Antimicrobial Activities of Ethanol Extract of Banana (*Musa sapientum L.*) Peels Against Organisms Associated with Urinary Tract Infection in Port Harcourt, Nigeria.

ABSTRACT

Banana peels refers to the outer coverage of the banana fruit. Apart from serving as a food for man and animals, the banana plant also has medicinal values. Urinary Tract Infection is an infection that affects any parts of the urinary system (upper or lower). Its treatment and diagnosis are carried out with urine sample and antibiotics. Increased bacterial resistance to the conventional antibiotics has led to the development of alternative treatment for bacterial disease infections. This study is aimed at assessing the antimicrobial properties of fresh unripe and dried unripe banana peels against selected isolates from Urinary Tract Infection sample. The isolates from culture were further identified using biochemical tests. 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 80% Ethanol solvent 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 showed that alkaloid, flavonoid, cardiac glycosids, phenols tannins and volatile oil were present. Musa sapientum L peels showed some effect on UTIs at 80% concentrations for ethanol. The peels of Musa sapientum exhibited some inhibitory activity on these selected UTIs isolates.

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

1.0 INTRODUCTION

Banana peels is described as the outer covering of the fruits. It serves as feed for animals (Happi *et al*., 2011). Banana fruit is one of the commonly consumed fruits in the world and the "an arch like shape'' of the fruit contributes to why the fruit appears appealing to human beings and animals (Happi *et al*., 2011). Virtually all parts of banana plant (stem, pulp, leaves, and flowers) have medicinal benefits (Imam & Akter, 2011). Shadma *et al*., (2014) in a study reported that the presence of certain bioactive compounds that confer antidiabetic, antimicrobial and anti-inflammatory properties in banana proves the therapeutic potential of the fruit.

The increasing therapeutic failure due to increasing microbial resistance to synthetic drugs or antibiotics has posed a serious public health concern and alternative studies on medicinal plants for potential treatment or support (Bhat *et al*., 2010). Studies have reported in time past the role of medicinal plants in infection treatment. In herbal medicine, the availability of the herbs are critical part to consider and could serve as a basis for choice for herbal treatment. (Blant *et al.,* 2010).

The infections that affect the urinary tract system are referred to as Urinary Tract Infections (UTIs) (Geerlings, 2014). Both male and females can contract UTI but females are more at risk of UTI vulnerability because of the shared proximity between the anus and the vagina (*Flores-Mireles et al., 2015)*. Infection can span from the urethral to the urinary bladder and in a more severe case, in the kidney. Usually treatment is done with antibiotics. The focus of this study was to assess the antimicrobial function of fresh unripe and dry unripe *Musa sapientum l.* peels on selected isolates from suspected UTI 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 primary occupations of the inhabitants are fishing and farming (Konne *et al*., 2018). The banana bunch was identified in the Department of Plant and Environmental Science, Faculty of Science, Rivers State University, Port Harcourt. The unripe banana 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. Thereafter, it was also being dried in the hot air oven at a temperature of 37^0 C for about five days to ensure total elimination of Water (Water Availability). 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 4^0C, with label and date (Ayuba *et al.,* 2016).

2.1.1 Preparation of Banana Peels Extract

150g of fresh unripe and dry unripe banana peels were coarsely chopped and blended into the solvent (80% Ethanol v/v) of 100mls 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 (Zainab *et al*., 2013). Thereafter, the powder (Dry unripe peels) mixture in ethanol solvent was filtered using a muslin cloth. The fresh peels were blended and dissolved in (Ethanol) and was filtered accordingly both the fresh unripe and dry unripe respectively (Ehiowemwenguan *et al*., 2014).

It was evaporated with waterbath to obtain the (semisolid or paste) crude extract from the ethanol solvent. The crude extract obtained was stored in a refrigerator until required for use (Ehiowemwenguan *et al*., 2014).

2.1.2 Preparation of Stock Solution from the Extract of Banana Peels

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

2.2 Tests for Phytochemicals

Phytochemical screening was performed to identify phytochemicals in the crude of the banana peels (fresh unripe) and the powder (dry unripe). 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 Ehiowenwenguan *et al.,* (2014).

