

Original Research Article

Comparative study on treatment using fresh and dry unripe banana peels in water solvent with contemporary antibiotics on UTI

ABSTRACT

Almost every part of a banana plant has use in medicine. Increased bacterial resistance to the conventional antibiotics has led to research into the development of alternatives treatments to conventional antibiotics. This study focuses on the antimicrobial properties of banana peels against selected isolates from Urinary Tract Infection sample. The isolates from culture was further analysed with agarose gel electrophoresis for the presence of 16SrRNA and Phylogenetic analysis revealed *Staphylococcus sciuri* strain, a coagulase-negative species, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Fresh unripe and dry unripe were used to determine the antimicrobial activities. A measure of 150grams of both fresh unripe and dry unripe was extracted with 100ml of both 70% ethanol and water solvent (sterile) respectively for about 2 days. The solvent extracts were concentrated separately under reduced pressure, 10g of each concentrated solvent extracts were dissolved in 5ml of sterile distilled water and used for antimicrobial assay using agar well diffusion method. The phytochemical analysis of fresh unripe and dry unripe revealed that alkaloid, flavonoid, cardiac glycosids, and phenols were present. The zone of inhibition recorded from the extracts on selected organism was concentration dependent (100%, 80%, 60%, 40% and 20%). The higher the concentration the wider effect on each of the test isolates. *Musa sapientum* L peels showed some effect on *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus sciuri*, *Klebsiella pneumoniae* and *Proteus mirabilis* at 80% concentrations for water. Dry unripe has advantage over fresh unripe banana peels and solvent type (water) also play a synergetic effect. The peels of *Musa sapientum* exhibited some inhibitory activity on these selected UTIs isolates, which could be attributed to the presence of certain secondary metabolites. Finally, when compared the peels extracts against the standard antibiotics drugs as the control, the water solvent extract were less effective.

Keywords; Fresh unripe banana peels; dry unripe banana peels; urinary tract isolates.

1. INTRODUCTION

Banana peel is the outer covering of a banana fruit. It is commonly used in animal feeds (Happiet *al.*, 2011). Banana is one of the most popular food all over the whole world and its name is derived from the Arabic word "banan" meaning "finger". It has a shape "like an arch" which also contributed to the appealing nature or appearance of the fruit especially to human beings and monkeys (Happiet *al.*, 2011). It is a tropical fruit, which belongs to the family, *Musaceae* and is grown in many countries of the world (Shadmaet *al.*, 2014). Almost every part of a banana (flower, pulp, stem & leaves) plant has medicinal and nutritional values (Imam & Akter, 2011). A study by Shadmaet *al.*, (2014) has shown that banana contain some bioactive compounds which shows that it contain some pharmacological effect like antioxidant, antidiabetic, anti-inflammatory and antibiotic properties.

The increased failure of some chemotherapy and the resistance of pathogen to conventional antibiotics have led to several screenings of medicinal plants for potential antimicrobial functions.

These medicinal plants have longer evolution of resistance against microbial agents, which has led to alternative directions in drug and chemotherapy development (Bhatet *et al.*, 2010). These therapeutic plants are found to have longer evolution of resistance against microbial agents, which has led to several tips in drilling and chemotherapy improvement. The availability of medicinal plants everywhere could also be another contributory factor for the choice (Blantet *et al.*, 2010).

Urinary Tract Infection or UTI is an infection of the urinary system for example the kidneys, bladder, ureters, and urethra. However, most urinary tract infections comprise the lower urinary tract like the bladder and the urethra (Geerlings, 2014). Females are susceptible to UTI when compared to their male counterparts. (Flores-Mireles *et al.*, 2015). Infection restricted to the bladder of the UTI can be so painful, annoying or bothersome. Nevertheless, severe penalties can occur if a UTI spreads to the kidneys. Usually treatment is done with antibiotics. This study is aimed at the antimicrobial assessment of fresh unripe and dry unripe *Musa sapientum l.* peels against selected isolates from urinary tract infection samples.

2.0 MATERIALS AND METHODOLOGY

2.1 Collection of Banana Peels

The unripe banana peels that were used in these investigations were obtained from the same bunch from Bori General Market in Khana Local Government Area of Rivers State. This region is marked by lengthy rainy seasons and short dry seasons with temperatures of 25-28°C. It is situated in the southern part of Rivers State in Nigeria. The people living in the area are known to have fishing and farming as their primary occupation (Konne *et al.*, 2018). The bunch of banana was identified in the Department of Plant and Environmental Science, Faculty of Science, Rivers State University, Port Harcourt. The banana (unripe) was washed thoroughly with water, air dried and peeled. One was prepared to shade-dried for few weeks in the Department of Medical Laboratory Science and the second was chopped fresh and blended. The dried banana peels were ground with a sterilized surface grinder, into powdery form and stored in some clean tight-capped bottles (Reagent bottle) at about 4°C, with label and date (Ayubaet *et al.*, 2016).

