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Sensitivity of *Salmonella* and *Shigella* to *Mangifera indica* Linn (Mango) Crude Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author FJO designed the study. The draft of the manuscript was written and literature searches were managed by authors FJO and OOA managed the statistical analyses and wrote the protocol of the study. All the authors were involved in carrying out the experiments, read and approved the final script.

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ABSTRACT

Aims: To study the antimicrobial activities of methanol, acetone and aqueous extracts of *Mangifera indica* Linn crude leaves on *Salmonella* and *Shigella* in order to provide natural therapy against them.
Study Design: *Salmonella* and *Shigella* isolates were obtained from twenty-five stool samples of hospital patients. Six isolates were identified and characterized. The six isolates were labelled accordingly and used in the study. The antimicrobial activities of the

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extracts preliminary screened for the presence of phytochemical compounds were tested on the six isolates.
Place and Duration of Study: The study was carried out in the Department of Microbiology, Delta State University, Abraka, Nigeria between October, 2012 and August, 2013.
Methodology: The test isolates (*Salmonella* and *Shigella*) were isolated from stool samples on *Salmonella/Shigella* agar and characterized. The phytochemical constituents of the leaves of *M. indica* were extracted by soaking in water, methanol and acetone. The extracts were investigated for antimicrobial activities on theisolates using the agar well diffusion method.
Results: Phytochemical analysis of the leaf extract showed that the extracts contained alkaloids, tannins, flavonoids, steroids, anthroquinones, reducing sugars, glycosides and phenols. The acetone and methanol extracts exerted the highest zone of inhibition (18.00mm and 15.00mm respectively) though there was a significant difference ($p<0.05$) in the activities of the extracts. There was no significant difference ($p>0.05$) when compared with that of the water extract. Salmonella was more susceptible to acetone extract than *Shigella* with a Minimum Inhibitory Concentration of 25.00 mg/ml while that on *Shigella* was 50.00mg/ml. The MIC of the ethanolic extract on *Salmonella* was 50.00mg/ml while that of *Shigella* was 100.00mg/ml.
Conclusion: The acetone and methanolextracts of the leaves of *M. indica* had high inhibitory effects on *Salmonella* and *Shigella* thus providing a basis for its recommendation in the treatment of diseases caused by these organisms.

Keywords: Mangifera indica L.; acetone; methanol; extracts; antimicrobial; Salmonella; Shigella.

1. INTRODUCTION

Natural plants as herbal remedies are being employed to prevent and cure several illnesses in different communities [1].

The advent of science into the search of antibiotics largely depends on some of these medicinal plants as raw materials. According to World Health Organization (WHO), a medicinal plant could be any plant thatcontains substances which can be obtained from its different parts and can be applied for beneficial purposes or can be predecessors for the production of useful drugs [2]. Plants are easily accessible and cost effective sources for alternative medicines [3-5]. *Mangifera indica* L. or commonly called mango tree, is aplant belonging to the family *Anacardiaeceae* which consists of about sixty genera and six hundred species [6]. It is one of the most popular tropical fruit- bearing trees in the world [7].

The aqueous leaf extract of *Mangifera indica* L. has been reported to be rich in polyphenols amongst which mangiferin has been extensively studied by several authors and proposed as the bioactive principle of *M. indica* stem, bark and leaf extracts, possess several pharmacological activities including antioxidants, analgesic, antidiabetic, anti-inflammatory, antitumor, immunomodulatory and anti-HIV effects [8].

In India and Nigeria, the infusion of the leaves singly orcombined with the leaves of *Citrus sinensis* is used in treating diarrhea, dysentery, gastrointestinal tract disorder, typhoid fever, sore throat and scurvy [9-11].

The bacterium *Salmonella typhi* is a Gram-negative, motile, non-sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits vegetables or via convalescent or chronic carriers, causing typhoid fever. Enteric fever is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate in developing countries [12].

Species of *Shigella* are Gram-negative, non-spore formers, rod-shaped, facultative anaerobic entero bacteria. *Shigella* species cause bacilliary dysentery with *Shigella dysenteriae* being the most virulent [13].

Based upon the information mentioned above, we realized the present study in order to investigate the antimicrobial activity of extracts of Mango plant leaves against *Salmonella* and *Shigella* in order to provide natural therapy against these organisms.

2. MATERIALS AND METHODS

2.1 Collection of Mango leaves

Healthy and mature fresh leaves of *Mangifera indica L.* were collected by cut off from the trees grown in premises of Faculty of Arts, Delta State University, Abraka, in a clean polythene bag. The plant species was identified and authenticated by the Department of Botany, Delta State University, Abraka, Nigeria.

