Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 5, Issue 3, pp: (26-31), Month: May - June 2018, Available at: www.noveltyjournals.com

EVALUATING THE ANTIBACTERIAL ACTIVITY OF *Musa acuminata* (BANANA) FRUIT PEELS AGAINST MULTIDRUG RESISTANT BACTERIAL ISOLATES

[1]LUCKY EVBUOMWAN, [2]IKECHUKWU BRIGHT JACOB,
[3]GIFT ONORIODE ONODJE, [4]CHUKWUKA EMEKA PATRICK

[1,3]Department of Microbiology, Faculty of Life Sciences, P. M. B. 1154, University of Benin,Benin City, Nigeria.
[2]Department of Virology, College of Medicine, University of Ibadan, Nigeria
[4]Laboratory Department, Maternity and Children Hospital, Al Mubaraz, Al Ahsa, Saudi Arabia
Corresponding Author Email Id: evbuomwanlucky1@gmail.com

Abstract: **This work was aimed at evaluating the antibacterial activity of banana peel using ethanol and aqueous as solvent of extraction. The antibacterial activity of both extract was assayed using agar well diffusion method. Clinical isolates including *Escherichia coli, Bacillus subtilis, Streptococcus mutans, Pseudomonas* and *aeruginosa* were used for the antibacterial analysis. The phytochemical screening revealed the presence of flavonoids, quinnones and alkaloid in both ethanolic and aqueous extracts. *Streptococcus mutans* was the most susceptible bacteria species with inhibition zones ranging from 8mm at 3.125mg/ml to 25mm at 100mg/ml of the ethanolic extract. *Pseuodomonas aeruginosa* and *Escherichia coli* were also observed to be very sensitive to the ethanolic extract and zones of inhibition ranged from 8mm to 24mm and 8mm to 25mm respectively at the same concentration range. The minimum inhibitory concentration of the ethanol and aqueous extracts was found to be 3.125 against all tested bacterial strains. Apart from *Streptococcus mutans* with minimum bactericidal concentration of 50mg/ml, all the other ted organisms had MBC of 100mg/ml in both the ethanolic and aqueous extract. The test organisms showed varying degree of resistance to conventionally used antibiotics and they were observed to be multiple drugs resistant. The most effective drugs were streptomycin and gentamicin while complete resistance was observed against the others. The sensitivity of these multidrug resistant pathogens to banana peel extract is of great importance to public health. Therefore, this extract should be seen as a potent antimicrobial agents.**

Keywords: **antibiotics, antibacterial, microorganisms, phytochemical, resistance, sensitivity.**

1. INTRODUCTION

Musa acuminata (banana) is the fourth most important food crop in developing countries, after rice, wheat, and maize, with nearly 90% of the crops being grown for small-scale consumption and local trade (Mumtaz *et al*., 2010). Bananas are an excellent source of vitamin B_6 and contain moderate amounts of vitamin C, manganese and dietary fibre.

Peels are often the waste part of various fruits. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their unknown benefit of commercial application. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fibre (50% on a dry matter

(DW) basis), proteins (7% DW), essential amino acids, polyunsaturated fatty acids and potassium. Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana peel ranges from 0.90 to 3.0 g/100 g DW (Sathya, 2014). Phytochemical analysis revealed the presence of Phytochemicals that maybe responsible for the activities displayed by the extracts. Phyto-analysis of extracts revealed the presence of chemicals such as alkaloids, flavonoids, glycosides, saponins, steroids, tannins and xanthoproteins (Rao *et al*., 2012). Tannin, Phenol and oil content were found in banana peel (Fapohunda *et al*., 2012). Phytochemical analysis of *Musa acuminata* flower extract by Sumathy *et al*. (2011) showed that it contains glycosides, tannins, saponins, phenols and flavonoids. Qualitative phytochemical analysis of the methanolic extract of Musa sapientum subsp. Sylvestris fruit peel, pulp and seed revealed the presence of carbohydrates, alkaloids, steroids and glucosides in all extracts. Flavonoids were detected in pulp, saponin in peel and seed and tannin in peel and pulp extracts (Zafar *et al*., 2011).