2.2 Preparation of Banana Peels Extract

One hundred and fifty grams of fresh unripe and dry unripe banana peels were coarsely chopped and blended into different solvents (Water) of 100mls each for extraction. This was allowed to stand for about 2-3 days at room temperature with agitation at intervals to allow for proper reaction of yellow-transparent colour, which will indicate complete reaction (Zainabet *et al.*, 2013). Thereafter, the powder (Dry unripe peels) mixture in water solvent and was filtered using a muslin cloth. The fresh peels were blended and dissolved in the solvent (water) and was filtered accordingly both the fresh unripe and dry unripe respectively (Ehiowemwenguanet *et al.*, 2014).

It was evaporated with waterbath (Electronic thermostat water tank, Model HH.W21.Cr4211) was used for the extract (water) to reduce or eliminate the solvents, to obtain the (semisolid or paste) crude extract from the water. The crude extract obtained was stored in a refrigerator until required for use (Ehiowemwenguanet *et al.*, 2014).

2.3 Preparation of Stock Solution from the Extract of Banana Peels

After the completion of solvents evaporation, each semisolid or paste obtained were freeze-dried (lyophilized), 10 grams of each crude extract (paste) was reconstituted in sterile distilled water of 5mls to obtain a stock solution of mg/ml (milligram per millilitre) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2003).

2.4 TESTS FOR PHYTOCHEMICALS

Phytochemical screening was carried out to identify phytochemicals in the crude of the banana peels (fresh unripe) and from dry unripe banana peels. Test for Alkaloids (Dragendorff test), Flavonoids detection (Shinoda's test), Tannis detection (Lead acetate test), Saponins detection (Frothing test), Cardiac glycosids detection, Steroids detection (Liebermann-Burchard test), Phenols, Terpenoid, Lignans and Volatile oil were carried out using standard method as described by Ehiowenwenguanet *al.* (2014).

2.5 Preparation of Media

Preparation of CHROMagar™ Orientation, Nutrient Agar and Peptone water for culture and sensitivity testing, appropriate quantities were weighed based on the required numbers of plates and manufacturers' instruction was strictly adhered to. After sterilization with autoclave (Eschmed Medical England, Model YX-280A) at 121°C (15 lbs of pressure) for 15 minutes. It was then allowed to cool at temperature of about 45 to 50°C, poured into Petri-dishes and was allowed to solidify for well diffusion (Merlionet *al.*, 1996, Cheesbrough, 2000).

2.6 Source of Isolate and Culture.

Urine samples were collected from patients visiting Braithwaite Memory Specialist Hospital with suspected cases of UTIs was involved in this study. A Mid-Stream Urine (MSU) samples were collected into sterile universal containers from the subjects who visited BMSH for Urinary Tract Infections and was cultured on CHROMagar™ Orientation mainly for detection and differentiation of urinary tract pathogens (Merlionet *al.*, 1996).

2.7 CONVENTIONAL IDENTIFICATION OF ISOLATES

Gram Staining Procedure

Thereafter, with the aid of a sterile wire loop, a loopful colony of the isolated microorganisms were picked and emulsified on a clean grease free glass slide to air dry. The smear was adequately stained with freshly prepared gram staining reagents. Finally, the back of the stained slides were wiped with a cotton wool and placed in a draining rack for the smear to air dry. It was examined microscopically using ×100 oil immersion objective (Gram negative retained the counter stain as pink or dark red, while gram positive retain the primary stain dark purple or violet colour) according to Cheesbrough, (2000).

3.0 RESULTS AND DISCUSSION

Qualitative Screening for Active Phytochemical Components of Water-based Banana Peel Extract and Control

The qualitative phytochemical screening of both fresh unripe and dry unripe banana peels using water solvent shows that the extracts contain some classes of compounds as shown in Table 3.1. The Saponins, Alkaloids, Flavonoid, Cardiac glycosids, and Phenol were present. Whereas, Steroids, Terpenoid and Lignans were absent. The control solvent (ethanol) shows no bioactive substance (Table 3.1).

Physicochemical Properties of Water used as Solvent.