2.2 Test Organisms

The test organisms (*Salmonella* and *Shigella* species) were isolated from stool samples collected from the Delta State University Health Center in sterile specimen bottles, which were transported to the laboratory immediately for analysis.

The samples were cultured using pour plate technique on a *Salmonella/Shigella* (SS) agar (LAB-M, UK) and incubated at 37ºC for 24 hours. The black and creamy colonies were sub cultured onto nutrient agar plates using Streak plate technique and incubated overnight at 37ºC. Pure colonies (6 colonies; 3 *Salmonella* and 3 *Shigella*) from the growth were transferred onto agar slants, and stored at 4ºC in the refrigerator until needed for further analysis.

2.3 Extract Preparation

Fresh leaves of *Mangifera indica L.* were thoroughly washed in distilled water andair- dried for five days and ground with a macerator blender Model 890968, Dallas. Five hundred grams of *Mangifera indica L.* leaves were dispensed into six sterile 500ml conical flasks for the aqueous, and acetone extraction of each sample. The flasks were properly labeled. Two hundred millimeters of each solvent (water, ethanol, and acetone) were dispensed into each flask, sufficient to cover the leaves. The flasks were left on a disinfected table for three days for extraction [14].

Following extraction, the content of each flask was filtered into a sterile conical flask through a funnel fitted with a Whatman No1 filter paper (England).

The filtrates were evaporated in water bath (Hospitex, England) at 80ºC. Homogenous mixture of the various extracts was prepared by mixing 5.0g of each extract in 200ml of distilled water. All the mixtures were stored in the refrigerator until ready for use.

2.4 Identification of Isolates

The pure colonies of isolates earlier saved were sub-cultured on fresh nutrient agar and used for the identification of the organisms. They were characterized and identified based on their morphological, cultural and biochemical properties as described previously [15,16]. *Salmonella* species are motile, spore producers, colorless with a black centre on Salmonella/Shigella agar, indole positive while *Shigella* species are colorless, non- motile, non- spore formers and indole positive or negative.

2.5 Determination of the Phytochemical Constituents of the leaf extracts

The phytochemical analysis of the leaf extracts of *Mangifera indica L.* was carried out according to the methods of Jigna [17]. The crude extracts were subjected to preliminary screening for the presence of alkaloids, saponins, tannins, anthraquinones, flavonoids, steroids, carotenoids, reducing sugar, glycosides, terpenes and phenols

2.6 Susceptibility Test

Test on the susceptibility of the isolates to the extracts was carried out using the modified agar diffusion technique.

A sterile cork borer was used to bore wells into the agar plates. The plates were inoculated with the test bacteria in six replicates for each. Sterile calibratedPasteur pipette was used to introduce 0.5ml of extract reconstituted in sterile distilled water for aqueous, ethanolic and acetone for the plant extracts. The plates were allowed to equilibrate after which they were incubated at 37ºCfor 24 hours. Zones of inhibition for the extracts on the organisms were measured using a calibrated ruler and recorded.

The concentrations of extracts used for the test were (200.00, 100.00, 50.00, 25.00 and 12.50)mg/ml.

2.7 Determination of Minimum Inhibition Concentration (MIC)

Different petri dishes containing sterile nutrient agar were inoculated with 0.1ml of *Salmonella and Shigella* species respectively, mixed thoroughly by swirling and left undisturbed on disinfected table for1 hour.

Wells with ten millimeter in diameter were bored on the surface of the agar using a sterile cork borer and 0.1ml of each of the aqueous, methanolic and acetone extracts was dispensed separately into the wells. The plates were incubated at 37ºC for 24hours. Each plate was observed and the diameter of zones of inhibition was measured using a calibrated ruler. The least dilution of the extract that inhibits the growth of the organisms is considered as the MIC.

2.8 Determination of Minimum Bactericidal Concentration (MBC)

The MBC was estimated as an adjunct to the Minimum Inhibition Concentration (MIC), but the MBC was performed after the incubation period of 48 hours. After 48 hours, the cultures were sub-cultured by collecting a loopful of broth culture from the test tubes that did not show any growth and inoculated onto sterile nutrient agar plates. The plates were incubated at 37ºC for 24 hours. The lowest concentration which failed to produce growth is the Minimum Bactericidal Concentration (MBC).

3. RESULT

The characterization of the six isolates showed that they were *Salmonella* and *Shigella* species (Table 1). Three were *Salmonella* species and coded as S1, S2 and S3 and three as *Shigella* species and coded as SH1, SH2 and SH3.

