



International Journal of Traditional and Complementary Medicine  
(ISSN: 2474-1361)



## Phytochemical Screening and Antimicrobial Activity of *Tamarindus indica*

Ushie O.A\*, Egwaikhide, P.A and Longbab B.D

Department of Chemical Science, Federal University, Wukari Nigeria

### ABSTRACT

The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. The phytochemical screening of crude yields of the chemical constituents of *Tamarindus indica* showed that alkaloids, flavonoids, and tannins are present in all the extracts are present in all the leaf extracts. Activity of the crude hexane, chloroform, ethyl acetate acetone and methanol extracts from the leaf of *Tamarindus indica* were tested on five clinical isolates; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *penicillium spp* Augmentin and mycotin were used as control drugs. All the crude extracts of the leaf inhibited or exhibited antibacterial activity against all the bacteria pathogens tested with a diameter that ranged between 8 – 26 mm. All the crude extracts of the leaf inhibited or exhibited antifungal activity against *Penicillium Spp* with a diameter that ranged between 8 - 13 mm but did not show significant inhibition against *A. niger*. The minimum inhibitory activity (MIC) of the extracts of *Tamarindus indica* against tested microbes ranges from 400 to 100 mg/ml in all the extracts against the tested bacteria. The minimum inhibitory activity (MIC) of the extracts of *Tamarindus indica* against tested microbes ranges from 400 to 200 mg/ml in almost all the extracts for the tested fungi.

### \*Correspondence to Author:

Ushie O.A

Department of Chemical Science, Federal University, Wukari Nigeria

e-mail: [afiushie@yahoo.com](mailto:afiushie@yahoo.com)

### How to cite this article:

Ushie O.A, Egwaikhide, P.A and Longbab B.D. Phytochemical Screening and Antimicrobial Activity of *Tamarindus indica*. International Journal of Traditional and Complementary Medicine 2016; 1(2): 0010 - 0017.

Published July 27 2016.

**eSciencePublisher**  
eSciPub LLC, Houston, TX USA.  
Website: <http://escipub.com/>

## Introduction

Most of the people in the rural areas of the world depend largely on herbs for treatment of several ailments because medicinal herbs constitute indispensable components of traditional medicine practice due to low cost, easy access and ancestral experience (Marini-Bettolo, 1980). There is evidence of herbs been used in the treatment of diseases and for revitalizing the body system in almost all ancient civilization (Manjula *et al.*, 2013). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. In developing countries majority of the people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases. Many indigenous plants are used as food plants or crops. They are sometimes added to food meant for pregnant and nursing mothers for medicinal purposes (Adamu *et al.*, 2015). It is a rich source of most of the essential amino acids and phytochemicals, and hence the plant is reported to *T. indica* has ameliorative effects on many diseases (Kuru 2014). It can also be preferred as a nutritious support for malnourished patients as it is cheap and easy to access. Throughout Southeast Asia, fruit of the tamarind is used as a poultice applied to foreheads of fever sufferers (Doughari, 2006) Kuru 2014 pointed out that *T. indica* is used for abdominal pain, diarrhea and dysentery, some bacterial infections and parasitic infestations, wound healing, constipation, possess antidiabetic, antimicrobial, antivenomic, antioxidant, antimalarial, cardioprotective, hepatoprotective, antiasthmatic, laxative and anti-hyperlipidemic activity and inflammation. The plant kingdom still holds many species of plants containing substances of medicinal value, which are yet to be discovered in Zing Local Government Area of Taraba State, Nigeria. *Tamarindus indica* leaf is one part of the plants which have been used in traditional medicine for many years. To the best of our knowledge from the available literature there is no work which has been done on the plant *Tamarindus indica* leaves, in Zing Local Government Area. The present work is designed to enrich the available scientific data on the phytochemistry and antimicrobial activities *T. indica* leaf extracts.

## MATERIALS AND METHODS

### Sample Collection and Preparation

*Tamarindus indica* leaves were collected from their natural habitat of Zing Local Government Areas of Taraba state, Nigeria. The samples were air-dried for two weeks and then milled into fine powder using a milling machine.

