



Phytochemical Screening and Antibacterial Activity of *Mangifera indica* Extracts

*¹Mohammed, A. H., ²Na'inna, S. Z., ³Yusha'u, M., ⁴Salisu, B., ⁵Adamu, U. and ⁶Garba, S. A.

¹Department of Biology, Science and Technical College, P.M.B. 008, Hadejia

²Department of Biological Sciences, Federal University, Kashere, P.M.B. 0183, Gombe state

³Department of Microbiology, Bayero University, P.M.B. 3011, Kano

⁴Department of Microbiology, Umaru Musa Yar'adua University, Katsina

⁵Medical Laboratory Department, Hadejia General Hospital, Jigawa State

⁶Chemistry Department, Sule Lamido University, Kafin Hausa

*Correspondence author email: khsabdul@gmail.com, khsabdul@yahoo.com

Abstract

Mangifera indica leaves are used for the treatments of various ailments in folklore medicine. This research was aimed to determine phytochemical composition and antibacterial activity of leaves extracts of *Mangifera indica*. Powdered leaves of *Mangifera indica* were extracted with water, ethanol and chloroform solvents via percolation method. The extracts were tested for antibacterial activity against clinical isolates of *Salmonella* Typhi, *Salmonella* Paratyphi A and *Salmonella* Paratyphi B using agarwell diffusion method. The extracts were further subjected to phytochemical tests for the presence of secondary metabolites using standard procedures. Results of the sensitivity test showed that highest zone of inhibition was observed in ethanolic extract with 13mm for *S. Paratyphi* A, 11mm for *S. Paratyphi* B, and 10mm for *S. Typhi*, followed by aqueous extract with 11mm for *S. Paratyphi* B and 10mm for *S. Typhi*. *S. Paratyphi* A, was resistant to both aqueous and chloroform extract of *M. indica* while *S. Paratyphi* B was only resistant to chloroform extract. Results of phytochemical screening indicated the presence of alkaloids, flavonoids, steroids, tannins and phenols.

Key words: Antibacterial activity, agar well diffusion, phytochemical tests, sensitivity and zone of inhibition.

INTRODUCTION

Plants have for generations been a source of various kinds of remedies and been used for medicinal purpose to cure different types of ailments and will continue to provide remedies for these ailments, especially in rural areas of developing countries. Consumption of medicinal herbs is tremendously increasing over past decades as an alternative approaches to improve the quality of life and to maintain good health (Pintu and Arna, 2014). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al.* 2006).

Mangifera indica is a large evergreen tree, with a heavy, dome-shaped crown. It belongs

to the family Anacardiaceae. It is found all over the tropical regions of the world where it is used as a horticultural and medicinal plant. Fruits contain protein, fat, carbohydrate, minerals, vitamins A, B and C and amino acids. The fruits also yield a resin that is said to contain mangiferene, mangiferic acid, resinol and maniferol and others (Wauters, *et al.*, 1995; Dweck, 2001). The leaves contain the glucoside mangiferine. The bark of the mango tree contains 16–20% tannin (Wauters *et al.*, 1995; Dweck, 2001).

Various parts of Mango plant are used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhea, dysentery, anaemia, asthma, bronchitis, cough,

hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles (Shah *et al.* 2010).

The aim of this research was to determine the phytochemical composition of the leaves extract of *M. indica* used as traditional medicine in northern Nigeria for the treatment of typhoid fever (either singly or in combination with other leaves) and also to assess its antibacterial potential against clinical isolates of *Salmonella* Typhi, *Salmonella* Paratyphi A and *Salmonella* Paratyphi B.

MATERIALS AND METHODS

(a) Collection and identification of Plant Materials

The plant leaves were collected from Bayero University, Old campus, Kano, Nigeria. Their identity was confirmed by a botanist, in the Department of Plant Biology, Bayero university, Kano and voucher specimen was deposited in the departmental herbarium. The leaves after collection were air-dried under shade. Dried leaves were then pounded to a fine powder for ease of extraction of active compounds (Aliyu, 2006).

(b) Extraction

Fifty grams (50g) each of the dried powder of the plant leaves was transferred into 3 different glass containers. The powdered leaves were sequentially extracted with 250ml each for Ethanol and chloroform. For water 500ml was used. The extraction method employed was percolation for a week, during which the bottles were undergoing shaking at regular intervals. The extracts were filtered using Whatman No. 1 filter paper. Each of the resulting filtrate was then concentrated by complete evaporation of solvent at room temperature except for aqueous extract which was evaporated in a water bath at 100°C. The filtrate was carefully labeled and stored in the refrigerator for further use (Fatope *et al.*, 1993).