These phytochemicals are responsible for the various biological properties of banana peel extracts. In recent years, there has been an upsurge in the emergence of drug resistant bacterial pathogens thereby rendering antibiotics inefficient in treatment. There is therefore the need to source for other efficient treatment options such as the use of plant extract. In a study, agar filter paper disc method showed that *Proteus vulgaris* KZN and *Klebsiella pneumonia* ATCC 13047 were the most susceptible to banana peel extracts. Extracts obtained from *Musa sapientum* displayed a good activity against *Proteus vulgaris* and *Klebsiella pneumonia.* It was also shown to be active against *Bacillus subtilis* (Fapohunda *et al*., 2012).

The antimicrobial activities of solvent extracts (aqueous, methanolic and ethanolic) of Musa spp. were evaluated by Kirby-Bauer method against bacteria viz, *Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli* (Rao *et al*., 2012). Extracts from organic solvents with higher polarity such as methanol and ethanol gave significant stronger inhibitory activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Vibrio parahaemolyticus*. The antimicrobial activity of the extracts increased as the polarity of the extracting solvents increased (Padam *et al*., 2012). Antimicrobial activity of extracts of *Musa sapientum* has also been shown to have antimicrobial effects against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Samonella enteritidis* and *Escherichia coli* (Chanda *et al*. 2010). Banana blossom extract also exhibited significant antimicrobial activity against *Staphylococcus aureus*, *Proteus mirabilis*, *Bacillu ssubtilis*, *Aspergillus niger*, *Candida albicans*, *Micrococcus* sp and *Salmonella* sp. In this study, antibacterial activity of aqueous and ethanol extracts of banana peels were assayed

2. MATERIALS AND METHODS

Collection and preparation of plant material:

Fresh and ripe bananas were obtained from New Benin market, Benin City. Bananas were washed in running tap water in laboratory. The fresh peels were taken and cut into smaller bits, 5mm × 5mm and allowed to air dry. The dried pieces were grinded by a blender. Thereafter, 50g of the powder was soaked in 250ml of sterile distil water and ethanol in order to prepare aqueous and ethanol crude extract respectively. After twenty four hours, the solution was filtered and the clear filtrate was evaporated to dryness using water bath at 40^{o}C. the dried matter was obtained and bottled. It was then kept in the refrigerator at 4^{o}C until when needed.

Test microorganisms:

Four bacteria cultures including two gram positive bacteria, *Streptococcus mutans* and *Bacillus subtilis* and two gram negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* were used to carry out the test. The organisms were obtained from Microbiology laboratory, University of Benin Teaching Hospital (UBTH). Each isolates was subjected to standard morphological and biochemical technique for identification in microbiology laboratory, University of Benin, Benin City. The morphological and biochemical tests included gram staining, motility test, oxidase test, catalase test, coagulase test and indole test.

Antimicrobial Susceptibility Testing:

Preparation of Different Concentrations:

100mg/ml of each peel extract solution was used as the highest extract concentration. This was obtained by dissolving 1g of the extract in 10ml ethanol (80%. BDH). 50mg/ml, 25mg/ml and 12.5mg/ml, concentrations were subsequently prepared by double serial dilution method.

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 5, Issue 3, pp: (26-31), Month: May - June 2018, Available at: www.noveltyjournals.com

Bacteria inoculum preparation:

The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37°C. After incubation, One milliliter of the cultures was inoculated onto solidify nutrient agar at 45°C using a Pasteur pipette.

Evaluation of antibacterial activity:

The antibacterial and antifungal activities of the test sample were done using the Kirby- Bauer method also known as disk diffusion method. The organisms were inoculated into the Petri dishes containing nutrient agar, potatoes dextrose agar was used for the fungus. The disks were then placed appropriately on the surface of the agar plate by using a sterile forceps. The plates were inverted and placed in an incubator at 350c within 15 minutes after disks were applied.

The plates were incubated aerobically and examined after 24 hours of incubation. Each plate was examined, and the diameter of the zones of complete inhibition was measured to the nearest centimeter using a ruler.

