient agar by streaking. Plates inoculated were incubated at 37°C for 24 hours. After incubation, the minimum concentration in which there was no visible growth was taken as the minimum bactericidal concentration (Doughari, 2006).

RESULTS AND DISCUSSION
The physical characteristics of the extracts (table 1) showed a black, sticky texture with a 28.36% yield for the ethanolic extract and a brown, powdery with a 30.09% yield for the aqueous extract. This is likely because solvents (water and ethanol) used for the extraction have the capacity to extract different phytoconstituents depending on their solubility in the solvent, mostly due to variation in polarity (Cowan, 1999). This may also be responsible for the variations observed in the physical properties of the extracts. In this study, ethanol has shown a higher solubility for more phyto-constituents than water, but the water was able to extract a higher quantity of those constituents they were both able to extract. This result is in contrast to what was reported by Yusha'u *et al.* (2014) who reported that the ethanolic extraction of stem bark of *T. indica* had 25.5% yield, whereas the aqueous extraction yielded only 3.5%.

The phytochemical screening of *T. indica* leaf extracts revealed the presence of alkaloids, tannins, saponins, steroids and flavonoids as shown in table 2. The presence of some of these phytochemicals in plants is thought to be responsible for its medicinal and antibacterial activity. The results of this study were in agreement with those of Sravanthi *et al.* (2017), which reported the presence of tannins, saponins, alkaloids, flavonoids, and steroids.

However, Yusha'u *et al.* (2014) reported the absence of flavonoids from all three extracts tested. This may be because the extracts were obtained from the stem back and not the leaves, as in this study. The number of phytochemicals detected was higher in the ethanolic extract than in the aqueous extract. This may be because the solvent ethanol used for the extraction was able to dissolve an appreciable number of the constituents. The absence of terpenoids was not in line with the work of Abdallah and Muhammad (2018) which reported the presence of terpenoids in the leaf extract of *T. indica.* This may be due to the fact that methanol was used in the extraction which may have a better solubility. Therefore, the use of methanol for extraction is recommended where terpenoid is of interest since it can extract it better than ethanol and water.

Both extract of *T. indica* has shown anti-bacterial activity against the two isolates although the ethanolic extract is showing a slightly greater activity (table 3). This is likely as a result of the ability of the solvent (ethanol) to dissolve more of the bioactive organic matter which lead to the extraction of more classes of bioactive substances. This suggest that ethanol has a better extraction efficiency when it comes to the antibacterial activity. It also indicates that certain bioactive components were more soluble in ethanol than in water. The ethanolic extract showed the highest zone of inhibition of 20mm against *E. coli* and 17mm against *Salmonella sp*. at 100mg/ml. The aqueous extract had 18mm as the highest zone of inhibition against *E. coli* and 15mm against *Salmonella sp*. The findings of this study indicated that *E. coli* was more susceptible to the extracts with the highest zone of inhibition of 20mm for ethanolic extract when compared to *Salmonella sp.* with the highest zone of inhibition of 18mm. This supported the findings of Daniyan and Muhammad (2008), who assessed the antibacterial activity of *T. indica* leaf extracts against some disease-causing bacteria, including *E. coli* and *Salmonella sp*. They reported that the zone of inhibition for *E. coli* was 20mm, and 18mm for *Salmonella sp.*

for ethanolic extract. The demonstration of antibacterial activity by aqueous extract provides the scientific basis for the use of water in the traditional treatment of diseases caused by *E. coli* and *Salmonella sp*, using *T. indica* leaves because most traditional herbalists use water as their solvent in which the concoctions are prepared (González-Lamothe *et al.*, 2009).

The MIC of the ethanolic extract of the leaf of *T. indica* was 50mg/ml and 75mg/ml for *E. coli* and *Salmonella sp.,* respectively. The MIC of aqueous extracts was 75mg/ml against both organisms (table 4). Both extracts have the same effect on *Salmonella sp.* because they have the same MIC value. On this basis, water can be recommended for the extraction of the leaf of *T. Indica* for the treatment of *Salmonella* infections because it is readily available and will not incur any cost compared with ethanol which is costly and does not bring in any additional benefits. MBC of ethanol extract was found to be 75mg/ml and 100mg/ml against *E. coli* and *Salmonella sp.* respectively. The aqueous extract was bactericidal at 100mg/ml against all the test organisms. The higher values of MIC and MBC for *Salmonella sp* reported in this study an indication that either the plant extracts are less effective on the organism or that the organism is less susceptible because it has the potential to develop some kind of resistance, whereas the low MIC and MBC values for *E. coli* are an indication of the effectiveness of the plant extracts against the organism.

Table 1: Physical properties of leaf extracts of *T. indica.*

Physical parameters	**Ethanol**	**Aqueous**
Weight (g)	42.54	49.64
Percentage yield (%)	28.36	30.09
Color	Black	Brown
Texture	Sticky	Powdery

Table 2: Phytochemical constituents of the *T. indica* leaf extract

Test	**Ethanolic extract**	**Aqueous extract**
Alkaloid	+	-
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Steroids	+	-
Glycosides	-	+
Phlobatannins	-	-
Terpenoid	-	-

Key: + = positive, - = negative

Table 3: Antibacterial activity of the leaf extract of *T. indica* against *E. coli* and *Salmonella sp*.

Concentrations (mg/ml)	**Zones of inhibition (mm)**			
	E. coli		***Salmonella sp***	
	EE	**AE**	**EE**	**AE**
100	20	18	17	15
75	17	14	14	11
50	13	10	10	8
25	8	6	7	-
Control (CPX 50mg/ml)	26		23	

Key:EE = ethanolic extract, AE = aqueous extract, - = no measurable zone

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extract of *T. indica on E. coli* and *Salmonella sp.*

Organisms	MIC (mg/ml)		MBC(mg/ml)	
	Ethanol	Aqueous	Ethanol	Aqueous
E. coli	50	75	75	100
Salmonellasp	75	75	100	100

CONCLUSION

From this study, it can be concluded that ethanol can be able to extract more of the antibacterially significant phytochemicals, but a larger quantity of the extracts was obtained using water, and both the aqueous and ethanolic extracts of *T. indica* have a good antibacterial potential. The current research recommends further studies on the activity of the extract at a lower concentration and the isolation of the active component responsible for antimicrobial activity as well as its toxicity and safety level.

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