(c) Phytochemical screening

i. Test for alkaloids

Aliquots of the extracts (0.1ml) were added in test tubes and then 2 to 3 drops of Dragendoff's reagent were added. An orange red precipitate indicated the presence of alkaloids (Ciulci, 1994).

ii. Test for flavonoids

To 4 mg/ml of each of the extracts, a piece of magnesium ribbon was added this was followed by concentrated HCl drop wise. A colour change ranging from orange to red indicated flavones while red to crimson indicated flavonoids (Sofowora, 1993).

iii. Test for saponins

Half gram of the extract was dispensed in a test tube. Five milliliters of distilled water was added to the tubes and it was stirred vigorously. A persistent froth that lasts for about 15 min indicated the presence of saponins (Sofowora, 1993).

iv. Test for steroids

Two milliliters of the extracts were taken into separate test tubes. The residues were dissolved in acetic anhydride and chloroform was then added. This was followed by the addition of concentrated sulfuric acid by the side of the test tubes using a pipette. A brown ring at the interface of the two liquids and a violet colour in the supernatant layer denoted the presence of steroids (Ciulci, 1994).

v. Test for tannins

Two milliliters of each aliquots of the extract was diluted with distilled water in separate test tube and 2 to 3 drops of 5% ferric chloride (FeCl₃) solution was added. A green – black or blue colouration indicated the presence of tannins (Ciulci, 1994).

vi. Test for glycosides

Ten milliliters of sulfuric acid (50% v/v) was added to 1 ml each of the *M. indica* leaf extracts in separate test tubes. The mixtures were heated for 15 min.

Ten milliliters of Fehling's solution was added to tubes and the mixture boiled. A brick red precipitate indicated presence of glycosides (Sofowora, 1993).

vii. Test for Terpenoids

To 0.5g of the plant extracts 2 mL of chloroform was added. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase formation show positive results for the presence of terpenoids (Ayoola *et al.*, 2008)

viii. Test for phenols

Extracts were treated with few drops of ferric chloride solution. Formation of bluish-black colour indicates the presence of phenols (Sowofora, 1993)..

BIOASSAY STUDIES

(a). Isolates collection and Biochemical tests

Clinical bacterial isolates comprising of *S. Typhi*, *S. Paratyphi A* and *S. Paratyphi B* were obtained from Aminu Kano Teaching Hospital, Kano, Nigeria and their identity was confirmed using biochemical tests for identification of catalase, oxidase, indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production (Cheesbrough, 2006).

(b). Media preparation

Mueller Hilton Agar and Nutrient broth were prepared according to manufacturer's specifications.

(c). Standardization of Inoculum

Using sterile inoculation wire loop, 3-4 colonies from an overnight culture of the test organism was transferred into a tube of saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

(d). Antimicrobial Susceptibility Test

The agar well diffusion method was used for the antimicrobial susceptibility test. Mueller Hilton agar was prepared according to manufacturer's specification. The media were autoclaved and dispensed into sterile petri-dishes and allowed to gel. Standardized inocula of each bacterial isolates were streaked on the agar plate. Four wells of 6mm each was made in each plate with a central well for control using a sterile cork borer. The wells were filled with 0.1ml of different concentrations (400 μ /ml, 200 μ /ml, 100 μ /ml and 50 μ /ml) of the extract with the aid of sterile pipettes per well. Likewise, 400 μ /ml, 200 μ /ml, 100 μ /ml and 50 μ /ml of the standard antibiotic (amoxicillin) were used in separate plates to serve as positive control. While sterile distilled water was used as negative control on separate plates. The plates were allowed to stand for 15 minutes on a table to allow free diffusion of the extracts. Diameters of zones of inhibition were measured using transparent plastic metre rule after 24 hours of incubation at 37°C (Dahiruet *al.*, 2013).

(e). Minimum Inhibitory concentration

MIC was determined by preparing various concentrations of the extracts by serial doubling dilution and incorporated into test tubes containing 2 ml nutrient broth. Standardized inocula of 0.1 ml of the isolates were inoculated and the tubes were incubated at 37°C for 24 h (NCCLS, 2008).

(f). Minimum Bactericidal Concentration (MBC)

Nutrient agar plates were inoculated with sample from each of the tubes that show no turbidity and the plates were incubated at 37°C for 24 h to determine the MBC. MBC was determined by inoculating samples from the MIC tubes that showed no bacterial growth on Mueller Hilton agar plates separately and then incubated at 37°C for 24 hours. After the incubation the plates were observed for presence or absence of growth. The least concentration of the extract that showed no bacterial growth was considered as the MBC (NCCLS, 2008).

RESULTS AND DISCUSSION

The physical properties and percentage yield of the aqueous, ethanolic and chloroform extracts of *M. indica* is shown in (Table 1). The highest percentage yield of the extract was observed in aqueous extract which was 13.24%w/w of the total sample extracted, followed by ethanolic extract with 6.76% w/w and lastly chloroform extract with the least of 2.46%w/w. This indicates that the plants components are more soluble in high polar solvents. It can therefore, be deduced that the amount of extracts recovery is polarity dependent.