2.3 Preparation of Media

Preparation of CHROMagar^{TM} 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 121^0C (15 lbs of pressure) for 15 minutes, it was then allowed to cool at temperature of about 45 to 50^oC, poured into Petri-dishes and was allowed to solidify for well diffusion (Merlion *et al*., 1996, Cheesbrough, 2000).

2.4 Source of Isolate and Culture

Mid-Stream Urine (MSU) samples from patients visiting Braithwaite Memorial Specialist Hospital (BMSH) with suspected cases of UTIs were collected. The samples were collected into sterile universal containers from the subjects who visited BMSH for UTI test. Samples were cultured on CHROMagar^{TM} Orientation mainly for detection and differentiation of urinary tract pathogens (Merlion *et al*., 1996).

2.4 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 dried. The smear was adequately stained with freshly prepared gram staining reagents. Finally, the back of the stained slides was 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).

Further identification of UTI was done using biochemical tests.

2.4 Statistical Analysis

The obtained data were entered and analyzed using Statistical Package for Social Science (SPSS) version 20. The descriptive data was presented as means ± standard deviation (SD) and pvalue less than 0.05 was considered significant.

3.0 RESULTS AND DISCUSSION

Table 1. Biochemical tests used in the identification of the bacterial isolates

Biochemical and Enzymes Test	***Escherichia Coli***	***Enterococcus faecalis***	***Klebsiella Pneumoniae***	***Staphylococcus sciuri***	***Proteus Mirabilis***
Indole Test	+	-	+	-	+
Catalase Test	-	+	+	+	+
Coagulase Test	-	-	-	-	-
Citrate Test	-	-	+	-	+

Key: (-) Negative, (+) Positive

3.1. Qualitative Screening for Active Phytochemical Components of Ethanol and Control of Banana Peels Extract.

The qualitative phytochemical screening of both fresh unripe and dry unripe banana peels using ethanol shows that the extracts contain some classes of compounds as shown in **Table 2.** The Saponins, Alkaloids, Tannins, Flavonoid, Cardiac glycosids, Phenol and Volatile oil were present in the fresh unripe peels whereas, Steroids, Terpenoid and Lignans were absent. In the dry unripe, only Alkaloids, Tannins, and Falonoid were present, while Saponins, Steroid, Cardiac glycosids, Phenols, Terpenoids, Lignans and volatile iols are absent. The control solvent (ethanol) shows no bioactive substance.

Table 2: Qualitative Screening for Active Phytochemical Components of Ethanol and Control of Banana Peels Extract.

Types of Solvents & Control	Ethanol Extract	Neg. Control

Active Components	FU	DU	Ethanol (80%)
Saponins	+	-	-
Alkaloids	++	++	-
Tannins	+	+	-
Steroids	-	-	-
Flavonoid	++	+	-
Cardiac glycosids	++	-	-
Phenols	+	-	-
Terpenoid	-	-	-
Lignans	-	-	-
Volatile oil	+	-	-

Key
++ Absolutely Detected
+ Moderately Detected
- Not Detected

FU = Fresh Unripe
DU = Dry Unripe

This study was concerned with evaluating the medicinal and pharmacological effects of extracts of *Musa sapientum L.* peels on organisms obtained from subjects who had urinary tract infection. Both fresh unripe and dried unripe forms of the peels were used. The peels were obtained from the same bunch of banana sample (*Musa sapientum L.*). Urinary tract infection has been shown to be a common illness which cuts across all ages affecting both male and female, although the female tend to show a higher occurrence due to their anatomy, which also include the close proximity between the anus and the vagina (Nicolle *et al*., 2005).

