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Research Article

Effect of fruits methanolic extracts on *Tamarindus indica* against some bacterial isolates causing urinary tract infection among pregnant women

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Abstract: The present study aimed to determine the sensitivity pattern of the isolates against the *Tamarindus indica* fruit methanolic extracts as well as to detect the mode of action of the extract. Phytoconstituents were obtained from the crude extracts through the process of qualitative mode of screening and antibacterial activity was evaluated by agar well diffusion method against the gram negative bacteria. The bioactive ingredients found were mainly; the alkaloid, flavonoid, tannin, saponins, phenol, and phytosterols were found in the extracts of methanolic leaves which showed sound activities against the tested organisms; *E. coli* and *Shigella*. The package used for the data analysis was (SAS) version 8.0.

Keywords: Medicinal plants, microbes, traditional medicine, isolates tamarind.

INTRODUCTION

Tamarindus indica being an indigenous to tropical Africa, but has been cultivated for so long on the Indian subcontinent that it is some time reported to be indigenous there, where its known as (Imli) in Hindu Urdu. It grows wild in Africa in local as diverse to Sudan, Cameroon, Nigeria and Tanzania. In Arabia it is found growing wild in Owon, especially Dhafur, where it grows on the sea-facing slope of mountain. It reaches south Asia likely through human transportation and cultivation several thousand years and the America, especially Mexico (Doughari *et al*., 2013). Nevertheless, the young green leaves and the isolated pule are component of a drink in Nigeria. Prepared by infusing *T. indica* dried pule. In some part s of West Africa non cereal plant contribute to the diets of local resident mainly during time of grain shortage. Moreover, fruit of *T. indica* (Tsamiya) contained a moderate portion of protein WHO, 2002. *T. indica* fruits also contained a reasonable amount of fatty acids which served as a supplier of some diets for a wellbeing of locals. In western Mali the nutritional importance of green leaves and fruits from *T. indica* were used in different season. Preferentially in rural region wild also gathered the foods are used as much as fresh cultivated food due to the presence of some important metabolites. In Nigeria *T. indica* was applied against worm infection, *Trypanasomiasis* in domestic as well as against guinea worm. (Chung *et al*., 2005., Garba *et al*.,2005 M *et al*., 2003, and Nassereddin., 2005).However, *T. indica* reported to have a wide spectrum of antibacterial activity. Some parts of it like leaves and fruits methanolic extracts revealed a wide potencies against the enteric bacterial isolates such as; *Klebsiella pneumoniae* and *E. coli* using Agar well diffusion method as well as compared with standard antibiotic disks Amikacin and piperacillin (Vaghasiya *et al.*, 2009). The methanolic and aqueous of *T. indica* revealed highest inhibition zones against isolates from both gram positive and gram negative bacteria (Doughari et *al.*, 2006). Other studies have suggested that *T. indica* has shown potential antibacterial activity. Ethanolic extracts of *T. indica* ripe fruits were pinpointed for the antibacterial potentialities against the bacterial isolates (Warda *et al.*, 2007). *T. indica* fruits extracts when soaked in water consumed by Fulani people in Nigeria, it cures diarrhoea (Lockett, *et al*., 2000). It has also been reported that ethanolic, aqueous and methanolic fruits of *T. indica* revealed a sound inhibition zones against some clinical isolates; *Escherichia coli*, *Klebsiela pneumonia*, *Salmonella*

paratyphi but resistant to some *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which was in line with the presence of some metabolites that allow the inhibitory actions perfectly well; alkaloids, saponin, flavonoid, and tannins (Daniyan *et al.,* 2008). The fruits part of *T. indica* possessed vitamin B, carotene and Vitamin C in a higher quantity. Subsequently, tannins and some minerals (P, K, Ca and Mg) were also found in a higher quantity. It served as an antioxidants, anti-inflammatory, antimicrobial and antifungal, it was in line with the properties possessed, recommended to be utilised traditionally as medicine for many ailments in many parts of Africa and beyond (Caluwe *et al*., 2010).

MATERIALS AND METHODS

Preparation of leaves and fruits extracts

The fresh leaves of *Tamarindus indica* were rinsed thoroughly in running tap water, chopped to tiny pieces and air dried at room temperature for a period of 14 days, and subsequently pulverised with a pestle and mortar. The fresh or pulp covering the seed was removed and dried as below. Approximately 60.0 gram of powered leaves pulp were each macerated in 500ml of distilled water and methanol for period of 24 hour at room temperature. The distilled water extraction and methanol of each of the two plant part was described by (Okoli *et al.,* 2000). Also 60.0g fruits of *Tamarindus indica,* pulp were each macerated in 500ml of hot water for period of 24 hours. The hot water extraction and methanol of each of the two plant part. Each preparation was filtered through a Whatman filter paper and filtrate evaporated to dryness in a steady air current after with all extract were stored in a sterile container and store at a room temperature (Azoro *et al*., 2000).