Determination of minimum inhibitory concentration (MIC):

The Nutrient agar was prepared and sterilized, then poured into sterile Petri dishes and allowed to solidify. The surface of the medium was inoculated with the test isolates. 0.2ml of different concentrations of the extract was placed in the well of the seeded nutrient agar. The plates were incubated at 37°c for 24 hours, after which they were examined for the presence of growth inhibition. The MIC was taken as the lowest concentration that prevented the growth of the test microorganisms.

Minimum bactericidal concentrations (MBC):

A loopful of the content of clear zones in each plate, which did not show any visible growth after the period of incubation was streaked unto freshly prepared Nutrient agar, to determine their MBC and then incubated at 37°C for 24 hours after which it was observed for visible growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration.

Antibiotics susceptibility pattern:

Antimicrobial disc tests of the isolates were performed according to the recommendations of the National Committee Laboratory Standards (NCCLS) [2000] using the following antibiotic discs: tetracycline (20µg), ampiclox (30µg), zinnacef (20µg), amoxicillin (30µg), rocephin (25µg), ciprofloxacin (l0µg), Nitrofurantin (20µg), streptomycin (30µg), erythromycin (l0µg), gentamycin (I0µg), septrin (30µg), chloramphenicol (25µg), perfloxacin (l0µg), and ofloxacin (30µg) and antibiotics resistance was interpreted by diameter of inhibition zones around the antibiotic discs.

3. RESULTS

Results obtained from this research work are summarized in the tables below.

Table 1 presents the different phytochemicals present in both ethanolic and aqueous extract of banana peels.

Table 2 shows the cultural, morphological and biochemical characterization of the bacterial isolates. The antimicrobial activity of the ethanolic and aqueous extract of banana peels are summarized in Tables 3 respectively. Table 4 outlines the minimum inhibitory and bactericidal concentrations of both extract against the test isolates. Finally, Table 5 shows the antibiotic sensitivity pattern of the test organisms.

Table 1: Phytochemical analysis of *ethanol* and *aqueous extracts of banana peel*

Phytochemicals	Solvents	
	Ethanol	**Aqueous**
Flavonoids	+	+
Tannins	-	-
Terpenoids	+	+
Saponins	-	-
Quinines	+	+
Alkaloids	+	+

Key: + = present

-= Absent

International Journal of Novel Research in Life Sciences
Vol. 5, Issue 3, pp: (26-31), Month: May - June 2018, Available at: www.noveltyjournals.com

Table 2: Antimicrobial activity (inhibition zones in cm) of ethanolic extract of Banana peel

Bacteria	Concentration (mg/ml)					
	100	**50**	**25**	**12.5**	**6.25**	**3.125**
Streptococcus mutans	25	25	8	8	8	8
Bacillus subtilis	23	20	12	11	10	8
Pseudomonas aeruginosa	24	12	10	8	8	8
Escherichia coli	25	8	8	8	8	8

Table 3: Antimicrobial activity of Aqueous extract of Banana peel

Bacteria	Concentration (mg/ml)					
	100	**50**	**25**	**12.5**	**6.25**	**3.125**
Streptococcus mutans	25	22	12	12	8	8
Bacillus subtilis	23	8	8	8	8	8
Pseudomonas aeruginosa	24	22	20	16	8	8
Escherichia coli	18	8	8	8	8	8

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of Banana peel extracts

Test organisms	**MIC(mg/ml)**		**MBC (mg/ml)**	
Bacteria	Ethanol	Aqueous	Ethanol	Aqueous
Streptococcus mutans	3.125	3.125	50.00	100
Bacillus subtilis	3.125	3.125	100.00	100.00
Pseudomonas earuginosa	3.125	3.125	100.00	100.00
Escherichia coli	3.125	3.125	100.00	100

Table 5: Antibiotics sensitivity pattern of bacteria isolates

Organisms	**Antibiotics**									
Gram positive	CPX	S	SXT	E	PEF	CN	APX	Z	AM	R
S. mutans	R	S	S	R	R	S	S	R	R	R
Bacillus subtilis	S	S	R	R	S	R	R	S	S	S
Gram –ve	TE	NB	AX	OF	C	CF	AM	N	CN	CPX
P. aeruginosa	R	S	S	R	R	S	S	S	R	R
E. coli	R	S	R	R	R	R	R	R	S	S

