

Antimicrobial Effect of Mango (*Mangifera Indica*) Leave Extract on Some Pathogenic Bacteria

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Abstract: *Mangifera indica*, commonly called mango, is an edible fruit that are also used for juice and wine production. Fresh leaves of *M. indica* were collected from the premises of Adekunle Ajasin University, Akungba Akoko Ondo State, Nigeria. Materials used, including Gram staining reagents, for the study are properly sterilized. Similarly, the culture media used, including Nutrient agar, MacConkey agar, Eosin methylene blue agar, Salmonella-Shigella agar, Mannitol salt agar, Mueller-Hinton agar and Nutrient broth, were properly sterilized. The bacterial isolates identified and used for the study were *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Citrobacter* spp. Antimicrobial susceptibility test of the Mango (*Mangifera indica*) leaves extract against the test bacteria shows that 200 mg/ml and 175 mg/ml extract of the plant source had observable effect against test organisms, particularly *Escherichia coli*, *Salmonella typhi*, and to a varying extent on others. The zones of inhibition formed at 200 mg/ml were 20 mm, 11 mm, 10.8 mm, 12 mm 16.3 mm and 7 mm for *Escherichia coli*, *Klebsiella* spp, *Staphylococcus aureus*, *Citrobacter* spp *Salmonella typhi* and *Pseudomonas aeruginosa* respectively. Thereafter, the negative control of the 25% DMSO used for dissolving the extract was done and the result was all negative compared with known active positive control – Ampliclox that is an effective antibiotics. With this current observation, Mango, apart from its nutritious component as food complement, coupled with its good fiber contents as fruit, is also found valuable for some antimicrobial purposes. Which means it can be formulated as food supplement or concentrates for some clinical and pharmaceutical purposes.

Keywords: Antimicrobials, Mango, *Mangifera Indica*, Nutrients, Pathogens

Introduction

Bacterial resistance is a public health problem that involves the world's population, this is because bacteria that cause infectious diseases become resistant to antibiotics (Kumar et al., 2020). The incorrect and indiscriminate antimicrobial use has led to the emergence of these multi-drug resistant bacteria (Liu et al., 2017). Some medicinal plants with antimicrobial attributes are capable of evading the activity of multi-drug resistant (MDR) microbes, which helps in withstanding antimicrobial resistance (Dzotam et al., 2017). However, as resistant pathogens emerge and spread, the efficacy of the antibiotics is reduced (Reddeman et al., 2019). The resistance of bacteria to the antimicrobial agents poses a serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs (Spellberg and Gilbert, 2014). Due to the report of increasing development of drug resistance in human pathogen as well as undesirable side effects of certain antimicrobial agents, it is essential to search for new agents that are better, cheaper and devoid of side effects for treating infectious diseases especially in developing countries and this has led to a re-evaluation of the therapeutic use of ancient remedies such as plants and plants products (Ouf et al., 2020).

Alternative herbal medicine has been used to treat various infections from centuries (Kubmarawa et al., 2007). Plants have aroused interest of both scientists and of the pharmaceutical industry (Mostafa et al., 2018). Plants possess limitless ability to synthesize aromatic secondary metabolites which include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and Coumarins. These compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms (Princwill-Ogbonna et al., 2019).

Mangifera indica (Family: Anacardiaceae) is commonly called Mango, and its leaves have been reported for various health and medicinal benefits like antioxidant, antimicrobials, anti-helminthic, anti-diabetic and anti-allergic (Batool et al., 2018). Distinct morphological parts of the mango plant like leaves, stem, kernel, seeds, and bark have been manifested to show antimicrobial activities against microbes like *Staphylococcus* sp., *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Shigella flexneri*, *Klebsiella pneumoniae*, and *Salmonella typhi* (Itoh et al., 2020). The fruits are consumed, and used for juice and wine production. Traditionally the mango plant has medicinal applications (Lauricella et al., 2017). In Côte d'Ivoire the leaf-decoction is used as a febrifuge and the bark infusion has been used as gargle to treat mouth infections in children ((Doughari and Manzara, 2008). In India and Nigeria, the infusion of the leaves combined with leaves of *Citrus sinensis* and *Sida acuta* is used in treating diarrhea, dysentery, malaria fever, gastrointestinal tract disorders, typhoid fever, sore throat and scurvy (Oboh et al., 2019). It is also sipped as tea for treating brain damage and as a relief from pains and joints inflammation in India (Ali et al., 2020). The fruits have also been consumed with salt and natural undiluted honey as source of vitamin A and for the treatment of blood disorders. Infusion of the ground seeds have been used as remedy for hyperglycemia (Campbell et al., 2018). Sap from the leaves and unripe fruits have been eaten as remedy to allergic reactions, scorpion and bee stings. The most essential of bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Bioactive