Phytochemical screening

The phytochemical screening purposed at standardized extraction procedure for crude drugs (Medicinal plant part) is to attain the therapeutically desired portion and to eliminate unwanted materials by treatment with a selective solvent known as menstrum. The extract medicinal agent as such in the form of tincture or fluid extracts or further processed to be incorporated in any dosage form such as tablet and capsules. The product contain complex mixture of many medicinal plant metabolite such as alkaloid, flavonoid, phytosterols, tannin, saponins and phenols (Prashant *et al*., 2011).

Phytochemical screening on leaves of *Tamarindus Indica*

Detection of flavonoids

Alkaline reagent test: Extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of diluted acid, indicates the presence of flavonoids. (Ncube *et al*., 2008).

Detection of saponin

From the Test: The extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutes. Formation of 1cm layer of foam indicate the presence of saponins. (Handa *et al*., 2008).

Detection of tannins

Braymers Test: 2ml of the extracts plus few drops (2-5) of 10% alcoholic ferric chloride solution. Formation of brown-reddish precipitate indicate the presence of tannin (Roy *et al*., 2005).

Detection of alkaloid

Wagner's test: Filtrate were wagners reagent (Iodine in potassium iodide). Formation of brown reddish precipitate indicates the presence of alkaloids (Parekh *et al*., 2010).

Detection of phenol

Ferric chloride test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicate the presence of phenol and the phenol is absent (Parekh et al., 2009).

Detection of phytosterols

Salkawskis test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentration sulphuric acid, shaken and allowed to stand, golden yellow colour appeared indicates the presence of phytosterols (Kumar *et al*., 2010).

Collection of sample for cultivation of test organisms

Stool samples were collected from microbiology laboratory unit, General hospital Potiskum, Yobe State. In sterile universal containers, preserved and transported to Yobe State University, Biology research laboratory under aseptic condition.

Media preparation/ XLD agar (Xylose, Lysine and Deoxycholate)

28g of the XLD agar was weighed into a conical flask and transferred into a conical flask containing 500ml of distilled water. The mixture was heated on hot plate at 75℃ for 1 hour. The media was allowed to cool, and poured into 20 petri-plates and allowed to solidify. The stool samples were inoculated on the media using sterile wire loop, incubated at 37℃ for 24 hours and observe the growth of *Shigella* (Abdallah and Ali, 2018).

MacConkey agar

28g of the macConkey was weighed into a conical flask and transferred into a conical flask containing 500ml of distilled water. Heated boiled and dissolved completely as well as Sterilized by autoclaving for 15 minute, at 121℃. The media was allowed to cool and transferred into a sterile petri dished up to the mark and to solidify (Abdallah and Ali, 2018).

Gram staining techniques

Thin smear of about 200mm in diameter were formed on grease free slides which were also fixed over a burning flame. A crystal violet solution was used to cover the smear for 60 seconds and after that, distilled water was applied to decolorized the stain and acetone was applied, lastly the safranin solution was applied for counter stain on the surface for a minute, washed and allowed to dry at room temperature, then the stains were observed under microscope with oil immersion (Mada et al., 2012).

Preparation of the well diffusion

The method was adapted by (Mada *et al*., 2012). Water extract and the fraction was carried out using agar well diffusion method. Five holes were made on sterilized Mueller Hilton agar contained in a sterilized petri dishes. The organisms *E.coli* and *Shigella* were inoculated with the aid of wire loop, by streaking with the wire loop containing the inoculums in 1-10 sterile petri dishes for *E.coli* and 10 sterile petri dish for *Shigella.* The plates were rotated by 60° and rubbing procedure was repeated two times, to ensure and even distribution of the inoculums, it was then allowed to surface dry for 3-5 minutes, to allow the absorption of excess moisture. The well diffusion was done by using cork borer and the stocked extract was poured into the hole to ensure complete contact between. It was then incubated at 30℃ 16-18 hours. Clear zone of inhibitions were measured after 24 hours of incubation. The effects were compared with that of the standard antibiotic used as control (Tetracycline) (Mada et al., 2012).

The diameter of the clear zone (zone of inhibition) was measured to the nearest millimeter using transparent ruler. This was taken as the degree of sensitivity of the test organisms to Methanolic extract of *Tamarindus indica* (Mada et al., 2012).

Determination of minimum inhibitory concentration (mic)

Minimum inhibitory concentration (MIC) was determined for each of the extract showing antibacterial activity against the test pathogens. The MIC value were taken as the lowest concentration of the extract. In the well of the test tubes that showed no turbidity after incubation. The turbidity of the well in the test tubes were interpreted as visible growth of microorganisms (Mada et al., 2012).

Statistical tool

The package used for the data analysis was Statistix (SAS). Version 8.0 so as to know the level of significance among the variables.

