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Antibacterial activity of leaves and fruits extract of *Tamarindus indica* against clinical isolates of *Escherichia coli* and *Shigella* at potiskum yobe state, Nigeria

Abstract

The study was conducted to determine the phytochemical composition and antibacterial activity of *Tamarindus indica* leaves and fruits extracts against clinical isolates of *Escherichia coli and Shigella* sp isolated from stool samples of pregnant women attending antenatal clinic Potiskum, Yobe State, Nigeria. Preliminary phytochemical analysis was conducted using laboratory method while agar well diffusion method was used to determine antibacterial activity of the extracts. The result of phytochemical screening of the extracts showed the presence of alkaloid, glycoside, saponin, tannin, anthraquinone and steroid, reducing sugar flavonoid, terpenoid and phenol. The result of the antibacterial efficacy of the extracts against the isolates indicated the extracts were active against the isolates with higher activity in methanol extract (with average zone of inhibition of 14.48mm) when compared to aqueous extract (12.52mm). The result of susceptibility of the isolates to the extracts showed *Escherichia coli* is more sensitive to the extract with average zone of inhibition of 14.62mm when compared to *Shigella* sp with average zone of inhibition of 11.47mm. The minimum inhibitory concentration (MIC) of the extracts showed that dilutions of various concentrations of aqueous and methanol extracts inhibit the growth of the isolates at a concentration of between 3.125–25mg/ml. Statistical analysis of the results indicated that there is significant different in the activity of the extracts against the isolates used at $p<0.05$. Findings from this work support the use of *Tamarindus indica* leaves and fruits extracts for medicinal purpose.

Keywords: antibacterial activity, *Escherichia coli*, extract, *Shigella*, tamarinds

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Abdallah MS,[1] Muhammad Ali[2]
[1]Desert Research Monitoring and Control centre, Yobe State University Damaturu, Nigeria
[2]Department of Microbiology, Federal University Gusau, Nigeria

Correspondence: Muhammad Ali Department of Microbiology, Federal University Gusau, Nigeria, Email alimahd4real@gmail.com

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Introduction

Herbs have been used as medicine since the history of man and herbal preparation have been a major component of all traditional medicine system particularly in Asia, south America and Africa.[1] Nevertheless, herbal medicine was the starting point for the western medicine before the latter greatly diversified. An estimate of 75–90% of rural population of the world still relies on herbs for their healthcare. Thus, in many village market places in Asia, Africa and Latin America, medicinal herbs are sold alone side vegetable and other wares.[2] However even in the western culture where herbal medicine seemed to have been forgotten for a long time in preference to synthetic drugs, there is a rethinking and resurgence of herbal remedies. The new direction has been necessitated by high rate of resistance to antibacterial drugs.[3]

Medicinal plants materials are considered safe and understandable so because most of them are known as metabolize human and animal food items. Several Nigerians, used plant for treatment of disease have been authenticated to have antibacterial activity using *in vitro* test method.[4] Moreover, *Tamarindus indica* (tamarind) belong to the dicotyledonous family *Leguminosae* and sub-family *Caesalpiniceae*.[5] Tamarind has been used for centuries as a medicine plant; it fruits are most valuable part which have often been reported as curative in several pharmacopoeias, the leaves have a proven hap to protective activity associated with the presence of polydroxylated compound with many of them of a flavonoid nature.[6] Leaves also present good level of protein, fat, fibber, and some vitamin such as thiamine, riboflavin, niacin, ascorbic acid and B-carotene.[7]

Flavonoids and polyphenols the metabolites found in leaves have recorded as antimicrobial agents in many other plants. Many studies have shown the antimicrobial activity of tamarind leaves against gram positive and negative bacteria. In Northern Nigeria, the fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorder, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser.[8] The study was aimed to investigate the phytochemical and antimicrobial activity of the leaves and fruit extracts of *Tamarindus indica* against clinical isolates of *Escherichia coli* and *Shigella* sp isolated from stool samples of pregnant women attending antenatal clinic Potiskum, Yobe State, Nigeria.

Materials and methods

Ethical approval

Ethical approval for the study was obtained from Ministry of health Damaturu, Yobe State based on the consent of the Potiskum hospital Ethical Committee.