### Method of Extraction

The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. The extracts of the leaves was prepared by soaking 100 g of each in 250 ml hexane for four days with frequent agitation until soluble matter is dissolved. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotatory evaporator, kept in a vacuum oven over night at room temperature to remove all the solvent and weighed. The procedure was repeated on the residue using the following solvents: Chloroform, ethyl acetate, acetone and methanol sequentially in order of polarity. The extracts were stored in a desiccator until required for testing.

### Phytochemical Screening Assay

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989), Harborne (1988).and Ushie *et al.*, 2013

### Test for Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue solution indicated the absence of tannins in distilled water and dark green colour indicating presence in methanol.

### Test for Saponins

About 0.2g of plant extract was mixed with distilled water and heated to boil. Frothing (appearance of creamy mix of small bubbles) showed the presence of Saponins in Methanol while red in Distilled water.

**Table 1: Phytochemical screening of *Tamarindus indica***

S/N	Phytochemicals	HE	CE	EAE	AE	ME
1	Alkaloids	+	+	+	+	+
2	Phlobatanins	-	-	-	-	-
3	Flavonoids	+	+	+	+	+
4	Saponins	-	-	-	-	-
5	Cardiac glycosides	-	-	-	-	-
6	Terpenoids	-	-	-	-	-
7	Steroids	-	-	-	-	-
8	Tannin	+	+	+	+	+

HE = Hexane extract, CE = Chloroform, AE = Acetone extract, EAE = Ethyl acetate extract,

ME = Methanol extract, L: Leaves, +: Present, -: Not present

**Table 2: Mean Zone of Inhibition of *Tamarindus indica***

Organisms	Conc. (Mg/ml)	HE	CE	EAE	AE	ME	C (+)	DMSO (-ve)
<i>Pseudomonas aeruginosa</i>	400	13	12	12	12	20	30	00
	200	11	08	12	12	14	22	00
	100	10	07	07	11	11	12	00
	50	00	07	06	08	08	10	00
<i>Staphylococcus aureus</i>	400	11	14	20	14	23	33	00
	200	09	10	14	12	11	21	00
	100	06	08	12	10	10	18	00
	50	00	00	07	06	08	12	00
<i>Escherichia coli</i>	400	11	27	28	11	26	32	00
	200	09	19	13	09	19	25	00
	100	05	14	14	05	13	18	00
	50	00	09	07	00	07	NA	00
<i>Aspergellius Niger</i>	400	06	06	06	07	06	NA	00
	200	03	03	02	05	05	NA	00
	100	04	05	03	03	03	NA	00
	50	00	00	00	00	00	NA	00
<i>Penicillium Spp</i>	400	12	11	13	09	19	NA	00
	200	08	07	09	05	13	NA	00
	100	07	05	05	05	09	NA	00
	50	00	00	00	00	00	NA	00

Key: HE = Hexane extract, CE= Chloroform, EAE= Ethyl acetate extract, AE = Acetone extract, ME = Methanol extract, Values greater than 7 mm indicate activity and 00 means no activity

### Test for Terpenoids

The extract (0.2g) was mixed with 2ml of chloroform, and 3ml of concentrated  $H_2SO_4$  was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids on both extract.

### Test for Steroids

Acetic anhydride (2 ml) was added to 0.5g of the extract in a test tube. It was then followed by the addition of 2 ml of sulfuric acid. A colour change from violet to blue or green indicated the presence of steroids on both extract.

### Test for Flavonoids

About 0.2g of the extract was dissolved in dilute sodium hydroxide solution, and equal amount of hydrochloric acid was added. A yellow solution that turned colourless indicated the presence of flavonoids on both extract.

### Test for Alkaloids

The aqueous (3ml) was stirred with (3ml) of 1% HCl on a steam bath. Meyer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as positive evidence of alkaloids

### Test for phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Disposition of red precipitate determines the presence of phlobatannins.

### Test for Anthraquinones

About 0.5g of the extract was boiled with 2ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10%  $NH_3$  solution was added to the mixture and heated. Formation of rose pink colour indicated the presence of anthraquinones on both extract.