This research work corroborates with similar findings including that of Pintu and Arna (2014) who reported that water extract of *Mangifera indica* young leaves contain tannins, alkaloids, steroid, carbohydrate, glycoside and flavonoid that may be responsible for the anti-diarrheal properties of the crude extract. Also Pritesh and Zara (2015) reported that saponins may help to prevent colon cancer. Flavonoids possess antiallergic, anti-inflammatory, antiviral and antioxidant activities and steroids are used to suppress various allergic, inflammatory and autoimmune disorders. Therefore the antimicrobial activity observed in *M. indica* extracts may be attributable to the presence of the above phytochemicals. Similarly, Harborne and Williams (2000) revealed that, Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids have been reported to possess many useful properties, including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activity. Flavonoids have been referred to as nature's biological response modifiers, because of inherent ability to modify the body's reaction to allergies. It possesses various pharmacological roles including anti-allergic, anti-inflammatory, cardio-protective, anti-microbial and anticancer activities (Duraipandiyani *et al.*, 2006).

The result of the antibacterial activity of *M. indica* is shown (Table 3). From the result highest zone of inhibition was observed in

ethanolic extract with 13.0mm for *S. Paratyphi A*, 11mm for *S. Paratyphi B*, and 10.0mm for *S. Typhi*. This is followed by aqueous extract with 10.5mm for *S. Paratyphi B* and 10mm for *S. Typhi*. *S. Paratyphi A*, was resistant to both aqueous and chloroform extract of *M. indica*. *S. Paratyphi B* was also resistant to chloroform extract. It can therefore, be deduced that, chloroform extract of *M. indica* was the least bioactive. It is pertinent however, to state that, lowest concentration of 50 and 100µ/ml showed little or no activity on the test bacteria. Thus, the bioactivity of the extracts followed the sequence: Combined Extract > Ethanolic Extract > Aqueous Extract > Chloroform Extract. While the hierarchy of the susceptibility pattern of the tested bacteria to the extract is: *S. Paratyphi A* > *S. Paratyphi B* > *S. Paratyphi C*.

In similar research study Odunbaku *et al.*, (2012) reported that the two trees extracts (*Mangifera indica* and *Citrus aurantifolia*) have considerable inhibitory effects on *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Aspergillus terreus*, *Aspergillus niger* and *Penicillium oxalicum*.

Shanjida *et al.*, (2015) reported that, different concentrations of the methanolic extracts of the leaves of *Mangifera indica* exhibited antimicrobial activities against all the isolates of bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*).

The MIC and MBC result is shown in (Table 4). From the result both aqueous, ethanolic and chloroform extracts indicated an MIC range of (12.5µg/ml - 50µg/ml) and MBC range of (50µg/ml - 100µg/ml, 25µg/ml - 400µg/ml and 25µg/ml - 100µg/ml) for aqueous, ethanolic and chloroform extracts respectively. While the standard antibiotic (amoxicillin) had MIC and MBC ranges of (6.25µg/m-25µg/m). However, from the finding of this study it is enough to state that ethanolic extracts had the lowest MIC values when compared to the other extracts. Ethanolic extracts similarly had the least MBC values.

RESULTS

Table 1: Physical Properties of the Extract

Solvent	Color	Odor	Texture	Weight of sample (g)	Quantity Recovered(g)	% Yield
Chloroform	Dark green	Odorless	Slightly sticky	50	1.23	2.46
Ethanol	Dirty green	Chemical	Slightly sticky	50	3.37	6.74
Aqueous	Reddish Brown	Odorless	Gummy	50	6.62	13.24

Table 2: Phytochemical composition of *M. indica* Extracts

EXTRACT	Alkds	Sap	Tan	Flavonoids	Steroids	Glycosides	Terpenoids	Phenols
Aqueous	+	-	+	+	+	-	-	-
Ethanolic	+	-	+	+	+	-	-	+
Chloroform	+	-	+	+	-	-	-	+

Key: + = present, - = absence, Alk = alkaloids, Sap = saponins, Tan = tannins

Table 3: Antibacterial Activity of *M. indica* Extracts

Bacteria	AE ($\mu\text{g/ml}$)				EE ($\mu\text{g/ml}$)				CE ($\mu\text{g/ml}$)				Amx ($\mu\text{g/ml}$)			
	Concentration/zone of inhibition															
	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400
STA	0	0	9	10	7	8	9	10	0	0	8	10	12	12	15	18
SPA	0	0	0	0	0	0	10	13	0	0	0	0	9	11	11	14
SPB	0	7	9	11	0	0	8	11	0	0	0	0	8	9	14	16