Note:

R= Resistance, S= Sensitivity, CPX-Ciprofloxacin, Ro-Rocephin, S-Streptomycin, TE-tetracycline

SXT-Septrin, NB-Nitrofurantin, E-Erythromycin, C-Chloramphenicol, PEF-Pefloxacin, CF- ciprofloxacin, CN-Gentamicin, N-Nalidixic, APX-Apmpiclox, AM-Amoxacillin, Z-Zinnacef

4. DISCUSSION

Banana is readily available and cheap fruit that is consumed by different people around the world. This is because of its high nutritional properties. It is usually consumed when fully ripened and during this process, the soft skin is usually peeled off and the edible part is consumed. In recent times, it had been reported that this peels are not altogether useless as many of the bioactive plant components reside in them (Venkatesh *et al*., 2013). Therefore this project work was undertaken to ascertain the antimicrobial efficacy of banana peels against clinical isolates.

The phytochemical screening of banana peel revealed the presence of flavonoids, quinnones and alkaloid in both ethanolic and aqueous extracts. Flavonoids are known to be synthesized by plants in response to microbial attack. Hence, it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms, when tested in-vitro. Their activity is probably due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria (Idris *et al*., 2009). Tannins are also reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phyto-therapeutically, tannin containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Westendarp, 2006).

The test microbial isolates were collected from University of Benin, Benin City and were identified using standard microbiological methods, to be *Escherichia coli*, *Bacillus subtilis*, *Streptococcus mutans* and *Pseudomonas aeruginosa.* These organisms, especially the bacteria, have over the years been reported as common nosocomial infectious agent in the clinical settings. They are also of public health importance in the community and have been major aetiologies of Urinary tract infections in both men and women.

The antibacterial potency of banana peel extract was observed to be considerably higher at higher concentrations but moderate at lower concentrations. *Streptococcus mutans* was the most susceptible bacteria species with inhibition zones ranging from 8mm at 3.125mg/ml to 25mm at 100mg/ml of the ethanolic estract. *Pseuodomonas aeruginosa* and *Escherichia coli* were also observed to be very sensitive to the ethanolic extract and zones of inhibition ranged from 8mm to 24mm and 8mm to 25mm respectively at the same concentration range. This is in line with the work of Zafar *et al.* (2011). All the organisms tested were found to be sensitive to the ethanolic extract of banana peels. It was observed in this work that the antibacterial potency of banana peel is concentration dependent and also on the nature of the test organisms. This is further confirmed by earlier research study by Evbuomwan and Inetianbor (2017) who reported the antimicrobial activity of *Persea americana* to be dependent on extracting solvent, extract concentration and nature of the microorganisms used.

The aqueous extract of the plant was found to be equally active against the test isolates, just like the ethanolic fraction. For example, at 100mg/ml, inhibition zone of 25mm was observed against *Streptococcus mutans* while at similar concentration, inhibition zone of 024mm was observed against *Pseudomonas aeruginosa.* Padam *et al*., (2012) reported similar findings on antimicrobial activity of aqueous extract of banana peel. This shows that the antimicrobial activity of banana peels also depend on the solvent of extraction.

The minimum inhibitory concentration of the ethanol and aqueous extracts was found to be 3.125 against all tested bacterial strains. Apart from *Streptococcus mutans* with minimum bactericidal concentration of 50mg/ml, all the other ted organisms had MBC of 100mg/ml in both the ethanolic and aqueous extract in accordance with the work of Rao *et al*. (2012). This shows similar antibacterial activity of both extracts.

The test organisms showed varying degree of resistance to conventionally used antibiotics and they were observed to be multiple drug resistant. The most effective drugs were streptomycin and gentamicin while complete resistance was observed against the others. These resistances may have been due to plasmid or chromosomal encoded resistant genes in these organisms. Comparatively, banana peel was more potent an antibacterial agent in this work than conventionally used antibiotics

The sensitivity of these multidrug resistant pathogens to banana peel extract is of great importance to public health. Therefore, this extract should be seen as a potent antimicrobial agents.