Table 1. Bacterial characteristics

Test	*Salmonella*			*Shigella*		
	1	**2**	**3**	**1**	**2**	**3**
Motility	+	+	+	–	–	–
Gram reaction	–	–	–	–	–	–
Aerobic growth	+	+	+	+	+	+
Shapes	Rod	Rod	Rod	Rod	Rod	Rod
Catalase	+	+	+	+	+	+
Gas production	+	+	+	+	+	+
H_2S production	+	+	+	–	–	–
Lactose	–	–	–	–	–	–
Glucose	+	+	+	+	+	+
Citrate	+	+	+	+	+	+
Urease	–	–	–	–	–	–
Oxidase	–	–	–	–	–	–
Endospore	–	–	–	–	–	–
Indole	–	–	–	+	–	–
Benzidine reaction	+	+	+	+	+	+

+ = Positive;-= Negative; H2S = Hydrogen sulphide

The phytochemical analysis revealed that *Mangifera indica L.* possesses the phytoconstituents such as alkaloids, tannins, steroids, flavonoids, reducing sugars, anthroquinones, phenols and cardiac glycosides as shown in Table 2.

The isolates of each organism showed similar susceptibility trend to the extracts. The susceptibility of the test organisms to the methanolic extracts of *Mangifera indica L.* shows that the extract exerted largezones of inhibition of 15.00mm and 12.00mm respectively on *Salmonella* and *Shigella* at 200.00mg/ml of the extract. Only *Salmonella* was susceptible to the extract at 12.50mg/ml concentration of the extract as shown in Table 2. The test organisms are highly susceptible to the acetone extract of *Mangifera indica L.* with the zones of inhibition ranging from 18.00-20.00mmand 12.00-18.00mm respectively for *Salmonella* and *Shigella* at 200.00mg/ml concentration of the extract.

The aqueous extract of *Mangifera indica L.* has no significant effect on the different isolates of the test organisms since it is inhibitory to *Salmonella* and *Shigella* with 2.00mm and 1.00mm zones of inhibition respectively for each organism at 50, 100 and 200mg/ml of the extracts. The extract did not show any effect on both organisms at 25.00mg/ml and 12.50mg/ml extract concentration.

The MIC and MBC of methanolic, acetone and aqueous extracts of *Mangifera indica L* are shown in Tables 3, 4, and 5. Both methanolic and acetone extracts are shown to be inhibitory to the test organisms while just slight inhibition was exhibited by the aqueous extract on these same organisms.

Table 2. Phytochemical constituents of leaf extract of *Mangifera indica* Linn

S/N	Constituents	A	B	C
1	*Saponins*	-	-	-
2	*Alkaloid*	-	-	+
3	*Tannins*	+	+	+
4	*Flavonoids*	+	+	+
5	*Steroids*	-	+	-
6	*Anthraquinones*	-	-	+
7	*Carotenoids*	-	-	-
8	*Reducing sugar*	+	+	+
9	*Phytotannins*	-	-	-
10	*Glycosides*	+	-	+
11	*Terpenes*	-	-	-
12	*Phenols*	+	+	+

+A= Water extract; B = Acetone extract; C = Methanol extract; -= Absent
+ = Present

The methanolic extract is inhibitory to the test organisms at 200.00mg/ml of the extract concentration while the acetone extract is efficacious to both organisms at 200.00mg/ml and 100.00mg/ml extract concentration.

The MIC of methanolic extract on *Salmonella* isolates was 50.00mg/ml while it was 100.00mg/ml on SH1and 2 and 200.00mg/mlfor SH3. The minimum bactericidal concentration was 100.00mg/ml for all Salmonella isolates and 200.00mg/ml for the *Shigella* isolates (Table 5). The MIC ofacetone extract on S2 and 3 was 25.00mg/mland 50.00mg/ml for *Salmonella* 1 while it was 50.00mg/ml for *Shigella 1* and 2 and 200.00mg/ml for Salmonella 3. The MBC was 50.00mg/ml and 100.00mg/ml respectively for each isolate. Only *Shigella*3 had MBC of 200mg/ ml.

Table 3. Susceptibility of test organism to the methanolic, acetone and aqueous leaf extract of *Magnifera indica* (Diameter of inhibition zone in mm)

Plant extract	Bacterial isolate	Concentration of plant extract (mg/ml)				
		200.00	100.00	50.00	25.00	12.00
Methanolic extract	* S1	15	13	10	8	5
	+ S2	14	12	10	6	6
	** S3	10	8	6	4	4
	++SH1	12	10	7	5	0
	*+*SH2	10	10	8	8	0
	*+SH3	14	14	12	10	0
Acetone extract	*S1	18	15	13	7	5
	+S2	20	20	18	13	10
	**S3	18	14	14	10	8
	++ SH1	13	11	9	5	0
	+ SH2	18	15	11	9	0
	*+SH3	15	10	8	6	6
Aqueous extract	*S1	2	1	1	0	0
	+S2	4	1	0	0	0
	S3	2	2	1	0	0
	SH1	1	1	0	0	0
	SH2	2	1	0	0	0
	SH3	0	0	0	0	0

