



Comparative Analysis of the Antibacterial Activity of Ethanolic and Silver Nitrate synthesized Extract of Mango Leaf and Bark against *Escherichia coli* and *Staphylococcus aureus* from Clinical Samples

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Abstract

Background: Nano-sized particles are recently being explored to improve delivery and efficacy of various medicinals. This study aim to determine and compare the antibacterial activity of the extracts of mango (*Mangifera indica*) leaf and bark of against two selected clinical isolates (*Escherichia coli* and *Staphylococcus aureus*). **Materials and Methods:** The active constituents from the powdered leaf and bark was extracted via use of ethanol (70%). Silver nanoparticles were prepared by green synthesis technique using *Mangifera indica* (Mango leaf and bark) while UV-Vis spectroscopy was used for its characterization. Antibacterial profile of the isolates with standard antibiotics as well as the antibacterial activity of the plant extracts was determined using disk and agar diffusion method respectively. **Results:** The results showed that the bark and leaf extracts of *M. indica* had significant antibacterial activity against the isolates when compared to standard antibiotics such as Cloxacillin, Augmentin, Ceftraxone and Ceftriazone while Ofloxacin had highest activity of 20 mm and 23 mm for *S. aureus* and *E. coli* respectively. Ethanol extracts showed highest inhibition at 23mm (bark) and 15mm (leaf). The band energy of the synthesized nanoparticle was calculated as 393 nm from UV-Vis, which confirmed the Ag nanoparticles. The Silver Nitrate ($AgNO_3$) displayed significant antibacterial activity against *E. coli* (10mm) and *S. aureus* (13mm). The result indicated that the $AgNO_3$ antibacterial activity was higher against *S. aureus* than against *E. coli*. **Conclusion:** This study reveals significant antibacterial activity of crude mango leaf and bark extract as well as silver nanoparticle against the clinical isolates in comparison to some standard antibiotics. Further study on the extraction of the bioactive agent in the plant is required to provide a variety of novel components for drug discovery.

Keywords: Nanoparticle, Mango, *E. coli*, *S. aureus*, Clinical

INTRODUCTION

Treatment of bacterial infections usually centers on the use of antibiotics however, due to emergence of antibiotic resistance, increased hospital stay, mortality and treatment cost has become an increasing global burden. Plant have been used and explored as crude drugs both traditionally and in modern research (Suleiman *et al.*, 2016). Mango (*Mangifera indica*), a member of the Anacardiaceae family is regarded as one of the most important tropical fruit crops in the world, both historically and economically (Barreto *et al.*, 2008). Apart from its well-known fruits, mango is an evergreen tree with a wealth of medicinal properties and applicability. Mango leaves (MLs) contain minerals such as nitrogen, potassium, phosphorus, iron, sodium, calcium, magnesium, and vitamins such as A, B, E, and C (Suleiman *et al.*, 2016).

Nanobiotechnology which involves using living organisms (such as animals, plants and microorganisms) in production of nanoparticles is a technology currently been explored for drug or bioactive component delivery (Shahcheraghi *et al.*, 2022). This is due to its nano-size thereby allowing for easier penetration in to microbial cell. This research aim to compare the antibacterial activity of crude ethanolic extract of mango and nano-synthesized particles of mango leaf and bark against commercially available antibiotics on selected clinical isolates as a baseline for further research in treatment interventions.

MATERIALS AND METHOD

Collection and identification of plant sample

Mango tree barks and leaves were collected from mango trees in Offa town. The samples were rinsed

under running water to remove filthy and undesirable piece/materials (Farouk, 2009). The collected samples were identified and deposited at the University of Ilorin Herbarium with the voucher number UILH/001/969/2021.

Preparation of Media

Nutrient agar for plating

Nutrient agar powder of 15.5 g was suspended in 500 ml of cold distilled water, the mixture was stirred and boiled to dissolve before sterilization by autoclaving at 121°C (15psi) for 15 minutes. After autoclaving the liquid agar was cooled to a temperature range of 44°C to 47°C and poured aseptically into sterile plates (90mm diameter). The agar was allowed to cool and solidify at room temperature. Labeled plates with nutrient agar were stored at 4°C. A single plate was examined for sterility by incubating at 35°C for 24 hours.

Mueller Hinton agar

Dehydrated medium weighing 9.5g was suspended in 250ml of distilled water. The mixture was brought to boil, with constant stirring until complete dissolution. Sterilization was done using an autoclave. Cooled Mueller Hinton agar was poured into sterile petri dishes on a level, horizontal surface to give uniform depth. Prepared media was allowed to cool and solidify at room temperature. Labelled plates with Mueller Hinton agar were stored at 4°C. Sterility was checked by incubating a plate at 35°C for 24 hours.