Isolation and identification of test isolates

Two (2) bacterial isolates recovered from stool samples of pregnant women attending antenatal clinic Potiskum, Yobe State, Nigeria namely; *Shigella sp* and *Escherichia coli* were used in this study. The bacteria isolates were characterized to species level by using different laboratory procedures including; Gram's stain, cultural characterization (Nutrient and MacConkey agar) and Biochemical tests include (Indole, Methyl red, Voges Proskauer, motility and

Citrate utilization) as described by Holt et al.,[9] Cheesbrough.[10] The isolates were maintained on Nutrient agar slants at 4^0C.

Collection and identification of plant materials

Tamarindus indica leaves and fruits were used in this study, which was collected from Yobe State University, Damaturu. Botanical identification and authentication of the plant material was done at Botanical garden in the Department of Biological Sciences, Yobe State University Damaturu. Voucher specimens were deposited there for future reference. The leaves and fruits were washed with water and removed dust and rinsed with distilled water, air dried for two-weeks and pulverized into powder form using sterile mortar and pestle under laboratory as described by Ali et al.[11] The powder samples were bagged in a black polythene bag and stored in air tight container for further work.

Preparation of leaves and fruits extracts

Approximately fifty (50) gram each of powered leaves and fruits were each macerated in 500ml of distilled water and methanol respectively for period of 24 hour at room temperature as described by Okoli et al.[12] Each preparation was filtered through a Whatman filter paper and the aqueous filtrate was evaporated to dryness in water bath at 40^0C while methanol extract in rotary evaporator at 50^0C. The residue obtained was further diluted using 10% Dimethylsulphoxide (DMSO) to produce 100 mg/ml of the extract from which various concentrations of 50, 40, 30, 20 and 10mg/ml were produced.[13]

Phytochemical screening

Qualitative method of screening was carried out so as to test the presence of the present bioactive ingredients, as being adopted by Kumar et al.[14] The presence or absence of the following phytochemicals was determined; Alkaloid, saponin, glycoside, reducing sugar, flavonoid, steroid, phenol, terpenoid, tannin and anthraquinone.

Antibacterial activity of the extracts

The sensitivity of each extracts was determined using the agar well diffusion method as described by Ahmed and Beg[15] with modifications. The prepared bacterial suspension equivalent to 0.5Mc Farland Standard (1.5 x 10^6 CFU) was inoculated into sterile Mueller-Hinton agar medium in a sterile Petri-dish and rotated at 60^0 to ensure and even distribution of the inoculums. A sterile 6mm diameter sterile cork borer was used to bore 6 wells into the agar medium. The wells were then filled up with approximately 0.1ml of the extract solution at a concentration of 10, 20, 30, 40 and 50mg/ml taking care to prevent spillage onto the surface of the agar medium. The plates were rotated allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37^0C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured. The experiment was conducted in triplicate and the average values were recorded. Ciprofloxacin 50mg/ml (Micro Lab limited) was served as a control (positive) for the experiment.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration MIC of the extracts was determined using broth dilution technique. Double fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37^0C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity.[15]

Statistical analysis

The data of average zone of inhibition produced by the isolates against the extracts used were analyzed using One-Way ANOVAs from statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Results

Identification of the isolates

The morphological and biochemical characterization of the isolates is presented in Table 1. Both the isolates are Gram negative rods, negative for Voges Proskauer and citrate utilization test while both positive for methyl-red test. *E. coli* is motile and lactose fermenter while *Shigella* is non motile and non-lactose fermenter.

Table 1 Morphological and biochemical tests for identification of the isolates
Key: +, positive; -, negative

S/N	Agar/ Biochemical test	*Escherichia coli*	*Shigella* sp
1	Nutrient agar	Whitish moist, smooth surface and opaque colony.	Translucent, opaque and glistening colony.
2	MacConkey agar	Non-mucoid dark pinkish colony	Transparent colourless colony with jagged edge
3	Gram staining/ shape	Negative/rod	Negative/rod
4	Indole test	+	-
5	Methyl-red test	+	+
6	Voges Proskauer test	-	-
7	Citrate utilization test	-	-
8	Motility test	Motile	Non-motile

Phytochemical screening

The phytochemical constituent of leaves and fruits extracts of *Tamarindus indica* is presented in Table 2. The preliminary phytochemical screening of the extracts revealed the presence of Alkaloid, saponin, glycoside, reducing sugar, flavonoid, steroid, phenol, terpenoid, tannin and anthraquinone. More phyto-constituents found in the fruits than the leaves extracts.