constituents have found applications as naturally occurring antimicrobial agents in the field of preservation, pharmaceuticals, phytopathology, etc. (Jantar et al., 2018).

The mechanism of exertion of antimicrobial activity by these compounds involves depleting intracellular ATP levels, depolarization of plasma membrane, cytoplasm leakage, damaging genetic material, and declining the concentration of microbial protein (Guo et al., 2020).

In view of the importance of *M. indica* in ethno botany as health remedy. This research was carried out to investigate the antibacterial activity of its leaf extracts against some pathogens.

Materials and Methods

Materials Used

Standard sterilizing laboratory equipments were appropriately used during the study. The culture media and reagent routinely used includes: Nutrient agar, MacConkey agar, Eosin methylene blue agar, Salmonella-Shigella agar, Mannitol salt agar, Mueller-Hinton agar and Nutrient broth. Similarly, Gram staining reagents (Crystal violets, Iodine, Safranin, Acetone), Distilled water, Normal saline, Methanol, Dimethyl sulfoxide (DMSO), Glucose, Mannitol, Sucrose, Lactose, Phenyl red (indicator) and Sodium chloride were adopted for conventional purpose in this study.

Source and Dilution of Standard Drugs

A 1000 mg concentration of Ampiclox (GSK, Plc.) was emptied into 10 ml sterile distilled water to make up the stock solution of 100 mg/ml. 0.5 ml (25 mg) of the standard drug was thereafter pipetted in the control well.

Source of Organism

The test bacteria were isolated from stool samples and wound swab of students of Adekunle Ajasin University, Akungba Akoko, Ondo state, Nigeria. The samples were cultured on media such as MacConkey agar (MCA), Eosin methylene blue agar (EMBA) and Nutrient agar (NA), Salmonella-Shigella agar (SSA), and Mannitol salt agar (MSA) using streaking and pour plate technique.

Identification of Test Organisms

All bacterial isolates tested were identified using standard microbiological measures. This includes Gram staining and sugar fermentation tests.

Source of the *Mangifera Indica* Leaves

Fresh leaves of *M. indica* were collected from the premises of Adekunle Ajasin University Akungba Akoko, Ondo state, Nigeria.

Preparation of Extracts

The fresh leaves were washed with distilled water to remove dirt and air-dried to constant weight for 5 weeks. The dried leaves were then blended using an electrical blender. The bioactive components were extracted using the methods of Akerele et al., (2008) with slight modification. One hundred milliliters (100 ml) each of methanol was added unto 500 g portions of the leaf powder in sterile conical flasks and allowed to soak at ambient temperature for 72 hours. The extracts were then filtered using Whatman no. 1 filter paper and the filtrates concentrated in vacuum and evaporated to dryness at 40° C using a rotary evaporator.

In-vitro Demonstration of Antimicrobial Activity of *Mangifera Indica* Leaves Extract

The evaluation of the antibacterial activities was carried out using the agar diffusion method as described by Lino and Deogracious (2006) and CLSI, (2016). The test bacteria were first inoculated into tubes of nutrient broth separately and incubated at 37° C for 4 hours. Each of the cultures was then adjusted to McFarland turbidity standard by diluting with normal saline and thereafter inoculate (0.2 ml each) onto Mueller Hinton agar (MHA, Oxoid) (Liasu and Ayandele, 2008). 1 gram (1000 mg) of the dried aqueous and organic extract was dissolved in 5 ml of 25% dimethyl sulfoxide (DMSO) to form a stock concentration of 200 mg/ml (100%). 150 mg/ml and 100 mg/ml concentrations were made from the aliquot to generate 75% and 50% concentrations respectively.