Preparation of Plant Material and Extraction procedure

The bark was cut into small pieces and then they were oven dried at 37°C for 3 days. This was pulverized using a mechanical grinder and the powdered outcome was kept in an air tight container until required for extraction. Twenty grams (20g) of the prepared plant material was weighed into 500 ml flask and 200 ml of ethanol was added, and then put in water bath for 1hour. The content was filtered using a whatman No 1 filter paper and concentrated using a rotary evaporator.

Phytochemical Screening

The extract was screened for the presence of some phytochemicals following Geetha and Geetha (2014) and Anibujuwon et al. (2020).

Collection of isolates

Identified clinical isolates namely *Staphylococcus aureus* and *Escherichia coli* were obtained from the Medical Microbiology Laboratory of University of Ilorin Teaching hospital, Ilorin.

Antibacterial susceptibility test

Filter paper disc containing varying concentration of plant extracts was prepared as described by Vineetha et al. (2014). The disc diffusion method was used to determine the antibacterial activity of *Mangifera indica*. The inoculum for each organism was standardized to 0.5 McFarland corresponding to 10^8 cells /ml after which a sterile Pasteur pipette was used to transfer 0.5 ml of the standardized inoculum onto the surface of Mueller Hinton agar plates. Filter paper discs impregnated with concentration of the crude extracts were then placed equidistant to each other using sterile forceps on the Mueller Hinton agar plates seeded with each test organisms. Plates were incubated at 37° C for 24 hours after which the mean diameter of the zone of inhibition of the extracts against each of the test organisms were measured.

Nano-synthesis

This was achieved using mango leaf and bark extract. The mango leaves and bark were collected and dried for some days at room temperature. The Mango leaf and bark broth solution was prepared by taking 5g of thoroughly washed and finely cut leaves in a 300 mL Conical flask with 100 mL of sterile distilled water and then boiled the mixture for 5 min before finally decanting it. They were stored at 4°C and used within a week. Typically, 10 mL of leaf and bark broth was added to 190mL of 1mM aqueous $AgNO_3$ solution for reduction of Ag^+ ions. The effects of temperature on synthesis rate and particle size of the prepared silver nanoparticles were studied by carrying out the reaction in water bath at 25–95°C with reflux. The concentrations of $AgNO_3$ solution and leaf and bark broth were also varied at 0.1–2 mM and 5–50% by volume, respectively. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min followed by redispersion of the pellet in deionized water. UV-vis spectra were recorded as a function of reaction time on a UV-1650CP Shimadzu spectrophotometer operated at resolution of 1 nm.

RESULT

Result of phytochemical screening of the extracts from *Mangifera indica* showed the presence of tannins, alkaloids, saponins, glycosides, flavonoids, phenol, steroid and reducing sugar.

Table 1 shows the activity of the test isolates in the presence of selected commercially available antibiotics as well as the respective plant extract. *S. aureus* showed resistance to Cloxacillin-CXC, Erythromycin-ERY, Augmentin-AUG, Ceftraxoline-CAZ and Ceftriazone-

CTR while *E. coli* showed resistance to AUG, CAZ and CTR. The extracted constituent from the leaf and bark of *Mangifera indica* had measurable effect on the isolates respectively.

The optical properties of the synthesized AgNO_3 were analyzed by UV-Visible absorbance spectroscopy. The UV-Visible absorption spectrum of AgNO_3 is shown in (Figure 1).

Figure 2 shows the respective zone of inhibitions as observed with the extract of ethanol and from the nanoparticle synthesis of the leaf and bark of *Mangifera indica*. All of the extract showed significant inhibition activity except nanoparticle extract from leaf against *S. aureus* and nanoparticle extract from bark against *E. coli* that showed resistance.

Table 1: Antibacterial activity for crude solvent extracts of *Mangifera indica* against test organisms

Parameters	INHIBITION ZONES (mm)	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Cloxacillin (CXC)	R	21
Ofloxacin (OFL)	20	23
Erythromycin (ERY)	R	13
Augmentin (AUG)	R	R
Ceftraxone (CAZ)	R	R
Gentamicin (GEN)	16	15
Ceftriazone (CTR)	R	R
Bark (Ethanol)	12	23
Leaf (Ethanol)	15	13

Key: R= Resistance

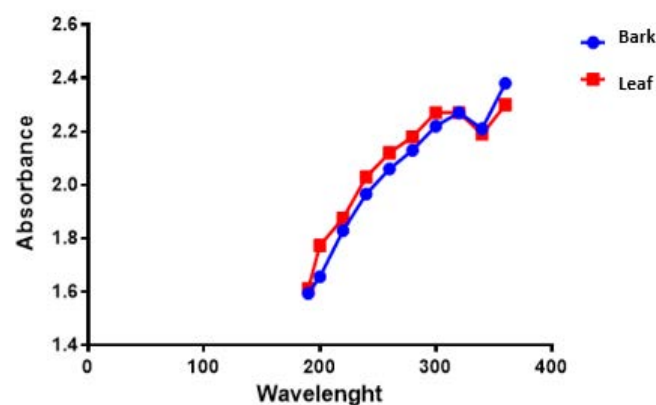


Figure 1: Result for UV- Vis Spectrophotometer of AgNO_3 nanoparticles.