Antibacterial activity of the leaves extracts

The antibacterial activity of *Tamarindus indica* leaves extracts against *Escherichia coli* and *Shigella* sp is presented in Table 3. The result showed that methanol extract is more effective with average zone of inhibition of 13.85mm than aqueous extract with average zone of inhibition of 9.57mm. Based on the result, *Escherichia coli* is more sensitive to the extract than *Shigella* sp. The zone of inhibition shown by Ciprofloxacin (25mg/ml) is 23 and 21mm for *Escherichia coli* and *Shigella* sp respectively.

Table 2 Phytochemical Screening of the plant materials
Key: +, presence of phytochemical; -, absent of phytochemical

S/N	Phytochemical	Leaves extract	Fruits extracts
1	Alkaloids	+	+
2	Saponin	+	+
3	Glycosides	-	+
4	Reducing sugar	-	+
5	Flavonoid	+	+
6	Steroids	+	+
7	Phenols	+	+
8	Terpenoid	+	-
9	Tannin	+	+
10	Anthraquinone	+	+

Table 3 Antibacterial activity of the leaves extracts against the isolates
Key: ALE, aqueous leaves extract; MLE, methanol leaves extract. Values having different superscript in the same row are considered significantly different at probability level of $p<0.05$.

Extracts	Conc. (mg/ml)	*Escherichia coli*	*Shigella* sp
	10	05.34±0.3^b	00.00±0.0^a
	20	09.00±0.0^b	00.00±00^a
ALE	30	12.67±1.2^b	07.34±0.8^a
	40	13.34±1.1^a	11.34±0.2^a
	50	17.00±1.8^b	13.67±1.3^a
	10	09.34±0.4^a	08.00±1.1^b
	20	10.67±0.5^a	09.67±0.4^a
MLE	30	13.67±0.7^a	13.67±0.9^a
	40	18.00±1.1^a	16.67±1.2^a
	50	21.00±1.3^b	17.34±1.8^a
Ciprofloxacin	25	23.34±1.3	21.00±0.0

Table 4 Antibacterial activity of the fruits extracts against the isolates
Key: AFE, aqueous fruits extract; MFE, methanol fruits extract. Values having different superscript in the same row are considered significantly different at probability level of $p<0.05$.

Extracts	Conc. (mg/ml)	*Escherichia coli*	*Shigella* sp
	10	08.34±0.1^a	07.67±0.0^a
	20	13.34±1.1^a	10.34±1.7^b
AFE	30	15.67±1.4^a	10.67±1.1^b
	40	17.67±1.3^a	15.67±1.5^a
	50	18.67±1.2^a	17.00±0.5^a
	10	12.00±0.6^a	10.34±1.8^a
	20	15.34±1.5^a	12.34±1.1^b
MFE	30	19.34 ±1.3^a	13.34±1.5^b
	40	19.67±1.7^a	16.67±1.9^b
	50	22.34±1.0^a	17.67±0.7^b
Ciprofloxacin	25	23	21

Antibacterial activity of the fruits extracts

The antibacterial activity of *Tamarindus indica* fruits extracts against *Escherichia coli* and *Shigella* sp is presented in Table 3,4. The result showed that methanol extract is more effective with average zone of inhibition of 15.95mm than aqueous extract with average zone of inhibition of 13.05mm. Based on the result, *Escherichia coli* is more sensitive to the extract than *Shigella* sp. The zone of inhibition shown by Ciprofloxacin (25mg/ml) is 23 and 21mm for *Escherichia coli* and *Shigella* sp respectively.

Minimum inhibitory concentration (MIC)

The minimum inhibitory Concentration of aqueous and methanol extract of leaves and fruits is represented in Table 5. The result showed dilutions of various concentrations of aqueous and methanol leaves and fruits extracts can inhibit the growth of the isolates. Lower MIC (3.125mg/ml) was shown by methanol fruits extract than aqueous extract with 6.25mg/ml.