A sterile cork borer of 6 mm diameter was then used to make five (5) wells for each of different concentrations of the extract, the positive control (Ampiclox), and the negative control (25% DMSO) on each of the plates containing cultures of the different test organisms. 0.5 ml of each of the extracts concentration was then introduced into the wells using sterile Pasteur pipettes. 0.5 ml of sterile dimethyl sulfoxide (25% DMSO) only was introduced in another well to serve as negative control. Wells containing the standard drug was included 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° C for 24 hours. After 24 hours, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm) (against the test organisms) around each of the extracts dilution, the negative control (DMSO) and the antibiotics (Lino and Deogracious, 2006).

Results

After 24 hours of incubation at 37° C, the isolates were identified by standard microbiological measures (Tables 1, 2 and 3 respectively). This identified isolates were *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli*, *Citrobacter freundii*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

Antimicrobial susceptibility test of the bacteria against the Mango (*Mangifera indica*) leaves extract was carried out and the results were shown on Table 4. Thereafter, the negative control of the 25% DMSO used for dissolving the extract was done and the result recorded. The results are represented on a multiple bar chart in figure 1. This shows the effectiveness of *Mangifera indica* leaves extracts at concentrations above 150 mg/ml against the test isolates. This result obtained is very encouraging because of high level of activity compared with the standard antibiotics used. Because, *Mangifera indica*, also compose some nutritive value which can enhance the human immune system boosting property of this plant source and related metabolic activity.

Table 1: The Cultural Characteristics of the Isolates on Solid Media

Samples Sources	NA	EMBA	MCA	MSA	SSA
Faeces	Pale cream and Mucoid colonies	Purple colonies that are small, circular, and convex	Pink colonies that round, Mucoid, opaque and glossy. Colourless colonies.	No growth observed	Colourless colonies with black center.
Wound Swab	Milky, Mucoid colonies	Purple colonies with green sheen	Bright pink halo bile precipitant around a pink colonies growth	Yellow colonies	Slight growth with pink and red colonies

NA= Nutrient Agar, EMBA= Eosin Methylene Blue Agar, MCA= MacConkey Agar, MSA= Mannitol Salt Agar, SSA= Salmonella-Shigella Agar

Table 2: Result of the Biochemical Test Carried Out for the Identification of the Organisms

Isolates	Gram Staining	Catalase	Indole	Oxidase	Methyl Red	Citrate
1	-	+	+	-	+	-
2	-	+	-	-	-	+
3	+	+	-	-	+	+
4	-	+	-	-	+	+
5	-	+	-	-	+	-
6	-	+	-	+	-	+

- = Negative Reaction, + = Positive Reaction

Table 3: Result of the Sugar Fermentation Test Carried Out on the Isolates

Isolates	Lactose	Mannitol	Glucose	Sucrose	Gas	Organisms
1	+	+	+	-	+	Escherichia coli
2	-	+	+	+	+	Klebsiella spp
3	+	+	+	+	-	Staphylococcus aureus
4	+	+	+	+	+	Citrobacter freundii
5	-	+	+	-	-	Salmonella typhi
6	-	+	-	-	+	Pseudomonas aeruginosa

- = Negative, + = Positive

Table 4: The Antibacterial Activity of *Mangifera Indica* Leaf Extract Against the Isolates

Isolates	200 mg/ml (mm)	175 mg/ml (mm)	100 mg/ml (mm)	Ampliclox 100 mg/ml (mm)	25% DMSO
Escherichia coli	20	10.6	NZ	15.7	NZ
Klebsiella spp	11	8	NZ	25	NZ
Staphylococcus aureus	10.8	8	5	15	NZ
Citrobacter freundii	12	8.2	3	7.5	NZ
Salmonella typhi	16.3	9	4	NZ	NZ
Pseudomonas aeruginosa	7	NZ	NZ	NZ	NZ

NZ = No Inhibition Zone, DMSO = Dimethyl Sulfoxide (Negative Control)

