Antibacterial Activity and Phytochemical Screening of *Mangifera indica* (Mango) Stem and Leaf Extracts on Clinical Isolates of Methicillin Resistant *Staphylococcus aureus'*

ABSTRACT

Background: Medicinal plants have been sources of a number of important compounds which have been discovered during last century. In the light of their established therapeutic efficacy, the pharmaceutical industries are using crude extracts of medicinal plants for manufacturing drugs.

Aim: The study was conducted to investigate the antimicrobial activities of *Mangifera indica* (leaves and stem) and its major antimicrobial constituents (phytochemicals).

Methodology: The aqueous and chloroform extracts from the leaves and stem of the plant was tested using well diffusion method for their antimicrobial activity against Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from wound, skin and urine samples of patients attending Muhammad Abdullah Wase specialist Hospital, Kano.

Results: The result shows that some extracts were active against the microorganisms tasted. Statistical analysis of the result shows that the extracts demonstrated antibacterial activity against the isolates tested with the highest zone of inhibition of 16mm, 14mm, 17mm, 15mm and 15.00mm for the five isolates used respectively. The chloroform extracts of the plant showed higher zones of inhibition compared to aqueous extracts. Preliminary phytochemical analyses of the plant showed that both stem and leaf extracts contain alkaloids, tannins, terpenoid, Anthraquinones, reducing sugar, amino acid, flavonoids, steroid, saponins, cardiac glycosides, resin and phenols.

Conclusion: *Mangifera indica* can be used as antibacterial agent against Methicillin resistant *Staphylococcus aureus*.

Keywords: Mangifera indica; phytochemicals; antibacterial activity; methicillin resistant Staphylococcus aureus; well diffusion.

1. INTRODUCTION

Plant derived products like gums, oils and extracts have been used for therapeutic purpose before the introduction of modern drugs [1] and continues to provide health coverage for over 80% of the world's population [2]. Serious attention is being given to medicinal plants as evidenced by the

recommendation given by the World Health Organization (WHO) in 1970. It gave emphasis on the need to include traditional remedies within national drug policies as these plants serve as the best sources of a variety of drugs? It is important to study plants so that a better understanding of their properties, safety and efficacy is derived for improved benefit. The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases [3]. Phytochemicals are naturally occurring compounds in the medicinal plants, [4]. Large populations of the world, especially in developing countries depend on the traditional system of medicine to treat variety of diseases [5]. Several hundred genera of plants were utilized traditionally for medicinal purposes. The world health organization [6] reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituents [7]. Mango (*Mangifera indica*), which belongs to the family Anacardiaceae, is commonly called Mango (English), Mongoro (Yoruba, Nigeria), Mangolo (Igbo, Nigeria) and Mangoro (Hausa, Nigeria) [8]. It grown naturally or cultivated mainly in tropical and subtropical regions and is one of the most popular edible fruits in the world. In India and Nigeria, the infusion of the leaves singly or combined with leaves of *Citrus sinensis* is used in treating diarrhea, dysentery, gastrointestinal tract disorders, typhoid fever, sore throat and scurvy[9].

In the present study, the extracts (Aqueous and chloroform) from leaves and stem of *M. indica* were screened for Antibacterial activity against Methicillin resistant *Staphylococcus aureus*. The phytochemical constituents of the extracts were also determined.

2. MATERIALS AND METHODS

2.1 Plant materials

The plant materials used in this study consisted of the leaves, stem bark and root of *Mangifera indica* plant which was collected from Bayero University, Kano old campus. Botanical Identification and Authentification of the plant materials was done at Herbarium unit by a staff of the department of plant Biology, Bayero University, Kano with the following Voucher specimen number: BUKHAN 0348. Voucher specimens were deposited there for future reference. The samples were washed with water and removed dust and rinsed with distilled water. Sample was air dried for two-weeks and pulverized into powder form using sterile mortar and pestle in the laboratory as described by Mukhtar and Tukur

[10]. The powder sample was bagged in a black polythene bag and stored in air tight container for further work.

2.2 Test organisms

Clinical isolates of *Staphylococcus aureus* were obtained from the laboratory of Muhammad Abdullah Wase Specialist hospital Kano for further experiment. Identification and characterization of the isolates was conducted there by using three procedures namely Gram staining, cultural characterization using selective or indicative media and biochemical characterization. Methicillin resistant *S. aureus* were determined by using Oxacillin 10µg sensitivity disc. The pure isolates of each of the test organism were inoculated in sterile slants containing Nutrient agar and transported to the department of Microbiology Kano University of Science and Technology, Wudil and refrigerated at 4°C before use.