Table 5 Minimum inhibitory concentration (MIC) and MBC of the extracts
Key: AFE, aqueous fruits extract; MFE, methanol fruits extract

	Leaves extract	fruits extract		
Isolates	**ALE (mg/l)**	**MLE (mg/ml)**	**AFE (mg/ml)**	**MFE (mg/ml)**
Escherichia coli	6.25	6.25	6.25	3.125
Shigella sp	25	6.25	6.25	3.125

Discussion

The Phytochemical screening of the *Tamarindus indica* leaves and fruits extracts indicated the presence of alkaloid, tannin, saponin, glycoside, flavonoid, anthraquinone, reducing sugar, terpenoid, and phenols. The presence of the above phytochemicals in the plant parts was responsible for its antibacterial activity. Flavonoids have been shown to possess anti-inflammatory, anti-hepatotoxic and antimicrobial activities.[16] Saponins are known to possess antibacterial activities[17,18] whilst tannins play an important role in wound healing and also possess some antimicrobial activities. According to this study, Alkaloid is also present in both the extracts. Alkaloid consists of large group of nitrogenous compound which are widely used as anticancer anesthetics and Central Nervous Stimulants. Alkaloids are known to play some metabolic roles and control development in living system. It also interferes with cell division, hence the presence of alkaloids in the *Tamarindus indica* leaves and fruits could account for their use as antimicrobial agents. The result of this study was inconformity with that of Sravanthi et al.[19] who reported that Results of the phytochemical studies revealed the presence of tannins, saponins, alkaloids and tri terpenoidal saponins and the extracts were active against both gram positive and gram negative bacteria.

The antibacterial activity of the plant showed that *Tamarindus indica* leaves and fruits extracts demonstrated an antimicrobial effect against the test isolate with higher activity in methanol extract compared to aqueous extract. The methanolic extract had total zone of inhibition of 14.86mm while 12.52mm for *S. typhi*, while aqueous extract. This may be due to the better solubility of the active components in the organic solvent (methanol) than water which leads to better efficacy of the methanol extracts. It suggests that the active component is more soluble in ethanol than in the other solvents. However, Doughari et al.[8] stated that the anti-microbial effect of the plant could be due to the bioactive compounds such as the phytochemicals constituent present in the plant. The results showed that the potency of the extracts on the test isolates had different hierarchy of susceptibility among the organisms. The findings of this study indicated that *E. coli* was more sensitive to the extracts with

average zone of inhibition of 14.62mm when compared to *Shigella* with average zone of inhibition of 11.47mm. The finding of this study supported the finding of Nwodo et al.[20] who assessed the antibacterial activity of *Tamarindus indica* fruit pulp, stem bark and leaves extracts against some bacterial isolates. They found that the fruit pulp extracts exhibited a wide spectrum of activity; the cold water extract against 95.5% of the test bacterial strains; and the hot water and ethanolic extracts against 90.9% and 86.4%, respectively. In contrast the cold water extract of the leaves and stem bark, each was active against 16.7%; while the ethanolic extract of each was active against 75% of the test strains. The minimum inhibitory Concentration of aqueous and methanol extract of leaves and fruits showed dilutions of various concentrations of aqueous and methanol leaves and fruits extracts can inhibit the growth of the isolates at 3.125mg/ml by methanol fruits extract and 6.25mg/ml for aqueous extract.

Statistical analysis of the result revealed that the fruit extract is more effective than the leaves extract. The fruits extracts has an average zone of inhibition of 14.70mm while leaves extract has an average zone of 11.39mm. There is considerable statistical difference on the activity of fruits and leaves extract tat $p<0.05$. Higher activity of fruits extracts can be attributed to higher number of phytochemical they contained when compared to leaves extracts. Nwodo et al.[20] found that *Tamarindus indica* fruits extract has better efficacy than stem bark and leaves extracts, this support the finding of the present study.

Conclusion

Phytochemical screening of *Tamarindus indica* leaves and fruits extracts indicate the presence of presence of alkaloid, tannin, saponin, flavonoid and phenols, terpenoid, glycoside anthraquinone and reducing sugar. The antibacterial activity of the extracts against *Escherichia coli* and *Shigella* sp showed that both the extracts demonstrated an antimicrobial effect against the isolates. The Minimum inhibitory Concentration (MIC) of aqueous and methanol extract of the plant showed dilutions of various concentrations can inhibit growth the isolates. Findings from this work support the use of *Tamarindus indica* leaves and fruits extracts for medicinal purpose.

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Conflict of interest

The authors declare that there is no conflict of interest.

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