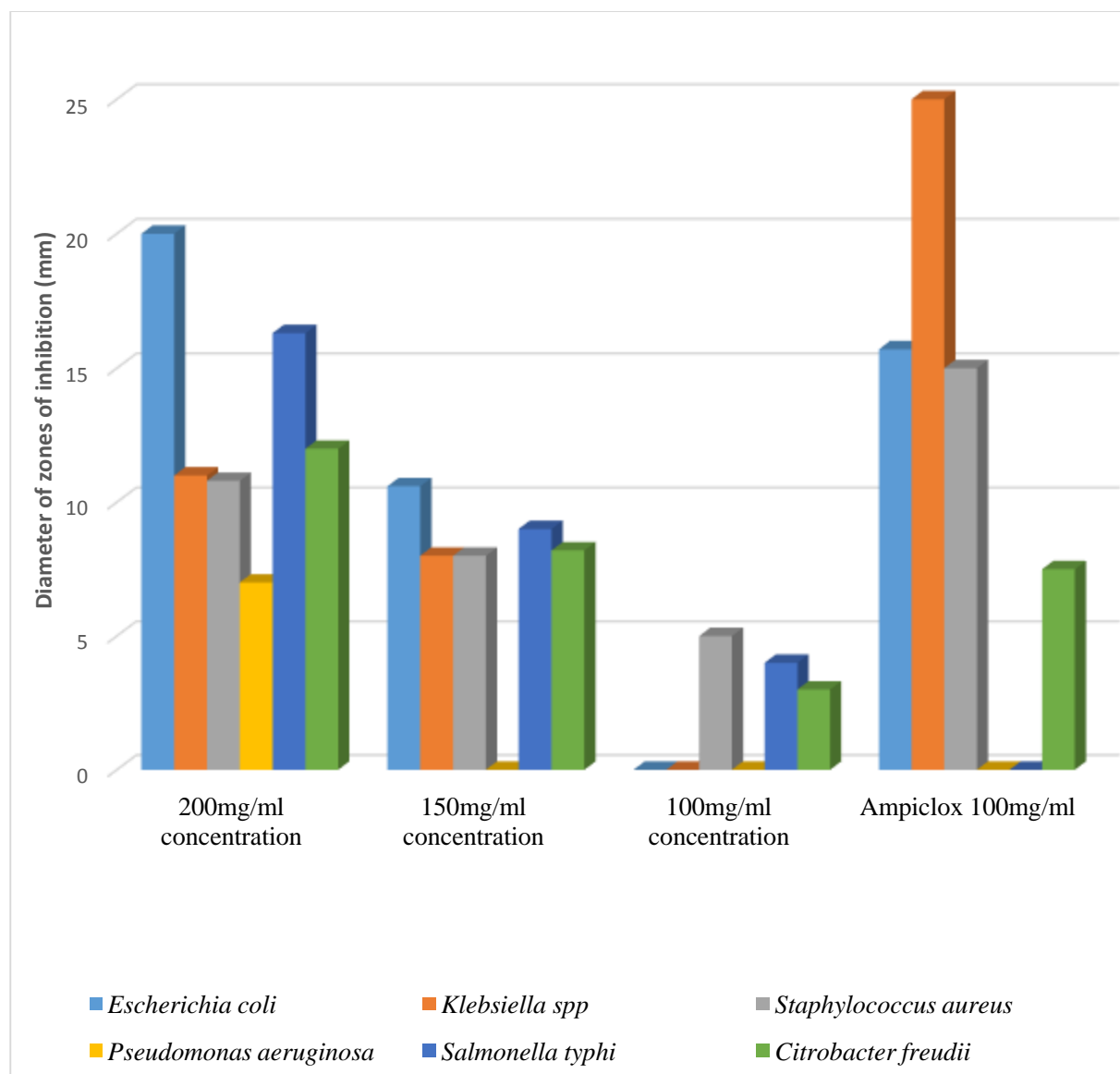


Figure 1: Different Concentration of Mangifera Indica Leaves Extract Against the Test Organisms

Figure 1 shows the antibacterial activities of different concentration of mango leaves methanolic extract against the bacteria isolates. It also shows the sensitivity pattern of the control antibiotics against the isolates. The test organisms' show varied susceptibility responses to different concentration of the extract (200 mg/ml, 150 mg/ml and 100 mg/ml).

