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Phytochemical Analysis and Antibacterial Efficacy of Ethanolic Extract of *Musa paradisiaca*

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Abstract

The antibacterial activities of the extract of *Musa paradisiaca* L. were evaluated against *Staphylococcus aureus, Bacillus subtilis, E. coli*. Ethanolic extract (MPE) of *Musa paradisiaca* L. were obtained by standard methods. The antibacterial activity was assayed using agar well diffusion method. The MPE exhibited antibacterial effects with inhibiting zones ranging from 3.09 ± 0.01 mm to 8.53 ± 0.01 mm. The extract showed appreciable quantity of total phenol (Gallic acid equivalent) of 23.12 Mg GAE/g. The higher phenolic content of MPE may be responsible for its antibacterial activity. This study shows that useful bioactives component that can be used in food processing industries.

1. Introduction

Plants have for generations been a source of various kinds of remedies and been used for medicinal purpose to cure different types of ailments and will continue to provide remedies for these ailments, especially in rural areas of developing countries. Consumption of medicinal herbs is tremendously increasing over past decades as an alternative approaches to improve the quality of life and to maintain good health (Pintu and Arna, 2014). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al.,* 2006).

Medicinal plants contain number of medicinal properties. One of such plant is *Musa paradisiaca*. *Musa paradisiaca* (family - Musaceae), also known as plantain, is a tropical plant that is native to India. It has been reported to have pharmacological activities such as antilithiatic, antioxidant, antibacterial, antidiabetic, antiulcer, antidiarrhoeal, hypocholesterolaemic, hepatoprotective, antisnake venom, wound healing, hair growth promoting, antifungal and antimenorrhagic activity (Ghani A., 2003). All parts of the Banana plant have medicinal uses. The flowers are used in treating bronchitis, dysentery, menorrhagia and ulcers. Cooked flowers are used to treat diabetes. The astringent plant sap is given in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, 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 roots are administered in digestive disorders, dysentery and other ailments. It also has anthelmintic property. Antifungal and antibiotic properties are found in the peel and pulp of fully ripe Bananas. The plant is also used in inflammation, pain and snakebite (Khare CP, 2007).

The peel of *Musa paradisiaca* is often ignored and considered as waste for possible utilization as livestock feeds. Literature reviews indicated that there are no reports available for antibacterial activities from *Musa paradisiaca* peel. This study was conducted due to lack of scientific data especially on the phytochemical compositions and antibacterial effects of *Musa paradisiacal* peel. In this context, the aim of this study was to investigate the composition and the antimicrobial capacities of extracts from *Musa paradisiacal*, which could be useful in the selection of materials for the production of bioactive compounds and nutraceuticals.

2. Materials and Methods

2.1 Collection of Plant Materials

The peels of banana fruits were collected from local market

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of Mayiladuthurai, Tamil Nadu. The peels after collection were air-dried under shade. Dried skins were then pounded to a fine powder for ease of extraction of active compounds.

2.2 Extraction

Fifty grams (50 g) each of the dried powder of the fruits was transferred into 3 different glass containers. The powdered leaves were sequentially extracted with 250 ml each for Ethanol and chloroform; for water 500 ml was used. The extraction method employed was percolation for a week, during which the bottles were undergoing shaking at regular intervals. The extracts were filtered using Whatman No. 1 filter paper. Each of the resulting filtrate was then concentrated by complete evaporation of solvent at room temperature except for aqueous extract which was evaporated in a water bath at 100 °C. The filtrate was carefully labeled and stored in the refrigerator for further use (Fatope *et al.,* 1993).

2.3 Phytochemical Screening

2.3.1 Test for Alkaloids

Aliquots of the extracts (0.1 ml) were added in test tubes and then 2 to 3 drops of Dragendoff's reagent were added. An orange red precipitate indicated the presence of alkaloids (Ciulci, 1994).

2.3.2 Test for Flavonoids

To 4 mg/ml of each of the extracts, a piece of magnesium ribbon was added this was followed by concentrated HCl drop wise. A colour change ranging from orange to red indicated flavones while red to crimson indicated flavonoids (Sofowora, 1993).