2.3 Preparation of extracts

The bioactive components were extracted using the methods of Akerele *et al*. [11] with slight modification. Two hundred and fifty milliliters (250 ml) each of chloroform and water were added unto 25g portions of leaves and stem bark powder in separate sterile conical flasks and allowed to soak at ambient temperature for 7 days. The extracts were then filtered using Whatman no. 1 filter paper and the filtrates concentrated at 70ºC using a rotary evaporator [11]. The solid residues obtained were reconstituted in DMSO at stock concentration, stored in the refrigerator at 4^0C until used.

2.4 Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins and phenols as described by Jigna *et al*. [12].

2.5 Assay for antibacterial activity

The antibacterial screening was carried out using the agar diffusion method as described by Lino and Deogracious [13]. The test bacteria isolates were first inoculated into tubes of nutrient broth separately and incubated at 37^0C for 24 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (0.1 ml each) onto Mueller Hinton agar (MHA, Oxoid) plates. A sterile coke borer was then used to make five wells (6 mm diameter each) for different concentrations

of the extract on each of the plates containing cultures of the different test isolate. The different concentrations of 0.1 ml of 30, 60, 90 and 120mg/ml of the extract were then introduced into four wells using sterile Pasteur pipettes. The fifth wells contain the standard antibiotics Gentamicin (62.5 mg/ml) which was used as positive control. The culture plates were allowed to stand on the working bench for 30 min for pre diffusion and were then incubated at 37℃ for 24 h. After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test isolate) around each of the extracts and the antibiotic [13].

2.6 STATISTICAL ANALYSIS

The data was analyzed using One-Way Anova and the 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 $p<0.05$.

3. RESULTS AND DISCUSSION

Results of preliminary phytochemical screening of the leaf and stem of *Mangifera indica* are presented in Table 1. Results showed the presence of alkaloids, anthraquinones, xanthoprotein, flavonoids, resins, saponin, amino acid, tannin and cardiac glycoside while steroid were absent in the leaf. On the other hand, anthraquinones was absent in the stem extract.

Table 1: phytochemical analysis of leaf and stem extract of *Mangifera indica*

Phytochemical	Leaves	Stem bark
Reducing sugar	+	+
Tannins	+	+
Anthraquinones	+	-
Steroids	-	+
Terpenoids	+	+
Saponins	+	+
Flavonoids	+	+
Alkaloids	+	+
Phenol	+	+
Xanthoprotein	+	+
Cardiac glycoside	+	+

Key: (+) Present, (-) Absent

Table 2 shows the results of antibacterial effects of extracts of the plant leaf and stem against the test isolates. Results showed that the activity of the extracts against the test bacteria increased with increase in the concentration with the chloroform stem extracts demonstrating higher activity (17 mm, 120 mg/ml,) than water extracts (15 mm, 50 mg/ml).

Table 2: Antibacterial activity of the extracts

ISOLATES	CONCENTRATION (mg/ml)/ ZONE OF INHIBITION (mm)					
	120	**90**	**60**	**30**	**CONTROL**	**EXTRACTS**
Isolate 1	14	13	07	06	19	LAE
	11	09	08	06		LCE
	16	12	10	06		SAE
	15	13	09	06		SCE
Isolate 2	06	06	06	06	06	LAE
	13	10	09	09		LCE
	06	06	06	06		SAE
	14	12	10	06		SCE
Isolate 3	10	08	07	06	17	LAE
	13	12	09	06		LCE
	17	13	10	06		SAE
	14	13	10	08		SCE
Isolate 4	13	13	08	06	15	LAE
	15	14	12	10		LCE
	12	12	11	06		SAE
	13	13	11	08		SCE
Isolate 5	12	10	10	06	17	LAE
	12	09	09	08		LCE
	13	12	08	06		SAE
	15	12	11	09		SCE

LAE= Leaf aqueous extract, LCE=Leaf chloroform extract, SAE= Stem aqueous extract, SCE=Stem chloroform extract

