IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 4, Issue 8 (August 2014), PP. 18-25

Antibacterial and phytochemical analysis of Banana fruit peel

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ABSTRACT: *The in vitro antibacterial activity of ethanolic and aqueous extract of banana (Musa sapientum) peels was investigated on both gram-positive and gram-negative bacteria using agar well diffusion technique . The ethanolic extract of the peels had MIC values ranging from 16mg/ml to 512.5mg/ml. The least MIC was 16mg/ml against Salmonella typhi while Bacillus subtilis and Staphylococcus aureus showed the highest MIC of 512.5 mg/ml. In the aqueous extract the MIC ranged between 512.5mg/ml to >1025mg/ml. Salmonella typhi, Micrococcus luteus and Staphylococcus aureus were not inhibited by the water extract. Phytochemical result showed ethanol to be a better solvent for the extraction of the bioactive agents in banana peels which include: glycosides, alkaloids, saponins, tannins, flavonoids and volatile oil.*

KEYWORDS: *Antibacterial qualities, Phytochemicals, Banana (Musa sapientum).*

I. INTRODUCTON

Banana is a tropical fruit grown in over 122 countries worldwide (Husain and William, 2010). Until 2004, the cultivated area of 3.8 million hectares and a total production of 56.4 million metric tones of the fruit were produced ranking it fourth behind rice, corn and milk (Chai *et al*., 2004; Arumugam and Manikandan, 2011). In recent times, Banana peel has been utilized for various industrial applications including bio-fuel production, bio-sorbents, pulp and paper, cosmetics, energy related activities, organic fertilizer, environmental clean up and biotechnology related processes (Morton, 1987; Gunaseelan, 2004; Bori *et al*., 2007).
Bananas are naturally slightly radioactive, more so than most other fruits, because of their potassium content and the small amounts of the isotope potassium-40 found in naturally occurring potassium (Amarnath and Balakrishnan, 2007).

Ripe bananas fluoresce when exposed to ultraviolet light. The study suggested that this allows animals which can see light in the ultraviolet spectrum (tetrachromats and pentachromats) to more easily detect ripened bananas (Ashraf *et al*., 2010). This property is attributed to the degradation of chlorophyll leading to the accumulation of a fluorescent product in the skin of the fruit. The chlorophyll breakdown product is stabilized by a propionate ester group (Anhwange *et al*., 2009). Banana-plant leaves also fluoresce in the same way (Ashraf *et al*., 2010). Green bananas do not fluoresce (Bhat *et al*., 2010[a]).

All parts of the banana plant have medicinal applications (Amit and Shailandra, 2006): the flowers in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics; the astringent plant sap in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers (Girish and Satish, 2008); the roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India (Bhat *et al*., 2010[a]).
Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas (Brooks, 2008). The antibiotic acts against Mycobacteria (Omojasola and Jilani, 2009). A fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants (Ponnuswamy *et al*., 2011). Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp (Ratule *et al*., 2007). The first two elevate blood pressure; serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines (Anhwang *et al*., 2009).

Some of the specific diseases known to be cured by banana are Anaemia: High in iron, bananas are believed to stimulate the production of haemoglobin in the blood and so helps in cases of anaemia (Amit and Shailandra, 2006). Blood Pressure**:** Banana is extremely high in potassium yet low in salt, making it the perfect food for helping to beat blood pressure (Debabandya *et al*., 2010). Depression**:** This is because bananas contain tryptophan, a type of protein that the body converts into serotonin known to make you relax, improve your mood and generally make you feel happier (Girish and Satish, 2008). Heartburn**:** Bananas have a natural antacid

effect in the body so if you suffer from heartburn, try eating a banana for soothing relief (Mokbel *et al*., 2005). Morning Sickness**:** Snacking on bananas between meals helps to keep blood sugar levels up and avoid morning sickness (Amit and Shailandra, 2006). Mosquito bites**:** Before reaching for the insect bite cream, try rubbing the affected area with the inside of a banana skin. Many people find it amazingly successful at reducing swelling and irritation (Odebiyi and Sofowora, 1978). Nerves**:** Bananas are high in B vitamins that help calm the nervous system (Singh and Bhat, 2003). Smoking**:** Bananas can also help people trying to give up smoking, as the high levels of Vitamin C, A1, B6, B12 they contain, as well as the potassium and magnesium found in them, help the body recover from the effects of nicotine withdrawal (Mokbel *et al*., 2005). Stress**:** Potassium is a vital mineral, which helps normalize the heartbeat, sends oxygen to the brain and regulates the body's water-balance (Girish and Satish, 2008).