The results obtained from the phytochemical test (**Table 1**) carried out reveals that banana peels of *Musa sapientum L.* contain some bioactive compounds. The various bioactive ingredients were found in banana peels of fresh unripe and dry unripe of *Musa sapientum L.* peel using ethanol (80%)**.** It was observed that different bioactive constituents of the banana peels were soluble based on the polarity of the solvent. In ethanol extract, the phytochemical contents are alkaloids, tannins, flavonoid, cardiac glycosids, phenols, and volatile oil, for fresh unripe. The dry unripe shows the presence of alkaloids, tannins, and flavonoids. Presence of secondary metabolites could also be responsible for the antimicrobial activity or function of the banana peels. Some medicinal plant and aromatic compounds had been found to be a good source of bioactive components responsible for inhibiting some bacterial pathogens by precipitating the bacterial proteins, which could affect the bacterial peptidoglycan (Ayuba *et al*., 2016). The fresh unripe banana peels show a greater level of active components in ethanol solvent, when

compared to the dry unripe in ethanol solvent which agrees with Singh & Singh, (2000), for the use of organic solvents to be more suitable for phytochemical extractions.

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

The zone of inhibition (mm) of the 80% extract on *Escherichia coli* were FU (11±1.0), DU (14±2.0), while those of standard drugs were Ciprofloxacin (14±2.0), Streptomycin (00), Chloramphenicol (21±1.5), Gentamycin (17±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 (10±1.1), DU (13±1.5), while those of standard drugs were Ciprofloxacin (15±0.5), Streptomycin (13±1.0), Chloramphenicol (13±1.0), Gentamycin (13±1.0), Erythromycin (00), Tarivid (15±1.0) and Pefloxacin (17±1.0). There was a significant difference in the zones of inhibition produced by these substances ($p<0.0004$). *Staphylococcus sciuri* were FU (00), DU (00), while those of standard drugs were Ciprofloxacin (17±0.5), Streptomycin (21±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 ($p<0.0001$). *Klebsiella pneumoniae* were FU (11±1.0), DU (10±1.5), while those of standard drugs were Ciprofloxacin (22±1.0), Streptomycin (00), Chloramphenicol (12±1.0), 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 (10±1.5), DU (10±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 (16±1.0). There was a significant difference in the zones of inhibition produced by these substances ($p<0.0001$). Posthoc Analysis of the data is presented in **Table 2**

Table 2: Comparison of Zone of Inhibitions (mm) of 80% Concentration Peels Ethanol-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**	***Escherichia coli***	***Enterococcus faecalis***	***Staphy. Sciuri***	***Klebsiella pneumoniae***	***Proteus mirabilis***
FU (mg/ml)	≤ 12	13	≥ 15	11±1.0 Resist.	10±1.1 Resist.	00 Resist.	11±1.0 Resist.	10±1.5 Resist.
DU (mg/ml)	≤ 12	13	≥ 15	14±2.0 Inter.	13±1.5 Inter.	00 Resist.	10±1.5 Resist.	10±1.5 Resist.
Ciprofloxacin (10µg)	≤ 15	16 – 20	≥ 21	14±2.0 Resist.	15±0.5 Inter.	17±0.5 Inter.	22±1.0 Suscep.	16±1.0 Inter.
Streptomycin (30µg)	≤ 12	13 – 15	≥ 16	00 Resist.	13±1.0 Inter.	21±1.0 Suscep.	00 Resist.	12±1.5 Inter.
Chloramphenicol	≤ 13	14 – 22	≥ 23	21±0.5	13±1.0	13±1.0	12±1.0	00

(30µg)				Suscep.	Inter.	Inter.	Resist.	Resist.
Gentamycin	≤ 12	13 – 14	≥ 15	17±0.5	13±1.0	00	00	12±1.0
(10µg)				Suscep.	Inter.	Resist.	Resist.	Inter.
Erythromycin	≤ 13	14 – 22	≥ 23	00	00	17±1.0	00	00
(10µg)				Resist.	Resist.	Inter.	Resist.	Resist.
Tarivid (10µg)	≤ 13	14 – 16	≥ 18	00	15±1.0	00	21±1.5	14±0.5
				Resist.	Inter.	Resist.	Suscep.	Inter.
Pefloxacin (10µg)	≤ 12	13 – 14	≥ 15	00	17±1.0	00	18±1.5	16±1.0
				Resist.	Suscep.	Resist.	Suscep.	Suscep.
F-value	-	-	-	20.56	6.097	89.39	46.66	8.506
p-value	-	-	-	<0.0001	<0.0004	<0.0001	<0.0001	<0.0001

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

CONCLUSION

This study reveals that the ethanol extract of dry unripe banana peel of *Musa sapientum* could be considered as a good complimentary antimicrobial agent for urinary tract isolates alongside with the conventional medicines. The extract of banana peels may have some significant inhibitory activities on urinary tract isolates.

