



Evaluation of Antibacterial Potential of Ethanol Fruit Peel Extract of *Mangifera indica* against Isolated UTI Pathogens

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ABSTRACT

Effective use of antimicrobial agents for treatment/management of infectious diseases is decreasing due to emergency of multi-drug and cross resistant strains of pathogenic microbes. Medicinal plants are now increasingly used alone or as an adjunct in the management of infectious diseases as a result of their claimed efficacy and safety. This research focused on evaluating the antimicrobial potential of ethanol fruit peel extract of *Mangifera indica* against isolated Urinary tract infection (UTI) pathogens. Urinary tract infection (UTI) bacterial strains (*staphylococcus aureus*, *escherichia coli* and *pseudomonas aeruginosa*) were isolated from midstream urine of infected students using standard procedures after which the isolated Urinary tract infection pathogens were subjected to antimicrobial susceptibility test by agar-well diffusion method (Cup plate method) and the mean diameter of growth inhibition zones (n=3) of the extract at different concentrations were compared against the controls (sterile water and ciprofloxacin). Ethanol fruit peel extract of *Mangifera indica* significantly and dose dependently inhibit the growth of all the isolated Urinary tract infection pathogens with *E.coli* exhibiting the highest inhibition zone ranging from 16.83 to 28.23 mm, this was followed by *pseudomonas aeruginosa* with inhibition zone of 24.33 mm and *staphylococcus aureus* least susceptible with 22.63 diameter of zone inhibition. Ethanol fruit peel extract of *Mangifera indica* demonstrated a dose dependent antimicrobial activity with more pronounced effect exhibited by *E. coli* suggesting that the extract is more effective against gram negative bacteria despite their permeability barrier, thus suggesting *Mangifera indica* fruit peel as a potential candidate for the management of bacterial infections especially those caused by gram negative organisms.

Keywords: Antimicrobial activity, Urinary tract infection, *Mangifera indica* fruit peel, Growth inhibition, *Escherichia coli*.

Introduction

Urinary tract infections (UTIs) are microbial infections involving the urinary system (the bladder, ureter or the kidneys) resulting in either

cystitis, urethritis or pyelonephritis in severe cases (CDC, 2015).

Urinary tract infection is a global problem and the 2nd most common type of infection occurring both

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in the community and within the health care system. It affects people of all ages and sexes. It is much more common in adults than in children, girls and women are more prone to urinary tract infections (UTIs) than boys and men due to their anatomical difference, hormonal effects and behaviour (Minardi *et al.*, 2011; Salvatore *et al.*, 2011). It accounts for over 8 million visits to hospitals every year (James, 2018) with approximately 150 million people having urinary tract infections in a given year (Flores-Mireless *et al.*, 2015). In Nigeria, more than 1.5 million cases per year of UTIs have been reported.

UTI is often caused by abnormal growth of pathogenic microbes especially gram negative bacteria such as *e.coli* which is responsible for more than 88% of cases (Abraham and Miao, 2015) and some gram positive bacteria such as *staphylococcus aureus* which is responsible for 5-10% of UTIs (Nicole, 2008), *pseudomonas aeruginosa*, *klebsiella pneumoniae*, *enterococcus* species in the urinary tract (Amdekar, 2011).

It may be acute uncomplicated or chronic complicated, asymptomatic or symptomatic UTIs and may occur due to susceptibility of the host to the organisms or due to pathogenicity of the organisms. Significant risks for recurrent UTIs have been linked to several factors which include age, gender, race, HIV status (Banu and Jyothi, 2013), diabetes, urinary catheterization (Mladenovic *et al.*, 2015), pregnancy state (Nelson and Good, 2015), genitourinary tract abnormalities (Yuyun *et al.*, 2004) among others.

Treatment of UTIs require the use of antibacterial agents such as nitrofurantoin, fosfomycin, cephalosporin, amoxicillin/clavulanic acid as well as fluoroquinolones among others (Zalmanovici *et al.*, 2010). Indiscriminate use and/or misuse of most of these antibiotics has resulted in these pathogenic microorganism developing resistant to most of the aforementioned drugs (FDA, 2018). Also, high cost of these drugs coupled with serious side effects (such as hypersensitivity, allergic

reactions etc) associated with their use has been a major global draw back in combating infectious diseases (Abraham and Okon, 2014) thereby making the need to search for alternative therapy a matter of urgency.