Strokes**:** eating bananas as part of a regular diet can cut the risk of death by strokes by as much as 40% (Amit and Shailandra, 2006). Temperature control**:** Many other cultures see bananas as a cooling fruit that can lower both the physical and emotional temperature of expectant mothers (Mokbel *et al*., 2005). Ulcers**:** The banana is used as the dietary food against intestinal disorders because of its soft texture and smoothness (Girish and Satish, 2008). And Warts**:** Those keen on natural alternatives swear that, if you want to kill off a wart, take a piece of banana skin and place it on the wart, with the yellow side out (Amit and Shailandra, 2006).
Alleged hallucinogenic effects of the smoke of burning banana peel have been investigated scientifically and have not been confirmed (Anhwange *et al*., 2009).
It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates (Singh and Bhat, 2003).

This study is aimed at investigating the antibacterial activity and phytochemical properties of Banana fruit (*Musa sapientum)* Peels on some bacterial isolates

II. MATERIALS AND METHODS

Sample Collection and Preparation

The banana peels used for the investigation were obtained from bananas bought from Uselu market, Benin City, Nigeria. They were air-dried for two weeks and ground into powder with a mechanical blender and sieved with a mesh of size 0.50mm. The powdered samples obtained were thereafter stored in clean brown bottles at room temperature ($28 \pm 2^{O}C$) until needed for use.

Preparation of Aqueous and Ethanol Extract

Ninety (90) grams of the powdered peels was dispensed in 900ml of distilled water in a 1L capacity conical flask. The mixture was stirred vigorously intermitently with a magnetic stirrer and then allowed to stand for 48h. It was stirred again and filtered through a Whatman filter paper lined funnel into a conical flask. The filtrate was evaporated at $40^{O}C$ with a water bath to obtain the solid crude extract.
The same procedure was carried out for ethanol extraction except that the crude solid extract was obtained by concentrating the filtrate with a rotary evaporator. All extracts obtained were stored in a refrigerator until required for use.

Phytochemical Analysis

The extracts of *Musa sapientum* peels were analysed for alkanoids, tannins, glycosides, steroids, flavonoids, saponins, volatile oil and resins using standard procedures.

Test for Glycosides: To 1ml of the extract was added 2ml of acetic acid and then cooled in an ice bath at $4^{O}C$. To this mixture 1ml of concentrated tetraoxosulphate (vi) acid (H_2SO_4) was added dropwise. The formation of an oil layer on top of solution indicated the presence of glycosides (Odebiyi and Sofowora, 1978).
Test for Alkaloides: To 3ml of the extract was added 1ml of 1% HCL. This resulting mixture was then treated with few drops of Meyer's reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids (Ogukwe *et al*., 2004).
Test for Saponins: Five drops of olive oil was added to 2ml of the plant extract and the mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins (Trease and Evans, 1996)
Test for Tannins: Two drops of 5% $FeCl_3$ was added to 1ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins (Trease and Evans, 1996)
Test for Flavonoids: To 1ml of the extract was added 3 drops of ammonia solution (NH_3^+) followed by 0.5ml of concentrated HCl. The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids (Odebiyi and Sofowora, 1978).

Test for Steroids: To 1ml of the plant extract was added 1ml of concentrated tetraoxosulphate (vi) acid (H_2SO_4). A red colouration confirmed the presence of steroids (Trease and Evans, 1996).

Test for Resins: To 5ml of the extract was added 5ml of copper acetate solution. The mixture was shaken vigorously and allowed to separate. The appearance of a reddish-brown precipitate indicated the presence of resins (Elmahmood and Doughari, 2008).

Source of Test Microorganisms

Pure cultures of pathogenic bacteria such as *Bacillus subtilis, Staphylococcus aureus, Micrococcus leutus, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* were obtained from the Department of Medical Microbiology, University of Benin, Teaching Hospital (UBTH), Benin City, Nigeria. They were gram stained and subjected to biochemical tests to confirm their identity (Cheesbrough, 2000). The organisms were subcultured in nutrient agar plates and stored in nutrient agar slants at $4^{O}C$ until needed for use.