5. CONCLUSION

The antibacterial properties of the ethanolic and aqueous extract of banana peels have found to be considerably high in this research work. The test organisms that were highly resistant to antibiotics were found to be susceptible to banana peel extract. Therefore, banana peel should be researched more into, for its possible synergistic or additive interactive antimicrobial efficacy against drug resistant pathogens.

REFERENCES

[1] S. Chanda, Y. Baravalia, M. Kaneria, and K. Rakholiya, “Fruit and vegetable peels–strong natural source of antimicrobics”. Journal of Agricultural and Food Chemistry Vol. 60, pp. 121-126, 2010

[2] L. Evbuomwan, and J. Inetianbor, “Antibacterial Activity of *Persia americana* Leaf Extracts Against Multidrug Resistant Bacterial Isolates”. *AASCIT* Journal of Bioscience vol. 3, No. 4, pp. 29-34, 2017

[3] S.O. Fapohunda, J.U. Mmom, and F. Fakeye, “Proximate analyses, phytochemical screening and antibacterial potentials of bitter cola, cinnamon, ginger and banana peel”. Academic Arena Vol. 4, No.8, pp. 8-15, 2012.

[4] S. Idris, G.I. Ndukwe, and C.E. Gimba, “Preliminary phytochemical screening and antimicrobial activity of seed extract of *persea Americana* (avocado pear)”. Bayero Journal of Pure and Applied Science, Vol. 2, No. 1, pp. 173-176, 2009.

[5] J. Mumtaz, M.K. Warsi, and F. Khatoon, "Concentration influence on antimicrobial activity of banana blossomextract-incorporated chitosan-polyethylene glycol (CS-PEG) blended film". Journal of Chemical and Pharmaceutical Research Vol. 2, No. 5, pp. 373-378, 2010.

[6] National Committee for Clinical Laboratory Standards NCCLS, "Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards- fifth edition. NCCLS document M7-A5. NCCLS: Wayne, PA, USA, 2000

[7] B.S. Padam, H.S. Tin, F.Y. Chye, and M.I. Abdullah, "Antibacterial and antioxidative activities of the Various Solvent Extracts of Banana (*Musa paradiasiaca* cv. *Mysore*) Inflorescences". Journal of Biological Sciences Vol. 12, No. 2, pp. 62-73, 2012.

[8] N.M. Rao, S.H.K.R. Prasad, and N. Jyothirmayi, "Efficacy of Ripened and Unripened Fruit Extracts of *Musa Paradisiaca* L. (Bontha Cultivar) Against Human Pathogens". International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 4, No. 1, pp. 455-460, 2012.

[9] M. Sathya, "Assaying the Antioxidant Activity of Banana Peel". American Journal of Bochemistry and Molecular Biology, Vol. 6, No. 5, pp. 1-6, 2014.

[10] V. Sumathy, J.S. Lachumy, Z. Zakaria, and S. Sasidharan, "*In Vitro* Bioactivity and Phytochemical Screening of *Musa Acuminata* Flower". Pharmacologyonline Vol. 2, pp. 118-127, 2011.

[11] V.K. Venkatesh, K.G. Kumar, K. Pradeepa, S.S.R. Kumar, and K. Vijay, "Anthelmintic Activity of *Musa paradisiacal* (L.). Puttabale". International Journal of Pharmaceutical Sciences and Drug Research Vol. 5, No. 2, pp. 67-69, 2013.

[12] H. Westendarp, 2006. "Effects of tannins in animal nutrition". Dtsch Tierarztl Wochenschr, Vol. ll3, No. 7, pp. 264-268, 2006.

[13] I.M. Zafar, A. Saleha, M.D. Mazumda, E. Hoque, and R.M. Sohel, "Antimicrobial and Cytotoxic Properties of Different Extracts of *Musa Sapientum* L. Subsb *Sylvestries"*. International Research Journal of Pharmacy Vol. 2, No. 8, pp. 62-65, 2011.