In the present research, *M. indica* leaf, and stem were screened for phytochemical analysis and antibacterial activities of the extracts against methicillin resistant *Staphylococcus aureus*. On phytochemical screening, the leaf extract posses the following phytochemicals reducing sugar, tannin, anthraquinones, terpenoid, saponin, flavonoids, phenol, xanthoprotein, however, steroid is absent. The stem bark extract possessed the following phytochemicals; terpenoid, saponin, flavonoids, alkaloid, phenol, xanthoprotein and cardiac glycoside while anthraquinones is absent. The result of phytochemical screening from this study shows similarities to several studies conducted by many researchers in an attempt to determine phytochemical constituents of different part of *M. indica.* The result was inconformity with that of by Doughari, and Manzara, [14] on *In vitro* antibacterial activity of crude leaf extracts of *M. indica,* the preliminary phytochemical analysis revealed the presence of tannins, glycosides, saponins and phenols. Another experiment conducted to determine the phytochemical constituents in *Mangifera indica* by Sanwaral and Susish [15] showed the presence of alkaloid, flavonoids, tannins, saponins, glycosides and anthraquinones. The above finding supported the result of the present study. This result is also supported by a study conducted by Aiyelaagbe and Osamudiamen [16].

The result of this study showed that the *M. indica* extract (chloroform and aqueous) posses antibacterial activity against MRSA. The results of antibacterial activity of the extracts of *M. indica* from this study were in conformity with several studies conducted by many researchers on antibacterial activity of *M. indica.* Experiment conducted by Chidozie *et al*. [17] on antibacterial activity of crude extract of *M. indica* shows that it is highly effective against some pathogenic bacteria namely *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Shigella spp,* on the other hand, the extract found to be non effective against *Streptococcus faecalis.* The aqueous and ethanol extract of leaves and stems of mango at 50 and 25 mg/ml has been found sufficient activity against bacteria; *Staphylococcus aureus*, *Streptococcus pyogenes, Streptococcus pneumoniae, Pseudomonas aeruginosa , Enterococcus faecalis* [18]. The above work supported this research that *M. indica* extract possessed antibacterial agent. This work is also inconformity with that of Vega-vega *et al*. [19] who found the antibacterial ability of extract against *Salmonella enterica*, *Listeria*

monocytogenes and *Escherichia coli*. Sahrawat *et al.* [20] also determines antibacterial activities of *M. indica* leaf on methanol, ethanol and benzene extract were studied against bacteria some as *Proteus vulgaris, Pseudomonas fluorescens*, *Shigella flexneri*, *Klebsiella pneumonia* and *Salmonella typhi* at100µl/ml concentration. Antibacterial activity of mango extracts upon gram-positive, gram-negative bacteria was also demonstrated [21] and it is thought that the antibacterial activity of mango extract is due to the presence of tannin and mangiferin.

In the study conducted by Majourie [22], the result shows that different extracts of *M. indica* had different compounds with antibacterial activity. This suggests that the antibacterial activity could be due to different classes of compounds. Some of the classes of compounds identified in the crude extract, such as alkaloids and terpenoid, have been reported to possess antibacterial activity [22]. The study conducted by Doughari and Manzara, [14] reveals that the active components of leaves of *M. indica* L. which were extracted using cold water and organic solvents (acetone and methanol) and were tested against *Staphylococcus aureus, Streptococcus pyogenese, Streptococcus pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas aerugenosa, Proteus mirabilis, Salmonella typhi* and *Shigella flexnerri* using the agar well (cup plate) diffusion method. Both the acetone and methanol extracts inhibited the growth of gram positive bacteria, with acetone extract exerting more activities on all the gram positive bacteria with zone of inhibition between 15 - 16 mm, and a gram negative bacterium *S. typhi* (14 mm) at 250 mg/ml. Whereas, water extract was not active on any of the bacterial pathogens tested at any of the concentration of the extract used. This also supported the present study.

4. CONCLUSION AND RECOMMENDATION

Results of the study showed that leaf and stem extracts of *Mangifera indica* possessed phytochemical substances that can be used as components of new antimicrobial agents. However, the chloroform extract demonstrated higher antibacterial property against the isolates compared to water. The finding of this study also shows that the stem back extract possessed higher antibacterial agents compared to leaf extract. It is recommended that there is need for further investigations in terms of toxicological studies and purification of active components with the view to using the plant in novel drug development. The study has also justified the traditional usage of this plant as health remedy.

ETHICAL APPROVAL

Ethical approval was obtained from Kano State Hospital Management Board based on the consent of Muhammad Abdullahi Wase Specialist Hospital ethical committee.

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