2.3.3 Test for Saponins

Half gram of the extract was dispensed in a test tube. Five milliliters of distilled water was added to the tubes and it was stirred vigorously. A persistent froth that lasts for about 15 min indicated the presence of saponins (Sofowora, 1993).

2.3.4 Test for Steroids

Two milliliters of the extracts were taken into separate test tubes. The residues were dissolved in acetic anhydride and chloroform was then added. This was followed by the addition of concentrated sulfuric acid by the side of the test tubes using a pipette. A brown ring at the interface of the two liquids and a violet colour in the supernatant layer denoted the presence of steroids (Ciulci, 1994).

2.3.5 Test for Tannins

Two milliliters of each aliquots of the extract was diluted with distilled water in separate test tube and 2 to 3 drops of 5% ferric chloride ($FeCl_3$) solution was added. A green – black or blue colouration indicated the presence of tannins (Ciulci, 1994).

2.3.6 Test for Glycosides

Ten milliliters of sulfuric acid (50% v/v) was added to 1 ml each of the *L. spericum* extracts in separate test tubes. The mixtures were heated for 15 min. Ten milliliters of Fehling's solution was added to tubes and the mixture boiled. A brick red precipitate indicated presence of glycosides (Sofowora, 1993).

2.3.7 Test for Terpenoids

To 0.5 g of the plant extracts 2 mL of chloroform was added. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase formation show positive results for the presence of terpenoids (Ayoola *et al.,* 2008).

2.3.8 Test for Phenols

Extracts were treated with few drops of ferric chloride solution. Formation of bluish-black colour indicates the presence of phenols (Sowofora, 1993).

2.4 Bioassay Studies

2.4.1 Isolates Collection and Biochemical Tests

Clinical bacterial isolates comprising of Gram-positive, gram-negative and fungal strains.

2.4.2 Media Preparation

Mueller Hilton Agar and Nutrient broth were prepared according to manufacturer's specifications.

2.4.3 Standardization of Inoculum

Using sterile inoculation wire loop, 3-4 colonies from an overnight culture of the test organism was transferred into a tube of saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

2.4.4 Antimicrobial Susceptibility Test

The agar well diffusion method was used for the antimicrobial susceptibility test. Mueller Hilton agar was prepared according to manufacturer's specification. The media were autoclaved and dispensed into sterile petri-dishes and allowed to gel. Standardized inocula of each bacterial isolates were streaked on the agar plate. Four wells of 6 mm each was made in each plate with a central well for control using a sterile cork borer. The wells were filled with 0.1 ml of different concentrations (400 µ/ml, 200 µ/ml, 100 µ/ml and 50 µ/ml) of the extract with the aid of sterile pipettes per well. Likewise, 400 µ/ml, 200 µ/ml, 100 µ/ml and 50 µ/ml of the standard antibiotic (amoxicillin) were used in separate plates to serve as positive control. While sterile distilled water was used as negative control on separate plates. The plates were allowed to stand for 15 minutes on a table to allow free diffusion of the extracts. Diameters of zones of inhibition were measured using transparent plastic metre rule after 24 hours of incubation at 37 °C (Dahiru *et al.,* 2013).

2.4.5 Minimum Inhibitory Concentration

MIC was determined by preparing various concentrations of

the extracts by serial doubling dilution and incorporated into test tubes containing 2 ml nutrient broth. Standardized inocula of 0.1 ml of the isolates were inoculated and the tubes were incubated at 37 °C for 24 h (NCCLS, 2008).

2.4.6 Minimum Bactericidal Concentration (MBC)

Nutrient agar plates were inoculated with sample from each of the tubes that show no turbidity and the plates were incubated at 37 °C for 24 h to determine the MBC. MBC was determined by inoculating samples from the MIC tubes that showed no bacterial growth on Mueller Hilton agar plates separately and then incubated at 37 °C for 24 hours. After the incubation the plates were observed for presence or absence of growth. The least concentration of the extract that showed no bacterial growth was considered as the MBC (NCCLS, 2008).