Table 1. Physical characteristics of both methanolic and aqueous extract of *E.Coli* and *Shigella*

Extracts	Weigh conc (G)	% Yield	Physical appearance	Characteristics texture
Methanolic extract of *Tamarindus indica* leaves	60g	60	Brown	Crystals
Aqueous extract *T. indica* leaves	60g	66.0	Brown	Crystals
Methanolic extract of *T. indica* fruit	60g	50.0	Dark brown	Jelly
Aqueous extracts of T. indica fruits	6og	70	Dark brown	Jelly

Table 2. Qualitative analysis of phytochemical screening

Formula = $\frac{\text{Initial weight of sample}}{\text{weight of extract}} \times 100$

Phytochemical screening	Status *T. Indica* leaves	*T. Indica* fruits
Saponinins	+	+
Flavonoid	+	+
Tannin	+	+
Alkanoid	+	+
Phenol	-	-
Phytosterol	+	+

Key: Positive = + and Negative = -

Table 3. Antibacterial activity of *T. indica* against the isolates

Extracts	Zone of inhibition (mm/dm) *E. coli*	*Shigella*
F (M) E		
10mg/ml	11.0	0.0
20mg/ml	14.0	0.0

30mg/ml	9.0	15.0
40mg/ml	20.0	28.0
50mg/ml	13.0	10.0

Table 4. Showing the zone of inhibitions in various extracts against the test organisms

Treatment	EC	SGL
Extract (ml)		
L(m)E	12.800[a]	5.800[ab]
L(m)E	3.6000[b]	3.600[ab]
F(m)E	13.400[a]	10.600[ab]
F(m)E	3.600[b]	1.2000[b]
S.E	3.8678	4.4045
Sig.	*	NS
Conc. Levels (mg)		
10	4.0000[a]	3.0000[a]
20	7.2500[a]	2.2500[a]
30	6.0000[a]	6.2500[a]
40	10.250[a]	9.5000[a]
50	14.250[a]	5.5000[a]
S.E	5.1454	5.4268
Sig.	NS	NS

Means within a column followed by the same letters are statistically not significant at 5% Level of probability using Duncan's multiple range test (DMRT)
*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = E-Coli, SGL = Shigella*

Table 5. Showing the minimum inhibitory concentration of methanolic extracts of fruit of Tamarindus indica on test organisms

Treatment	Cex
Test Organism	
EC	0.2960[a]
SHG	0.1060[a]
S.E	0.1605
Sig.	NS
Conc. Levels (mg)	
10	0.0550[a]
20	495.07[a]
30	0.5250[a]
40	0.2400[a]
50	0.1150[a]
S.E	313.02
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)
*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = E-Coli, SGL = Shigella, Cex = Concentration of Extract*

Table 6. Minimum inhibitory concentration (MIC) of aqueous extract fruits of *Tamarindus Indica* on test organisms.

Test organisms	Concentration of extracts (mg/ml)				
	10	**20**	**30**	**40**	**50**
E. coli	0.0	0.0	0.0	0.0	0.13
Shigella spp	0.0	0.0	0.5	0.0	0.1

Formula: MIC = $\frac{\text{potency} \times \text{weight}}{\text{concentration}}$

Table 7. Showing the minimum inhibitory concentration of aqueous extracts of fruit of Tamarindus indica on test organisms

Treatment	Cex
Test Organism	
EC	0.0260[a]
SHG	0.1200[a]
S.E	0.1004
Sig.	NS
Conc. Levels (mg)	
10	0.0000[a]
20	0.0000[a]
30	0.2500[a]
40	0.0000[a]
50	0.1150
S.E	0.1584
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)
*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = E-Coli, SGL = Shigella, Cex = Concentration of Extract*

DISCUSSION

The study established the effects of the methanolic and aqueous extract of *Tamarindus indica*. The result of the phytochemical analysis revealed the presence of Alkaloids, sapanins, flavonoid, phytosterols, tannins, and phenol (kubmarawa *et al*., 2008). Consistently reported phytochemical bioactive ingredients of *T. indica* to be tannins, alkaloid, saponins, flavonoids, phenol and phytosterols. Therefore, the result of phytochemical analysis of the extracts of *T. indica* obtained in this study conforms to the previous report as shown in the table 2. The antibacterial effects of the leaves extracts of *T. indica* were determined in the comparison with the standard antibiotic (Tetracycline) against the test organisms as shown in the table 4, 5, 6 and 7. There was a significant difference between the zone of inhibitions by the extract and the antibiotic (Control), which agreed with that of (Abdallah and Ali, 2018).The effect of the extracts on the isolates were due to the presence of the phytochemical components of the extracts as reported in the previous by (Kubmatawa *et al*., 2008). The methanolic leaves extracts had a higher activity on *E.coli* followed by *Shigella*. The present findings agreed with the work of (Kubmatawa *et al*., 2008) and (Doughari et *al.,* 2006), that reported the inhibitory effect of the methanolic leaves of *T. indica* on the isolates. While the methanolic leaves extracts also show more activity in all the isolates as in aqueous extracts with slight differences among them. The methanolic leaves extract had better activity than the aqueous extracts. This shows that methanol extracted the bioactive ingredients than that of aqueous in this study.
Conclusion.

Plants extracts generally are utilized in treating so many ailments most especially in rural areas based on their beliefs. However, *T. indica* has been identified as active plants in curing many microbes.

Conflict of interest

As far as this research is concerned, there was no conflict of interest.

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