### Test for Cardiac glycosides

10  $cm_3$  of 50%  $H_2SO_4$  was heated in boiling water for 5 min. 10  $cm_3$  of Fehlings solution (5  $cm_3$  of each

solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

### Bioassay

This is the study of antimicrobial activity of the crude or purified extracts against micro-organism. It was used as a guide to determine the active components of the leaves of *Ximenia americana*. The crude extracts were tested for antibacterial and antifungal activities. The test organisms were collected from Bauchi Specialist Hospital, Bauchi State, Nigeria. The antibacterial assay was carried out using methods described by Ochi *et al.*, (2015) with modifications.

### Preparation of varying concentrations of the extracts

Various concentrations of the extracts were prepared ranging from 50 to 400 mg/mL; this was obtained by measuring 1 mg of the extract and dissolved in 10 mL dimethyl sulphur oxide (DMSO), a solvent that dissolved the extract (100 mg/mL). A serial dilution of the dissolved extract (100 mg/mL) was carried out into three different bottles containing DMSO to obtain concentrations of 400, 200 100 and 50 mg/mL respectively.

### Sensitivity test of the crude extract using Agar Well Diffusion Method

The organisms used were standardized using McFarland turbidity standard scale I, to obtain a bacterial cell density of  $10^6$  colony forming unit per millilitre (cfu/mL). The standardized inoculate were uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextrose agar plates respectively for the bacterial and fungal growth. Five wells were made on the inoculated plates with a cork borer (8 mm in diameter). The wells were properly labeled according to different number of the concentrations prepared. The wells were then filled up with the extracts about 0.2 mL per well. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37°C for three days while the potato dextrose agar plates for fungi were incubated at room temperature (drawer) for three days. At the end of incubation period, all plates were observed for any evidence of inhibition, which will appear as clear zones that were completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured with a transparent ruler

**Table 3: Showing Minimum Inhibitory concentration (MIC) and Minimum Bacteriacidal Concentration in milligram per millilitre (mg/ml) of Sample B**

Organism	Method	MIC AND MBC (mg/ml)										Msc	OVC
		Chloroform		Acetone		Hexane		Ethylacetate					
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC		
<i>P. aeruginosa</i>												+	+
		100	200	100	200	100	200	100	200	100	200		
<i>S. aureus</i>		100	400	100	200	100	200	100	400	200	400	+	+
<i>E. Coli</i>		100	200	100	200	100	200	00	200	100	200	+	+
<i>A. Niger</i>		200	400	200	400	200	400	200	400	200	400	+	+
<i>Penicillium Spp</i>		100	200	100	400	100	400	100	400	200	400	+	+

Key :- Msc = Media Sterility Control OVC =Organism Viability Control



calibrated in millimeter (mm).

### Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB). Each tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism was used as a control. All tubes were then incubated at 37°C for 24 hours. After the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

### Determination of Minimum Bactericidal Concentration (MBC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the Minimum Bactericidal Concentration (MBC). A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test, it was then streaked onto a freshly prepared sterile nutrient agar plates. The plates were incubated at 37°C for 24 hours. After the incubation period the plates were then examined for the presence or absence of growth. This was done to determine if the antimicrobial effect of the extract was bactericidal or bacteriostatic.

## RESULTS AND DISCUSSION

### Results

Table 1 presents the results of phytochemical screening of leaf solvent extracts of *Tamarindus indica*. The phytochemical screening of crude extracts of *T. indica* revealed the presence of alkaloids, flavonoids and tannins in all the extracts, while other phytochemicals screened for were not dictated.

### Discussions

The phytochemical screening of crude yields of the chemical constituents of *Tamarindus indica* showed that alkaloids, flavonoids, and tannins are present in all the extracts are present in all the leaf extracts. These classes of secondary metabolites are known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993). These classes of phytochemical compounds are known to show curative activity against several bacteria and it is not surprising that these plant extracts are used traditionally by herbalist to cure bacteria related ill-health (Njoku and Obi 2009). *Tamarindus indica* can be used as an analgesic, anaesthetic and as social drugs since it contains alkaloids. The alkaloids contained in plants are used in medicine as anaesthetic agents (Herourat *et al.*, 1988). Harborne (1988) also reported on analgesic properties of alkaloids. Alkaloids has contributed to the majority of the poisons, neurotoxins and traditional psychedelics and social drugs [e.g. nicotine, caffeine, methamphetamine (ephedrine) cocaine, and opiates] consumed by humans (Zenk and Juenger 2007).