The physicochemical properties of the water that was used as the solvent, shows normal levels of pH, Salinity, Chloride, Total Alkalinity and Temperature, when compared with World Health Organisation (WHO) permissible limit. The Magnesium content was 0.46 mg/L, (WHO Standard <50mg/L), pH 7.0 (WHO Standard 6.5-8.0), Calcium 155mg/l (WHO Standard 100-200mg/l), Turbidity dissolved solid 259mg/l (WHO Standard <500mg/l), Chloride 197mg/l (WHO Standard 250mg/l). Others are Total dissolved solid 259mg/l (WHO Standard 500mg/l), Nitrate 0.33mg/l (WHO Standard 50mg/l), Sulphate 450mg/l (WHO Standard 400-500mg/l). The WHO permissible limit shows that the water sample does not contain any antimicrobial properties as a solvent (Table 3.2).

Table 3.1:Qualitative Screening for Active Phytochemical Components of Water and Control of Banana Peels Extract.

Types Solvents Control	Water Extract			Negative Control
	DU	FU	Water	
Active Components				
Alkaloids	++	+	-	
Flavonoid	+	+	-	
Cardiac glycosids	+	+	-	
Phenols	++	-	-	

Control of Banana Peels Extract

Key = (++) Absolutely Detected, (+) Moderately Detected, (-) Not Detected

This study focuses on evaluating the medicinal and pharmacological effects of *Musa sapientum* L. peels extracts on organisms obtained from subjects with urinary tract infection. Fresh unripe and dried unripe forms of the peels were used. The peels were gotten from a bunch of banana sample (*Musa sapientum* L.).Urinary tract infection on the other hand, appears to be the most common illness across all ages and genders, although it is more prevalent in the females due to

their anatomy, which also include the close proximity of the anus to the vagina (Nicolle *et al.*, 2005).

The phytochemical test carried out (**Table 3.1**) reveals that banana peels of *Musa sapientum*L. contains some bioactive compounds. The various bioactive ingredients were found in banana peels of fresh unripe and dry unripe of *Musa sapientum*L. peel using water as shown in table 3.1. Water extract shows the presence of alkaloids, flavonoids,cardiacglycosids, for both fresh unripe and additionally phenols for dry unripe. The dry unripe banana peels extract with water solvent has more of the bioactive compounds when compared to the fresh unripe. Some medicinal plant and aromatic compounds had been found to be a good source of bioactive component that are responsible for inhibiting some bacterial pathogens by precipitating the bacterial proteins, which could affect the bacterial peptidoglycan (Ayuba *et al.*, 2016). This indicates a little presence of active component in water solvent, which agrees with the results of Singh & Singh, (2000), for the use of organic solvents to be more suitable for phytochemical extractions.

Table 3.2: Physicochemical Properties of Water used as Solvent

Parameters	Water Sample	(WHO) Permissible Limit
pH	6.5	6.5 -6.9
Electrical conductivity	378	-
Turbidity (NTU)	0 unit	≤5units
Salinity %	0.19	-
Total dissolved solid mg/L	259 mg/l	<500mg/l
Phosphate PO ³⁻ ₄ (mg/L)	<0.05	-
Sulphate SO ²⁻ ₄ (mg/L)	450	400-500
Nitrate NO ⁻ ₃ (mg/L)	0.33	50
Chloride CL ⁻ (mg/L)	197mg/l	250mg/l
Total Alkalinity (mg/L)	4	-
Total Hardness as CaCO ₃	5.67	-
Calcium content	155	100-200 mg/l
Magnesium content	0.46	50mg/l
Temperature	29.7	-

Comparison of Zone of Inhibitions (mm) of 80% Concentration Banana Peels Water-Soluble Extract on UTIs Isolates.