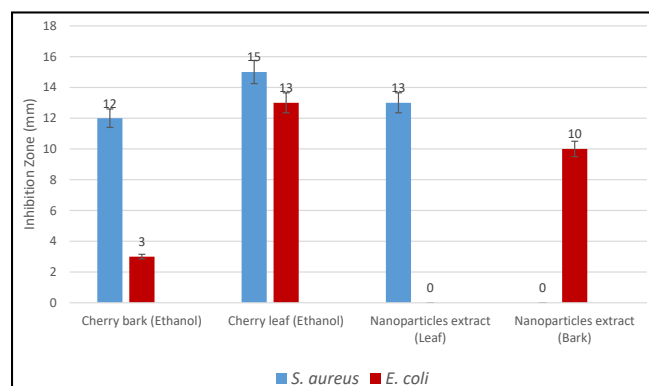


Figure 2: Antibacterial activities of ethanolic and AgNO_3 nanoparticle extract of *Mangifera indica* against *Staphylococcus aureus* and *Escherichia coli*

DISCUSSION

The basic parameters influencing the quality of an extract are plant parts used as a starting material, the solvent used for extraction, the extraction technology and sterilization method (Ncube *et al.*, 2007). These findings on extraction potential of the different solvents are consistent with previous investigation, in which the percentage yield of ethanol extract was high (Dieu-Hien, 2019).

Phytochemical analysis conducted on *M. indica* extracts revealed the presence of tannins, flavonoids, steroids, saponins, glycosides and resins among others. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms. Thus, *M. indica* containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (Kumar and Pandey, 2013). They also serve as health promoting compounds as a result of their anion radicals (Panche *et al.*, 2016). These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds. Alkaloids were also detected and their common biological property is cytotoxicity (Al-marzook and Omran, 2017).

Ethanol are polar solvent and their extraction capability has been adduced to the nature and potentiality of biologically active components (alkaloids, flavonoids), which could be enhanced in the presence of Ethanol (Jeyachandran *et al.*, 2010; Dieu-Hien, 2019). The leaf-bark extracts of *M. indica* had significant antibacterial potency against *Staphylococcus aureus* and *Escherichia coli*. This result may suggest that all extracts of *M. indica* possess compounds with antimicrobial properties which

can be used as antimicrobial agents in new drugs, for therapy of infectious diseases in human. The ethanol extracts had an inhibition zone diameter which was close to result from standard antibiotic, suggesting their effectiveness as antimicrobials.

Although, the components in the crude extract may be acting synergistically to produce antimicrobial effects, the disparity between the activities of the extracts and the standard antimicrobial drug, may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotic (Olajuyigbe et al., 2012). This is supported by the higher zone of inhibition reported with OFL at 23mm.

The optical properties of the synthesized AgNO₃ were analyzed by UV-Visible absorbance spectroscopy because measuring the band gap is an important parameter in Nano material industry. The term band gap refers to the energy difference between the top of the valence band and the bottom of the conduction band which are able to jump from one band to another (Jordan et al., 2015). The silver ion had been reported to show high biocidal effect on different types of bacteria including *E. coli* and *S. aureus* (Woo, 2008). Although the synthesized AgNO₃ solution displayed prominent antibacterial activity against *E. coli* and *S. aureus*, their effects differed because *S. aureus* was probably more sensitive to AgNO₃ than *E. coli*.

The antibacterial activity against *S. aureus* was generally slightly higher than *E. coli*, which was consistent with the results of previous studies (Xing et al., 2021). It seemed possible that the variety of cell walls between *S. aureus* and *E. coli* was a major cause. A vital function of the outer membrane is acting as a protective barrier, preventing or slowing the entry of antimicrobial agents and other toxic substances that may kill or harm the bacteria. The *E. coli* cell wall is known to be composed of an outer membrane of 50% lipopolysaccharides, 35% phospholipids, and 15% lipoprotein at about 6–18 nm thick. This outer film could act as a protective mechanism against antimicrobial agent. Consequently, absence of this in *S. aureus* could have influenced the higher inhibitory activity that was recorded (Hajipour et al., 2012).

CONCLUSION

The result of this study shows that the leaf and Bark extracts of *Mangifera indica* exhibit antimicrobial activity on the pathogenic organisms, therefore *Mangifera indica* leaf and bark parts have a promising use as an antibacterial agent to compliment treatment of various diseases caused

by *Escherichia coli* and *Staphylococcus aureus*. AgNO₃ were successfully prepared using different mango extract and it demonstrated significant antibacterial activity against *S. aureus* than *E. coli*. Research into isolation and identification of the active compounds in the bark and leaf of *M. indica* is encouraged for potential ingredient in drug discovery.

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