Okoli and Okere (2010) pointed out that flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth. The beneficial effects of fruit, vegetables, and tea or even red wine have been attributed to flavonoid compounds rather than to known nutrients and vitamins (Félicien, 2008). Saponins causes complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection. (Francis *et al.*, 2002). There is tremendous, commercially driven promotion of saponins as dietary supplements and nutraceuticals. There is evidence of the presence of saponins in traditional medicine preparations (Xu, *et al.*, 1996).

Activity of the crude hexane, chloroform, ethyl acetate acetone and methanol extracts from the leaf of *Tamarindus indica* were tested on five clinical isolates; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *penicillium spp* Augmentin and mycotin were used as control drugs. The measured zones of inhibition of the pathogens by the crude extracts are presented in the Tables 2 and 3. All the crude extracts of the leaf inhibited or exhibited antibacterial activity against all the bacteria pathogens tested with a diameter that ranged between 8 – 26 mm. All the crude extracts of the leaf inhibited or exhibited antifungal activity

against *Penicillium Spp* with a diameter that ranged between 8 - 13 mm but did not show significant inhibition against *A. niger*. The minimum inhibitory activity (MIC) of the extracts of *Tamarindus indica* against tested microbes ranges from 400 to 100 mg/ml in all the extracts against the tested bacteria. The minimum inhibitory activity (MIC) of the extracts of *Tamarindus indica* against tested microbes ranges from 400 to 200 mg/ml in almost all the extracts for the tested fungi.

The activities of these extracts against tested pathogens could not be unconnected to the presence of the plant secondary metabolites contained in the plant. The various concentrations were visibly active on the tested bacterial isolates due to the combinative therapeutic actions of the various secondary metabolites contained in the plants (Ushie *et al.*; 2013). The ability of the extracts to inhibit the growth of *E. coli* indicates that this plant drug can be used in the treatment of gastroenteritis that has been associated with *E. coli* (Etani *et al.*, 1999). Also the inhibition of growth of *S. aeruginosa* which are aetiology agents of urinary tract infection (Latta *et al.*, 1998, Tolson, 1997) have shown that the active components of the plant leaf extract can cure any disease caused by these organisms. The inhibition the growth of *Pseudomonas aeruginosa* showed that the active component of the plant drug can cure any disease such as blood stream infections associated with the organism (Okoro 2012).

## Conclusion

The phytochemical screening of crude extracts of *Tamarindus indica* showed that alkaloids, flavonoids and tannins in all the extracts, while other phytochemicals screened for were not detected. Tannins were detected in all the extracts, hence, *T. indica* can be used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoids, and diarrhea, and as antidote in heavy metal poisoning. Activity of the crude hexane, chloroform, ethyl acetate acetone and methanol extracts from the leaf of *T. indica* were tested on five clinical isolates; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Penicillium spp* Augmentin and mycotin were used as control drugs. All the crude extracts of the leaf inhibited or exhibited antimicrobial activity against all the pathogens tested. The minimum inhibitory activity (MIC) of the extracts of *T. indica* against tested microbes ranges from 400 to 100 mg/ml in almost

all the extracts and in few instance 50 mg/ml against the tested bacteria. The minimum inhibitory activity (MIC) of the extracts of *T. indica* against tested microbes ranges from 400 to 200 mg/ml in almost all the extracts for the tested fungi. These could explain the rationale for the use the plant in the treatment of the various conditions in traditional medical practice.

## Discussions

## Acknowledgments

The tertiary education trust fund (TETFUND INSTITUTION-BASED RESEARCH INTERVENTION) and Federal University Wukari (FUW) are thanked for providing financial support.