The zone of inhibition (mm) of the 100% extract on *Escherichia coli* were FU (9.3±1.5), DU (8.6±0.5), while those of standard drugs were Ciprofloxacin (15±1.0), Streptomycin (00), Chloramphenicol (21±1.0), Gentamycin (16±0.5), Erythromycin (00), Tarivid (00) and Pefloxacin (00). There was a significant difference in the zones of inhibition produced by these substances ($p < 0.0001$). *Enterococcus faecalis* were FU (8.6±0.5), DU (9.0±1.0), while those of standard drugs were Ciprofloxacin (15±1.1), Streptomycin (14±1.0), Chloramphenicol (13±1.1), Gentamycin (11±1.0), Erythromycin (00), Tarivid (15±1.7) and Pefloxacin (16±1.1). There was a significant difference in the zones of inhibition produced by these substances ($p < 0.0001$). *Staphylococcus sciuri* were FU (00), DU (00), while those of standard drugs were Ciprofloxacin (15±1.5), Streptomycin (18±1.0), Chloramphenicol (13±1.0), Gentamycin (00), Erythromycin (17±1.0), Tarivid (00) and Pefloxacin (00). There was a significant difference in the zones of inhibition produced by these substances ($p < 0.0133$). *Klebsiella pneumoniae* were FU (8.0±1.0) and, DU (10±1.0), while those of standard drugs were Ciprofloxacin (22±1.0), Streptomycin (00), Chloramphenicol (12±1.2), Gentamycin (00), Erythromycin (00), Tarivid (21±1.5) and Pefloxacin (18±1.5). There was a significant difference in the zones of inhibition produced by these substances ($p < 0.0001$). Finally, *Proteus mirabilis* were FU (8.6±0.5), DU (9.3±1.5), while those of standard drugs were Ciprofloxacin (16±1.0), Streptomycin (12±1.5), Chloramphenicol (00), Gentamycin (12±1.0), Erythromycin (00), Tarivid (14±0.5) and Pefloxacin (17±1.5). There was a significant difference in the zones of inhibition produced by these substances ($p < 0.0001$). Post hoc Analysis of the data is presented in **Table 3.3** below

Table 3.3: Comparison of Zone of Inhibitions (mm) of 80% Concentration Peels Water-Soluble Extract on UTIs Isolates and CLSI Standard Reference Range for Sensitivity.

UTIs isolates/Banana Peel Extracts and Sensitivity Reference Range.	Resistance (mm) CLSI	Intermediate (mm) CLSI	Susceptibility (mm) CLSI	<i>Escherichia coli</i> (Gram-ve)	<i>Enterococcus faecalis</i> (Gram+ve)	<i>Staphy. Sciuri</i> (Gram+ve)	<i>Klebsiella pneumoniae</i> (Gram-ve)	<i>Proteus mirabilis</i> (Gram-ve)
FU (mg/ml)	≤ 12	13	≥ 15	9.3±1.5 Resist.	8.6±0.5 Resist.	00 Resist.	8.0±1.0 Resist.	8.6±0.5 Resist.
DU (mg/ml)	≤ 12	13	≥ 15	8.6±0.5 Resist.	9.0±1.0 Resist.	00 Resist.	10±1.0 Resist.	9.3±1.5 Resist.
Ciprofloxacin (10µg)	≤ 15	16 - 20	≥ 21	15±1.0 Inter.	15±1.1 Inter.	15±1.5 Inter.	22±1.0 Suscep.	16±1.0 Inter.
Streptomycin (30µg)	≤ 12	13 - 15	≥ 16	00 Resist.	14±1.0 Inter.	18±1.0 Suscep.	00 Resist.	12±1.5 Inter
Chloramphenicol (30µg)	≤ 13	14 - 22	≥ 23	21±1.0 Inter.	13±1.1 Inter.	13±1.0 Inter.	12±1.0 Resist.	00 Resist.
Gentamycin (10µg)	≤ 12	13 - 14	≥ 15	16±0.5 Suscept.	11±1.0 Resist.	00 Resist.	00 Resist.	12±1.0 Inter.

Erythromycin (10µg)	≤ 13	14 - 22	≥ 23	00	00	17±10	00	00
Tarivid (10µg)	≤ 13	14 - 16	≥ 18	00	15±1.7	00	21±1.5	14±0.5
Pefloxacin (10µg)	≤ 12	13 - 14	≥ 15	00	16±1.1	00	18±1.5	17±1.5
F-value	-	-	-	58.87	26.34	6.358	77.44	28.32
p-value	-	-	-	<0.0001	<0.0001	<0.0133	<0.0001	<0.0001

Key: p-value less than 0.05 (p<0.05) is considered significant

KEY:(CLSI) – Clinical and Laboratory Standard Institute, (FU)- Fresh Unripe banana peels, (DU)- Dry Unripe banana peels, (Suscept) –Susceptibility, (Inter) – Intermediate zone, (Resist)- Resistance and
00 – Absent.

CONCLUSION

This study has shown that the aqueous extract of both fresh unripe and dry unripe banana peel of *Musa sapientum* could be considered as a good antimicrobial agent for urinary tract isolates alongside synthetic medications. The presence of some secondary metabolites, in water extract of banana peels shows some significant inhibitory activities on urinary tract isolates. Finally, there was a synergetic effect from *Musa sapientum* peels and 70% ethanol on the urinary tract isolates.

RECOMMENDATION

Study on macro and micro mineral constituents of the peels would be a good aspect to consider.

The use of banana peels on fungi using different types of solvents should also be encouraged.

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