Discussion

This study helps to evaluate and shows that Mango (*Mangifera indica*) leaves possess antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Citrobacter freundii* (Table 4).

Ediriweera et al., (2017), reported that the phytochemical analysis of mango leaves contain secondary metabolites such as alkaloids, tannins, flavonoids, and saponins which have an antibacterial mechanism of action. Alkaloids as an antibacterial act by way of interference with the components of the peptidoglycan of bacterial cell and as a result the lining of the cell walls are not fully formed (Luca et al., 2020). Flavonoids as an antibacterial is that it can damage the permeability of the cell walls of microbes. Saponin as an antibacterial is that it can cause leakage of proteins and enzymes from within the cell. Because the surface-active ingredient saponin is similar to detergent, so it will reduce the surface tension of the bacterial cell wall and damage membrane permeability (Klein-Júnior et al., 2019).

Norrby et al., (2017), also reported that the bioactive component extracted from *M. indica* is reported to possess remarkable anti-influenza properties. The activity against both gram-negative and gram-positive bacteria tested may suggest a broad spectrum of activity. The leaves extract at 100% concentration (200 mg/ml) has antibacterial activities against all the test bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Citrobacter freundii*) at a diameter of inhibition of 20.0 mm, 10.8 mm, 11.0 mm, 16.3 mm, 7.0 mm and 12.0 mm respectively (Table 4). The 150 mg/ml concentration is active against the test bacteria except *Pseudomonas aeruginosa* which shows no zone of inhibition. From the result represented on table 4, it shows that the diameters of zones of inhibition increases with an increase in the concentration of the extract. This study

is in agreement with a Nigerian study that showed inhibition zones of 4.0 mm, 7.0 mm, 9.0 mm, 12.0 mm, 14.0 mm at concentrations of 50, 100, 150, 200 and 250 mg/ml respectively (Doughari and Manzara, 2008). The *M.indica* extract was effective to *Pseudomonas aeruginosa* at 100% concentration (7.0 mm), and the other diluents shows no activity against the organism at lower concentration. The extract at all the concentrations tested shows significant activities against *Staphylococcus aureus*, *Salmonella typhi* and *Citrobacter freundii*. This corroborates with the studies of Agedah et al. (2019) who reported similar observation.

In comparison with the control (Ampliclox 100 mg/ml), the extract at 200 mg/ml produce more zones of inhibition against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Citrobacter freundii* with a diameters of inhibition zone of 20.0 mm, 7.0 mm, 16.3 mm, and 12.0 mm respectively (Table 4). This agrees with a similar study in Vietnam, whereby antibacterial effect of polyphenol mangiferin obtained from mango leaves was screened against *Salmonella* species (Navarro et al., 2018). The pure bioactive agent showed remarkable zones of inhibition at 15, 20, 25 mg/ml of mangiferin that were 22.2 mm, 26.7 mm, and 29.6 mm respectively (Table 4 and Fig 1). The activities against the test bacteria gives scientific evidence for the ancient usage of this plant in the treatment of various disease. This demonstrates that there is a need to examine the chemical component of mango leaves and to explore the most powerful agents used for its antibacterial properties.

The methanol extract of *Mangifera indica* leaves in this study shows antibacterial activity against both Gram positive and Gram negative bacteria. This also demonstrate the broad spectrum property of this extract. Hence, it can be used to complement antibiotic substances for drug development to control bacterial infections caused by this aetiologic agents. It is important to further discover the effects of the compounds on animals and human cells, including the toxicity and the mode of action, to make sure that the compounds are safe and has no bad effects on the health. Further investigations of its activity against a wider range of bacteria and fungi, identification and purification of its chemical composition, and toxicological assay of the plant extracts should be carried out with a view to developing novel drugs for human consumption.

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Conflicts of Interest Statement

The authors declare that there are no conflicts of interest.

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