## References

- Adamu, H.M, Ushie, O.A, Ogah E, Longbap B and Dawud AU (2015). Phytochemical Screening and Antimicrobial activities of the Leaf extracts of *Mormodica balsamina*. *Ew J Microb Res*1(1): 16-19
- Doughari, J. H. (2006). Antimicrobial Activity of *Tamarindus indica*. *Tropical Journal of Pharmaceutical Research*.5 (2): 597–603.
- Félicien Breton (2008). «Health benefits of oligomeric proanthocyanidins». <http://www.frenchscout.com/polyphenols#procyanidins>.
- Etani . E.; Agai.M.; Tsukamoto, T, Ohta .M. (1998). Antibacterial action of Vinegar against food:borne pathogenic bacteria including *Echerchia coli* *Journal of food protection* 61(8) 953-959
- Francis, George; Zohar Kerem, Harinder P. S. Makkar and Klaus Becker (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition* 88 (6): 587–605.
- Harborne, J.B. (1988): Introduction to Ecological Biochemistry.3<sup>rd</sup> edition. Academic Press, London. 10-15.
- Herourat D, Sangwin R S, Finiaux M A, Sangwan-Norrel B S (1988). Variations in the leaf alkaloid content of androgenic diploid plants of *Daturinnoxia*, *Planta medica J. Med. Plant Res*.54:14-20.
- Kuru Pinar (2014) *Tamarind indica* and its health related effects *Asian Pacific Journal of Medicine* 4(9), 676-681
- Latta R.K., Schu M.J., Tolson D.E (1998) The effect

of growth conditions on in vitro adherence invasion and expression by proteus mirabilis 7570. *Canadian journal of microbiology*, **44**(9): 896-904.

Manjula Gupta, Ankur Kashyap, A.K.Mishra and M. Serajuddin (2013). Studies on Insecticidal Efficacy of some Plant Product for the Control of Predatory Aquatic Insects. *Research Journal of Life Sciences*, **01**(01), 1-5

Marini-Bettolo, GB (1980). Present aspects of the use of medicinal plants in traditional medicine. *J. Ethnopharmacol* **2**. 5-7.

Njoku O.V and Obi.C (2009). Phytochemical constituents of some selected medicinal Plants. *African Journal of Pure and Applied Chemistry* **3**(11), 228-233

Ochi I.O. Ekirigwe O.C, Longbap B.D., Abiazim C.V., Tabe N.T. (2015) Phytochemical analysis and antimicrobial screening of dried root extracts of *Alchornea cordifolia* *Ewemen Journal of microbial research* **1**(1): 25 -30

Okoli BJ, Okere OS. (2010) Antimicrobial Activity of the phytochemical constituents of *Chrysophyllum albidum* G.Don-Holl (African star apple) plant. *Journal of Research in National development*. **8**(1):356.

Okoro I.S (2012) Antimicrobial Effects of Blood Tree (*Harugana madagascariensis* lam ex. Pior) on some Human Pathogens. *Journal of Medical and Applied Sciences* Cenresin Publications **4**, 78-86

Sofowora, A. (1993) Medicinal Plants and Traditional Medicines Africa, Spectrum Books Ibadan

Tolson R.A.; Latta H.; R.A Lewe KK.; Altman E.; (1997) The expression of nonagglutinating fimbriae and its role in proteus mirabilis adherence to epithelial cells *Canadian journal of microbiology* (43) 8:709-717

Trease.G.E. and Evans.W.C (1989) Pharmacognsy 14<sup>th</sup> ed Baillere.Tindall London pp 176-180

Ushie O A, Neji, P.A and Nsor, G.E (2013) Phytochemical Screening and Antimicrobial Activities of *Phyllanthus Amarus* Stem Bark Extracts” *International Journal of Modern Biology and Medicine*, **3**(3): 101-112

Ushie O A, H.M. Adamu, O. J Abayeh and I.Y. Chindo (2013). Antimicrobial Activities of *Chrysophyllum albidum* Leaf extracts. *International Journal of Chemical Sciences* **6**(1), 69-76

Xu R. Zhao W. Xu J. Shao B, Qin, G. (1996). Studies on bioactive saponins from Chinese medicinal plants. *Advances in Experimental Medicine and Biology*. **404**, 371-82

Zenk MH, Juenger M (2007). Evolution and current status of the phytochemistry of nitrogenous compounds. *Phytochemistry*. **68**: 2757–72