**, **, +, ++, *+, *+* = Statistical Significant difference noticed at P<0.05 level of significance*
0 = No visible growth; S = Salmonella isolate; SH = Shigella isolate

Table 4. Minimum Inhibitory Concentration (MIC) of methanol, acetone and aqueous leaf extracts of *Mangifera indica* Linn

Plant extract	Bacterial Isolate	Concentration of plant extract(mg/ml)				
		200	100	50	25	12
Methanolic extract	S1	_	_	*	+	++
	S2	_	_	*	+	++
	S3	_	_	*	+	++
	SH1	_	*	+	++	++
	SH2	_	*	+	++	++
	SH3	*	+	++	++	++
Acetone extract	S1	_	_	*	+	++
	S2	_	_	_	*	++
	S3	_	_	_	*	+
	SH1	_	_	*	+	++
	SH2	_	_	*	+	++
	SH3	*	+	++	++	++
Aqueous extract	S1	+	+	+	++	++
	S2	+	+	++	++	++
	S3	+	+	++	++	++
	SH1	+	+	++	++	++
	SH2	++	++	++	++	++
	SH3	+	++	++	++	++

- = No growth; = MIC; + = Light growth; ++ = Heavy growth*
S= Salmonella; SH= Shigella

Table 5. Minimum Bactericidal Concentration (MBC) of the extracts on the test organisms

Plant extract	Bacterial Isolate	Concentration of plant extract (mg/ml)				
		200	100	50	25	12
Methanol extract	S1	_	(d)	+	++	++
	S2	_	(d)	+	++	++
	S3	_	(d)	+	++	++
	SH1	(d)	+	++	++	++
	SH2	(d)	+	++	++	++
	SH3	(d)	++	++	++	++
Acetone extract	S1	_	_	(d)	+	++
	S2	_	_	(d)	+	++
	S3	_	_	(d)	++	++
	SH1	_	(d)	+	++	++
	SH2	_	(d)	++	++	++
	SH3	(d)	+	++	++	++
Aqueous extract	S1	+	+	++	++	++
	S2	+	++	++	++	++
	S3	+	++	++	++	++
	SH1	+	++	++	++	++
	SH2	+	++	++	++	++
	SH3	+	++	++	++	++

- = No growth; (d) = MBC; + = Light growth; ++ = Heavy growth
S = Salmonella isolate; SH = Shigella isolate

4. DISCUSSION

This study revealed that the extract of the leaves of *Mangifera indica* L. is inhibitory to *Salmonella* and *Shigella.* Activities of extracts of plants against pathogens have been reported previously. The antimicrobial activity of *Annato bixa orellana* extracts on some bacterial isolates had been reported. Doughari and Manzara [5] and Masibo and He [18] reported the antimicrobial activity of crude leaf extract of *M. indica L*. against some bacterial pathogens.

The leaves *of M. indica* L. were known to be rich in compounds which have been used for curinga ailments in both man and animals. The presence of these phytoconstituents in these leaf extracts may have been responsible for their antimicrobial activities [19].

This study also revealed that the acetone extract exerted the widest zone of inhibitionrespectively on *Salmonella* and *Shigella* at extract concentration of 200.00mg/ml. It has been documented that different solvents have diverse solubility capabilities for different phytoconstituents [19]. The wider zone of inhibition exhibited by acetone extract against these organisms is an indication that acetone is a suitable solvent for the extraction of the antibacterial phytoconstituents of the mango plants. The absence of inhibition effects of aqueous extract of the mango leaves on the test organisms demonstrated the poor extractive capability of water for the antibacterial phytoconstituents in Mango leaves. *Salmonella* is more susceptible than *Shigella* to the extracts, which is similar with the work of [20].

The MIC and MBC values were in the range of 25.00 to 200 00mg/ml (ethanol and acetone) with the lowest MIC and MBC values of 25.00 and 50.00mg/ml in each case recorded by *Salmonella.* This observed MIC and MBC values against these bacteria means that the plant extracts have the potential to treat any ailments associated with these test organisms effectively.

5. CONCLUSION

The results from this study have revealed that the leaves of the mango plant have the potential for the traditional application as ethno-medicine. Its inhibitory effects on *Salmonella* and *Shigella* provides basis for its recommendation for its use in the treatment of gastroenteritis, typhoid fever, shigellosis and other related diseases. It is, therefore, recommended that further investigation be carried out on this plant inorder to fully discover its antimicrobial activities on wider zones of pathogenic microorganisms for the benefit of man.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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