The promising activities of a number of medicinal plants against multiple disease causing microorganisms has led to the documentation of their application in biomedicine (Chikezie *et al.*, 2015; Ogbole *et al.*, 2018). Many medicinal plants have been reported to possess bactericidal or bacteriostatic effect against many pathogenic bacteria. One of such is *Magnifera indica*, a flowering plant belonging to the family of *Anacardiaceae* family. It is a large tropical fruit tree capable of growing to a height of 10-40meters (Litz, 2009) with bark usually dark-grey brown to black and leaves 15-45cm in length alternately arranged. It has fleshy fruits which varies considerably in size, shape, colour, flavor and taste (Masud- Parvez, 2016), fruit peel which can be green, yellow, red, or orange in colour (Sivakumar *et al.*, 2011), seeds which can be ovoid or oblong shaped with hard endocarp. It is widely grown in many parts of the world and highly noted for its immersed health benefits to human race both in the Ayurvedic as well as in indigenous medical system. Its various parts (leaves, stem, bark, fruit peel, flesh, root, flowers) have been studied and reported to contain several bioactive chemical constituents such as flavonoids, polyphenols, terpenes, sterols, vitamins, tannins, gallic acid, amino acid etc (Ignat, 2011; Kabir *et al.*, 2017) and also to possess antidiabetic (Reda, 2010), anti-ulcer (Neelima *et al.*, 2012), anti-diarrheal (Alkizim *et al.*, 2012), antimicrobial and antioxidant (Sultan *et al.*, 2012; Vega-Vega *et al.*, 2013), anticancer (Alli *et al.*, 2012; Timsina *et al.*, 2015), as well as anti-inflammatory activities (Dhananjaya and Shivalingaiah, 2016). Anti-proliferative effects of the fruit peel have been reported (Abbasi *et al.*, 2017) but its antimicrobial potential on isolated UTI pathogens has not been investigated to the best of my knowledge. Thus, the need to investigate the

antimicrobial activity of ethanol fruit peel extract of *Mangifera indica* on isolated UTI pathogens.

Materials and Methods

Drugs and chemicals

All drugs and chemicals used were of Analytical grade.

Plant collection and Identification

Fresh semi ripe fruit of *Mangifera indica* were collected from Sagamu, Ogun state in March, 2018. The plant was identified and its botanical identity was confirmed and authenticated at the Herbarium section of the Department of Botany, University of Lagos, Akoka, Lagos, Nigeria. Voucher specimens (LUH 8742) were preserved and stored at the herbarium for future reference.

Extract Preparation

The fresh fruit peel of *Mangifera indica* were air dried at room temperature. The dried fruit peel were then reduced to coarse powder using grinding machine and weighed. 500g of the powdered plant was extracted with 4litres of 90% Ethanol in a cold maceration process for 72 hours and then filtered. The filtrate was evaporated to dryness at 40°C under reduced pressure to give a greenish extract which was stored in refrigerator in air tight container (universal bottle) until required.

Sample collection

One hundred (100) mid-stream urine (MSU) samples were randomly collected from consented female students of Department of Pharmacology, Olabisi Onabanjo University, Sagamu Campus that were not on any drug(s); aged between 18 to 22 years into a separate sterile wide mouthed container and conveyed to the microbiology laboratory unit of Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus within two hours for culturing and processing. Thirty (30) urine samples out of the 100

samples collected were infected and was used for the study.

Isolation of UTI associated Bacteria

The urine samples were inoculated on Nutrient agar medium, MacConkey agar medium and Cystine Lactose Electrolyte Deficient (CLED) agar medium. The inoculated plates were incubated at 37°C for 18 - 24 hours after which the colonies observed on the plates were subjected to morphological and biochemical characterization to identify the isolates.

Characterization and Identification of the Bacteria Isolates

The bacterial isolates were characterized based on their morphological and biochemical characteristics as described by Chessbrough, (2006).

Evaluation of antibacterial activity

The antibacterial activity of the extract was determined using agar-well diffusion method (Cup plate method) according to method described by Alsadig *et al.*, 2016 with slight modification.

Mueller Hinton agar media was sterilized by autoclaving and cooled, with the aid of a sterile cotton swab mildly soaked with the individual isolated bacteria, the isolates were inoculated on the surface of Mueller Hinton agar by surface spreading and allowed to set, wells of approximately 6 mm in diameter, 2.5 mm deep and 2cm apart were made on the surface of the solid medium using a sterile 6 mm cup-borer and the well labelled. Each well was then filled with 0.1ml of the reconstituted ethanol crude extracts serially diluted to four different concentrations (100%, 50%, 25% and 12.5%) each with sterile distilled water. Sterile distilled water was used as negative control while Ciprofloxacin was used as positive control. The inoculated plates were maintained at room temperature for 2 hours enabling the diffusion of the extracts into the medium. The bacterial plates were then incubated at 37°C for 24 hours.

The antimicrobial activity tests were undertaken under aseptic conditions and in triplicates. With the aid of a transparent ruler the diameters of zones of inhibition around the wells were measured in millimeters (mm) and the average of the triplicate's measurements recorded.

Statistical analysis

Data analysis was performed using Graph pad (version 6). Results obtained were expressed as mean ± standard error of mean (SEM). The data was compared using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparism. Results were considered as statistically significant at P<0.0001.

Results

The morphological and biochemical characteristics of the bacteria isolates obtained are shown in Table1. The isolates obtained consist of 1 gram positive and 2 gram negative organisms. The isolated organisms were identified based on the various characteristics obtained to include *staphylococcus aureus*, *escherichia coli* and *pseudomonas aeruginosa*.