RECOMMENDATION

Further study should be encouraged on any possible toxic effect from consuming these peels on human using laboratory rats and appropriate dose recommend for therapeutic consumption. The use of banana peels on fungi using different types of solvents should also be encouraged.

REFERENCES

Ayuba, S. B., Ikya, H. P., Ifeanyi, T. O., Oluboyo, B. O., Akele, Y. R., Akinseye, J. F. & Adewumi, A. F. (2016). Antibacterial Activities of Selected Fruit Peels against Organisms Associated with Urinary Tract Infection. *International Journal of Current Microbiology and Applied Sciences*, 5 (12), 265-272.

Bhat, M. S., Prabhakar, A., Rama, K. R. & Madhu, S. M. (2010). Optimization of Process Variables for Amylase Production Using Agro Residual Wastes by Faguchi Methodology. *Chemical of Production Processing Model,* 5, 4 – 7.

Bhat, R., Rai, R.V. & Karim, A.A. (2010). Mycotoxins in Food and Feed: Present Status and Future Concerns. *Comprehensive* Reviews in *Food Science and Food Safety,* 9, 57-81.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries*, Part 11, (2nd Edition). Cambridge, UK. Cambridge University Press.

Ehiowemwenguan, G., Emoghene, A. O. & Inetianbor, J. E. (2014). Antibacterial and Phytochemical Analysis of Banana Fruit Peel. *International Organization of Scientific Research,* 4, (8) 18-25.

Happi, E.T., Bindelle, J., Agneesens, R., Buldgen, A., Wathelet, B. & Paquot, M. (2011). Ripening Influences Banana and Plantain Peels Composition and Energy Content. *Tropical Animal Health Production Journal*, 43(1), 171 – 177.

Imam, M. Z & Akter, S. (2011). *Musa paradisiaca* and *Musa sapientum*. Phytochemical and Pharmacological Review. *Journal of Applied Pharmaceutical Science*, 1, 14 – 20.

Konne, F. E., Nwokah, E. G. and Wachukwu, C. K. (2018). Antimicrobial Assessment of Fresh Ripe and Dry Ripe *Musa sapientum L.* Peels against Selected Isolates Associated with Urinary Tract Infection in Port Harcourt, Nigeria. *Journal of Medical Science and Clinical Research*, 6(12), 420-430

Merlion, J., Ginn, A., Zong, Z., Wiklendt, A., Thomas, L., Gottlieb, T., van Hal, S., Harkness, J., Macleod, C., Bell, S., Partridge, S. & Iredell, J. (1996). Evaluation of CHROMagar Orientation for Differentiation and Presumptive Identification of Gram Negative *Bacilli* and *Enterococcus species*. *Journal of Clinical Microbiology*, 34, 1788-1793.

Nicolle S., Lindsay, E., Bradley, R., Colgan, S. & Hooton, M. (2005). Infectious Diseases Society of America Guidelines for the Diagnosis and Treatment of Asymptomatic Bacteriuria in Adults. *Clinical Infectious Diseases*, 40(5)2, 643–654.

Shadam, A., Sundaram, S. & Rai, G. K. (2014). Nutraceutical Application and Value Addition of Banana Peel. A Review. *International Journal of Pharmaceutical Science*, 6, 81 – 85.

Singh, I. & Singh, V.P. (2000). Antifungal Properties of Aqueous and Organic Solution Extracts of Seed Plants against *Aspergillus flavus* and *A. niger*. *Phytomorphyology*, 50,151-157.

Zainab, A. G., Chabuck,1., Alaa, H., Al-Charrakh, 1., Nada, K. H. & Shatha, K. H. (2013). Antimicrobial Effect of Aqueous Banana Peel Extract, Iraq. *Pharmaceutical Sciences*, 1, 73-75.