3. Results and Discussion

For the past few decades, extracts derived from various parts of plants such as roots, stems, leaves, barks and fruits are investigated for its various pharmacological activities due to its widespread, cost, nontoxic, easy availability and affordability. India has been considered as medicinal garden and having a very old traditional knowledge and folk medicine. Frequency of intake of antibiotics can increase the expensiveness as well as its side effects which make the scientist to focus on herbal drugs. The present study was undertaken to explore the phytochemicals and antibacterial activity from *Musa paradisiaca.*

The physical properties and percentage yield of the aqueous, ethanolic and chloroform extracts of banana peel is shown in (Table 1). The highest percentage yield of the extract was observed in aqueous extract which was 15.5% w/w of the total sample extracted, followed by ethanolic extract with 8.96% w/w and lastly chloroform extract with the least of 3.5% w/w. This indicates that the plants components are more soluble in high polar solvents. It can therefore, be deduced that the amount of extracts recovery is polarity dependent.

Table 1: Physical Properties of Extract

Solvent	Colour	Odour	Texture	Wt of the sample (g)	Quantity recovered	% yield
$CHCl_3$	Dark green	Odourless	Slighhtly sticky	50	1.2	2.46
Ethanol	Dirty green	Chemical	Slighhtly sticky	50	3.3	6.79
Aqueous	Reddish brown	Odourless	Gummy	50	6.6	13.24

Tannins in plants have been shown to confer anti-diarrhoeic and anti-haemorrhagic properties on plants (Asquith and Butler, 1986). This is consistent with the traditional use of the sap of *Musa paradisiaca* for the treatment of diarrhoea, fresh wounds, cuts and insect bites. Saponins have been reported to have antifungal properties (Osuagwu *et al.,* 2007) as well as serve as an expectorant and emulsifying agent (Edeoga *et al.,* 2003). Alkaloids, flavonoids and tannins have been known to show medicinal activity as well as exhibiting physiological activity (Sofowora, 1993). Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion and progression of tumors (Kim *et al.,* 1994). The presence of these phytochemicals in the sap of *Musa paradisiacal* confers medicinal properties on the plant and this explains the use of this plant for treatment of different ailments. The findings of this study isconsistent with reports of the presence of these phytochemicals in various parts of the *Musa paradisiacal* plant as documented by Akpuaka and Ezem (2011) and Akpabio *et al.* (2012) (Table 2).

Table 2: Phytochemical Composition

Extract	Alkaloid	Saponin	Tanin	Flavanoids	Steroids	Glycosides	Terpenoids	phenols
Aqueous	+	-	+	+	+	-	-	-
Ethanol	+	+	+	+	+	-	+	+
$CHCl_3$	+	-	+	+	-	-	-	+

"+" indicates presence, "-" indicates absence

The result of the antibacterial activity of *Musa paradisiacal* is shown (Table 3). From the result highest zone of inhibition was observed in ethanolic extract with 13.0 mm for *E. coli*, 1 mm for *B. subtilis* and 10.0 mm for *S. aureus*. This is followed by aqueous extract with 10.5 mm for *B. subtilis* and 10 mm for *S. aureus. E. coli* was resistant to both aqueous and chloroform extract of *Musa paradisiaca. E. coli* was also resistant to chloroform extract. It can therefore, be deduced that, chloroform extract of *Musa paradisiaca* was the least bioactive. It is pertinent however, to state that, lowest concentration of 50 and 100 µ/ml showed little or no activity on the test bacteria. Thus, the bioactivity of the extracts followed the sequence: Combined Extract > Ethanolic Extract > Aqueous Extract > Chloroform Extract. While the hierarchy of the susceptibility pattern of the tested bacteria to the extract is: *E. coli* > *B. subtilis* > *S. aureus.*

Similar study conducted by Karadi *et al.* (2011) studied the *in vitro* antimicrobial effect of crude extract of *Musa paradisiaca*