The results obtained from the susceptibility test showed that all the concentrations of the extract had inhibitory effects on all the isolated organisms from the infected students with *E. coli* most susceptible to the extract having diameter of zone inhibition ranging from 16.83 to 28.23mm, this was followed by *pseudomonas aeruginosa* having 24.33 diameter of zone inhibition and *staphylococcus aureus* least susceptible with 22.63 diameter of zone inhibition (Table 2).

Table 1: Biochemical characteristics of the Bacteria Isolates obtained from the Infected Students.

S/N	Gram stain	Cell type	Motility	Catalase	Coagulase	Citrate	Urease	Methyl red	Indole	Organism
1	-	R	+	-	-	+	-	+	-	<i>P.aeruginosa</i>
2	+	S	-	+	+	+	-	-	+	<i>S.aureus</i>
3	-	R	+	+	-	-	-	+	+	<i>E.coli</i>

Key: R = Rod, S = Spherical, += Positive, -= Negative

Table 2: Antibacterial Activity of Ethanol Fruit Peel Extract of *Mangifera indica* against Isolated UTI Pathogens

Bacteria Isolates	Concentrations of the Extract (mg/ml)				
	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	Cipro.
<i>E.coli</i>	28.23±0.81****	23.33±0.88****	19.37±0.67****	16.83±0.33****	25.31±0.44
<i>P.aeruginosa</i>	24.33±0.33****	20.10±0.06****	18.00±0.68****	15.33±0.17****	24.57±0.83
<i>S.aureus</i>	22.63±0.33****	19.33±0.33****	16.33±0.67****	14.53±0.33****	23.83±0.17

Values are expressed as Mean ± SEM, Data analyzed by one-way ANOVA followed by Dunnet's Post hoc test. ****P<0.0001 compared to the control. n=3

Discussion

Increased resistance of pathogenic microorganisms to newer or modern antibacterial agents has limited their effective use, thus becoming a global concern (Valarmathy *et al.*, 2010). Various medicinal plants have been successfully used for the treatment of many infections and diseases owing to their availability, cheapness and fewer side effects (Meran *et al.*, 2017).

The antibacterial study of *Mangifera indica* fruit peel extract showed that the extract exhibited a significant ($P < 0.05$) dose dependent inhibitory effect against all the isolated UTI pathogens with the highest inhibition at 500mg/ml. The highest inhibitory effect was produced against *E. coli* at all the concentrations of the extract used indicating that *E. coli* is the most susceptible organism and *S. aureus* being the least organism having the lowest effect.

The inhibitory activity of the extract against *E. coli* as revealed by the diameter of cleared zone produced is higher than that obtained from the standard antibiotics (Ciprofloxacin) used. This may be due to the mixture of bioactive compounds present in the extract which may be working synergistically to produce the antimicrobial effect as against the standard antibiotic that has pure compound only (Olajuyigbe and Afolayan, 2012).

The strong inhibitory activity of the extract against *E. coli* agrees with the findings by Mutua *et al.*, 2017 in which four (4) varieties of *Mangifera indica* used also elicited a strong inhibitory effect against *E. coli*. The antibacterial activity exhibited by the extract could be attributed to the presence of secondary metabolites with potential medicinal value in the extract. These include tannins, saponins, flavonoids, steroids, terpenoids and alkaloids to mention a few (Falusi *et al.*, 2019).

Reports have shown that flavonoids and tannins possess antimicrobial activity which may be due

to the ability of flavonoids to complex with extracellular and soluble protein as well as with bacterial cell wall and tannins' ability to inactivate microbial adhesions, enzymes and cell envelope proteins (Veerachari and Bopaiah, 2011; Olajide *et al.*, 2013). Tannins are also known to possess good anti-ulcer, anti-diarrhoea, anti-diabetic, anti-tumor, antimicrobial, anti-inflammatory, antiseptic as well as wound healing activities (Olajide *et al.*, 2013). Flavonoids have also been reported to elicit several pharmacological activities which include anti-hypertensive, anti-rheumatic, antimicrobial, anti-diuretic as well as antioxidant properties (Esseiett *et al.*, 2010; Veerachari and Bopaiah, 2011).

Alkaloids have many therapeutic activities including anti-arrhythmic effect, anti-hypertensive activity, antimicrobial activity, anti-cancer activity, anti-plasmodial activity. Terpenoids on the other hand was noted for its anti-carcinogenic, antimalarial, antiulcer, antimicrobial and anti-diuretic activities (Cushnie *et al.*, 2014).

It is therefore possible that the antimicrobial and antioxidant effects of these bioactive constituents present in the fruit peel of *Mangifera indica* could be responsible for the antibacterial activity against UTI pathogens.

Conclusion

Based on the results obtained from this study, it can be seen that the extract of *Mangifera indica* fruit peel showed antibacterial activity against all the isolated UTI pathogens with more pronounced inhibitory effect against *E. coli*, thus suggesting *Mangifera indica* fruit peel as a potential candidate for the management of bacterial diseases especially those caused by gram negative organisms.

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Competing interests

The authors declare no conflict of interest.

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