Antibacterial Activity Assay

The determination of antibacterial activity was done using the agar well diffusion technique (Cheesebrough, 2000).The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24h at $37^{O}C$, a loop of inoculum was transferred into 5ml of nutrient broth, incubated for 2h at $37^{O}C$. This served as fresh suspension inoculum. Wells (5mm diameter) were made in sterile nutrient agar plate using a sterile cork borer (flame sterilized) and inoculum containing 10^7 CFU/ml of test bacteria were spread on solid plates with the aid of sterile swab moistened with the bacterial suspension. Then 50µl of aqueous extract or ethanol extract of banana peels were placed in the wells made in inoculated plates. Controls were set up with 50µl of sterile distilled water or ethanol. The plates were incubated at $37^{O}C$ for 24h and zones of inhibition if any around the well were evaluated in millimeters (mm) (Girish and Satish, 2008).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICs)

Determination of the minimum inhibitory concentration (MIC) of the extracts was carried out using the tube-dilution technique described by Cheesebrough (2000). A double fold serial dilution was made using Muller Hinton broth (MHB).The following concentrations were obtained: 1025mg/ml, 512.5mg/ml, 256mg/ml, 128mg/ml, 64mg/ml, 32mg/ml, 16mg/ml and 8mg/ml. Equal volume of extract and Muller Hinton broth (2ml) was dispensed into sterilized test tubes. A quantity (0.1ml) of standardized inoculum (1.25×10^7 cfu/ml) was added to each of the test tubes which were incubated aerobically at $37^{0}C$ for each 24h. A tube containing broth and inoculum without extract served as organism control. The tube with broth and extract without inoculum served as extract control. The lowest concentration of the extracts which inhibited microbial growth (no turbidity) was recorded as the minimum inhibitory concentration(MIC).

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION. (MBC)

Sterile Muller Hinton agar plates were inoculated with samples from each of the test tubes that showed no visible growth from the MIC test. The plates were then incubated at $37^{0}C$ for 24h. The lowest concentration of the extract yielding no growth was recorded as the minimum bactericidal concentration (MBC).

III. RESULTS

PHYTOCHEMICAL PROPERTIES OF *Musa sapientum* Peels

The different phytochemical constituents present in the peels of *Musa sapientum* is shown in Table 1. It was observed that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity. In the ethanol extract, the phytochemicals present include : alkaloids, glycosides, saponins, tannins and flavonoids. Of the phytochemicals assayed for, only two: glycosoides and alkaloids where found in the water extract (Table 1).

ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *Musa sapientum* Peels

The results of the antibacterial activity of the aqueous and ethanolic extracts of *Musa sapientum* peels against the test organisms are shown in Tables 2,3, and 4. The zone of inhibition of the growth of the isolates was found to be a function of the relative antibacterial potency of the extracts. Thus zones of inhibition decreased as the concentration of the extracts decreased.(Tables 2 and 3). At a concentration of 1025mg/ml, the highest zone of clearance was obtained from ethanol extract against *Klebsiella pnuemoniae* with a diameter of 38mm. This was followed by *Pseudomonas aeruginosa* (33mm), *Salmonella typhi* (30mm) and *Escherichia coli* (26mm) respectively. The lowest zone of inhibition at this concentration was 8mm against *Staphylococcus aureus.*

Higher growth inhibition was obtained with the ethanol extract compared with aqueous extracts. In table 3 the antibacterial activity of the aqueous extract of *Musa sapientum* peels revealed the highest zone of inhibition to be 24mm against *klebsiella pnuemoniae* compared to 38mm of ethanolic extract at the same concentration (1025mg/ml). The bacteria *Staphylococcus aureus, Micrococcus leutus,* and *Salmonells typhi* were not inhibited with the water extract and thus showed no zone of inhibition. The lowest zone of clearance in this experiment was 2mm against *Escherichia coli* using water extract at a concentration of 64mg/ml.

In table 4 is shown the results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Musa sapientum* peels on the test bacteria. The ethanolic extract of the peels had MIC values ranging from 16mg/ml to 512.5mg/ml. The least MIC was 16mg/ml against *Salmonella typhi* while *Bacillus subtilis* and *Staphylococcus aureus* showed the highest MIC of 512.5mg/ml. The MBC values of the ethanol extract ranged between 32mg/ml to >1025mg/ml. The MIC and MBC values of the aqueous extract ranged between 0-1025mg/ml and 0 to >1025mg/ml respectively.In the water extract *Escherichia coli* showed the least MIC of 64mg/ml and the highest was 1025mg/ml against *Bacillus cereus.*

Table 1: Phytochemical characteristics of the whole extracts of *Musa sapientum* peels

Phytochemical constituent	Ethanol Extract	Water Extract
Glycosides	+	+
Alkaloids	+	+
Saponins	+	-
Steroids	-	-
Tannins	+	-
Flavonoids	+	-
Resins	-	-
Volatile oil	+	-

+ = present - = Absent.