Key: AE = Aqueous Extract, EE = Ethanolic Extract, CE = Chloroform Extract, Amx = Amoxicillin, STA = *S. Typhi*, SPA = *S. Paratyphi A*, SPB = *S. Paratyphi B*

Table 4: MIC and MBC of *M. indica* Against the TestBacteria

Test Organisms	Aqueous Extract		Ethanolic Extract		Chloroform Extract		Amoxicillin	
	MIC $\mu\text{g/ml}$	MBC $\mu\text{g/ml}$	MIC $\mu\text{g/ml}$	MBC $\mu\text{g/ml}$	MIC $\mu\text{g/ml}$	MBC $\mu\text{g/ml}$	MIC $\mu\text{g/ml}$	MBC $\mu\text{g/ml}$
<i>S. Typhi</i>	50	100	50	100	50	100	12.5	25
<i>S. Paratyphi A</i>	25	50	12.5	400	25	50	6.25	25
<i>S. Paratyphi B</i>	50	100	12.5	25	25	50	6.25	12.5

REFERENCES

- Aliyu, B. S. (2006): *Some ethno-medicinal plants of the Savannah Regions of West Africa Description and phytochemicals*. Triumph publishing company. Pp 135-152.
- Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. and Atangbayila, T.O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7, 1019-1024.
- Brain, K.R. and Turner, T.D. (1975): *The practical evaluation of phytochemicals*. Wright Sciencetehna, Bristol: Pp.57 – 58.

- Cheesbrough, M. (2006): *Biochemical tests to identify bacteria*. District Laboratory practice in Tropical Countries. Cheesbrough M (ed.), Cambridge, Part II: Pp 63-90.
- Ciulci, I. (1994): Methodology for the analysis of vegetable drugs. Chemical industries branch, Division of industrial operations. UNIDO, Romania: Pp.24 - 67.
- Dahiru, D., Malgwi, A. R. and Sambo, S. H. (2013): Growth Inhibitory Effect of *Sennasiamea* Leaves Extracts on Selected Microorganisms. *American Journal of Medicine and Medical Sciences*, **3**(5): 103-107.
- Duraipandiyar, V., M., Ayyanar L. and Ignacimuthu S. (2006): Antimicrobial activity of some ethnomedicinal plants. *Asian Journal of Microbiology*; **5**: 334-337.
- Dweck, A.C. (2001): Article for cosmetics and toiletries magazine ethnobotanical plants from Africa. Black Medicare Ltd, Itshire, UK.
- Fatope, A., O., Ibrahim, H. and Takeda, Y. (1993): Screening of higher plants reputed as pesticides using brine shrimp lethality bioassay. *International Journal of Pharmacognosy*, **31**: 250-256.
- Harborne, J. B. and Williams, C.A. (2000): Advances in flavonoid research since 1992. *Phytochemistry Journal*; **55**: 481-504.
- National Committee for Clinical Laboratory Standards (NCCLS, 2008): Performance standards for Antimicrobial Susceptibility Testing: Ninth Informational Supplement. NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, PA.
- Odunbaku, O. A. and Ashidi, J.S. (2012): Antimicrobial effect of ethanol leaf extracts of selected medicinal plants on some human pathogenic microbes. *International Journal of Science and Advanced Technology*; **2**:8.
- Pintu, K. D. and Arna, P. (2014): Effects of aqueous young leaves extract of *Mangifera indica* on (-) microorganisms causing gastrointestinal disorders. *Asian Journal of Plant Science and Research*; **4**(1): 23-27.
- Pritesh, R. D. and Zara S. (2015): Preliminary studies on phytochemicals and cytotoxic activity of methanolic rhizome extract of *Hedychium coronarium*. *Journal of Pharmacognosy and Phytochemistry*; **4**(1): 136-139
- Sandhya, B., Thomas, S., Isabel, W. and Shenbagarathai, R. (2006): Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (reserved forest). *Journal of Complementary and alternative medicines*, **3**: 101-114.
- Shah, K., Patel, M., Patel, R. and Parmar, R. (2010): Pharmacognosy Review, **4**: 42-48.
- Shanjida, I., T., Hossain, M. I. and Tasneema, I. (2015): Antimicrobial activities of *Psidium guajava*, *Carica papaya* and *Mangifera indica* against some gram positive and gram negative bacteria. *Journal of Pharmacognosy and Phytochemistry* **3**(6): 125-129.
- Sofowara, A. (1993): *Medicinal plants and traditional medicine in Africa*. Spectrum Books. Nigeria. 2nd Ed. Pp 10-158.
- Wauters, G., Charlier, J. and Janssens, M. (1995): Agglutination of pYV + *Yersinia enterocolitica* strains by agglutinin from *Mangifera indica*. *Journal of Clinical Microbiology*, **33**: 772-774.