Table 2: The antibacterial activities of the ethanol extract of *Musa sapientum* peels.

	Zone of inhibition (mm) of bacterial isolates						
Conc. (mg/ml)	B1	B2	B3	B4	B5	B6	B7
1025	12	08	14	38	33	26	30
512.5	08	05	10	34	28	23	24
256	0	0	04	30	20	18	21
128	0	0	0	26	15	12	16
64	0	0	0	24	0	08	10
32	0	0	0	10	0	0	08
16	0	0	0	0	0	0	06
8	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0

KEY
B1=*Bacillus subtilis* B2=*Staphylococcus aureus*

B3=*Micrococcus luteus* B4=*Klebsiella pneumonia*

B5=*Pseudomonas aeruginosa* B6=*Escherichia coli*

B7=*Salmonella typhi*

Table 3: The antibacterial activities of water extract of *Musa sapientum* peels.

	Zone of inhibition (mm) of bacterial isolates						
Conc. (mg/ml)	B1	B2	B3	B4	B5	B6	B7
1025	08	0	0	24	06	12	0
512.5	0	0	0	18	04	08	0
256	0	0	0	15	0	06	0
128	0	0	0	12	0	04	0
64	0	0	0	0	0	02	0
32	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0

KEY
B1=*Bacillus subtilis* B2=*Staphylococcus aureus*

B3=*Micrococcus luteus* B4=*Klebsiella pneumonia*

B5=*Pseudomonas aeruginosa* B6=*Escherichia coli*

B7=*Salmonella typhi*

Table 4: The Minimum Inhibitory and Bactericidal Concentrations of both Ethanol and Water extracts of *Musa sapientum* Peels on bacterial isolates

	Isolates	Ethanol Extract		Water Extract	
		MIC	MBC	MIC	MBC
B1	*Bacillus subtilis*	512.5	>1025	1025	>1025
B2	*Staphylococcus aureus*	512.5	>1025	Nil	Nil
B3	*Micrococcus leutus*	256	256	Nil	Nil
B4	*Klebsiella pneumonia*	32	64	128	512
B5	*Pseudomonas aeruginosa*	128	128	512	1025
B6	*Escherichia coli*	64	256	64	512
B7	*Salmonella typhi*	16	32	Nil	Nil

KEY
B1=*Bacillus subtilis* B2=*Staphylococcus aureus*

B3=*Micrococcus luteus* B4=*Klebsiella pneumonia*

B5=*Pseudomonas aeruginosa* B6=*Escherichia coli*

B7=*Salmonella typhi*

IV. DISCUSSION

The preliminary phytochemical screening carried out showed *Musa sapientum* peels contain some secondary metabolites such as glycosides, alkaloids, saponins, volatile oil, flavonoids and tannins.
In general secondary metabolites present in plants have been reported by Rabe (2000) to be responsible for their therapeutic activity. Singh and Bhat (2003) reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants.

Plant essential or volatile oils and their individual components have been used in traditional systems of medicines for a variety of bacterial infections for centuries. Furthermore, it has been demonstrated that antibacterial properties of these oils can be attributed to their hydrocarbon and terpene constituents (Amit and Shailendra, 2006). The presence of glycosides and alkaloids in *Musa sapientum* peels may be attributed to their use by traditional medicine practitioners in healthcare systems in the treatment of some bacterial infections such as cough, fever, cold and venereal diseases. The results of this research highlights the fact that the organic solvent (ethanol) extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted more or only through the organic solvent medium. This observation agrees with the report of other investigators of medicinal plants that organic solvents are more suitable for extraction of phytochemicals. (Singh and Singh, 2000; Natarajan *et al*., 2005).

Microorganisms vary widely in their degree of susceptibility to anti-microbial agents. A high MIC value indicates low activity and vice versa. In this study the gram- negative organisms had the lowest MICs and MBCs. This suggest their higher susceptibility to the extract of the peels. On the basis of the result obtained in this investigation it can be conclude that ethanol extract of *Musa sapientum* peels had significant *in vitro* broad spectrum antimicrobial activity. Thus extracts from the plant can be used to control infections caused by *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus.* Opportunistic infections

such as bronchopneumonia, bacterial endocarditis and meningitis caused by *Micrococcus Spp.* and *Pseudomonas aeruginosa* will also find treatment with the extracts of this medicinal peel. The results obtained in this study justify the use of banana peel by traditional medical practitioners.

V. CONCLUSION

Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms pose an enormous threat to public health. The use of medicinal plants with antimicrobial activity need to be given more attention to arrest the situation.

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