Table 3: Antibacterial Activity

Bacteria	AE (µg/ml)				EE (µg/ml)				CE (µg/ml)				AMOX (µg/ml)			
Concentration of Zone inhibition	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400
E. coli	0	0	9	10	7	8	9	10	0	0	8	10	12	12	15	18
S. aureus	0	7	9	0	0	0	0	10	13	0	0	0	0	9	11	14
B. subtilis	0	0	0	11	0	0	8	11	0	0	0	8	9	11	14	16

Where, AE: Aqueous extract; EE: Ethanolic extract; CE: Chlorofrm extract; Amox: Amoxicilin

and *Cocos nucifera* on bacteria *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and fungi Candida albicans, Candida tropicalis* & *Aspergillus niger.* Both the plants extract showed inhibitory effect on test organisms. The extract of *Musa paradisiacal* produced wider zones of inhibition against *Candida albicans*, than the crude extract of *Cocos nucifera.*

Jawla *et al.,* (2012) also confirmed that the antimicrobial and antihyperglycemic activities of *Musa paradisiaca* flowers. The EtOH and EtOH: water (1:1) extracts of *Musa paradisiaca* flowers were screened for antibacterial activity against standard strains of *Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus, Salmonella typhimurium* and *Candida albicans* against amikacin and clotrimazole respectively.

Ponmurugan and Mustaffa (2013) investigated different Musa species leave extracts of hexane, ethyl acetate and methanol of *Musa acuminate Colla, Musa troglodytarum, Musa sapientum* and *Musa paradisiacal* for antibacterial activity against multi-drug resistant pathogens causing nosocomial infection by agar well diffusion method. Antibacterial susceptibility test, minimum inhibitory concentration and minimum inhibitory bacterial concentration were determined. All the Musa sp. extracts showed moderate antibacterial activities expect. *Musa paradisiaca* with the inhibition zone ranging from 8.0 to 18.6 mm. Among four species, ethyl acetate extracts of *Musa paradisiaca* showed highest activity against tested pathogens particularly *E. coli, P. aeruginosa* and *Citrobacter species*. The minimum inhibitory concentrations were within the value of 15.63-250 µg/mL and minimum bactericidal concentrations were ranging from 31.25-250 µg/mL. The study concluded that among the different Musa species, *Musa paradisiaca* displayed efficient antibacterial activity followed by *Musa acuminata* against multi-drug resistant nosocomial infection causing pathogens.

The MIC and MBC result is shown in (Table 4). From the result both aqueous, ethanolic and chloroform extracts indicated an MIC range of (12.5-50 µg/ml) and MBC range of (50-100 µg/ml, 25-400 µg/ml and 25-100 µg/ml) for aqueous, ethanolic and chloroform extracts respectively. While the standard antibiotic (amoxicillin) had MIC and MBC ranges of (6.25-25 µg/m). However, from the finding of this study it is enough to state that ethanolic extracts had the lowest MIC values when compared to the other extracts. Ethanolic extracts similarly had the least MBC values.

Table 4: MIC and MBC

Bacteria	AE		EE		CE		Amox	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
E. coli	50	100	50	100	50	100	50	22.5
S. aureus	28	50	12.5	400	25	50	6.25	25
B. subtilis	50	100	12.5	25	25	50	6.25	12.5

Where, AE: Aqueous extract; EE: Ethanolic extract; CE: Chlorofrm extract; Amox: Amoxicilin

4. Conclusion

Frequent intake of allopathic drugs for the treatment of bacterial infections results in the generation of adverse side effects. Phytomedicines from herbals have been considered safer than the allopathic drugs. Banana is a tropical fruit cultivated all over the world and all the parts of the banana have assorted medicinal applications. The present study has explored the phytochemicals and antibacterial activity of ethanolic extract of *Musa paradisiaca* in a dose dependent manner. The results concluded that *Musa paradisiacal* peel can be used for treating bacterial infections and its utilisation for this purpose should be encouraged, thereby enhancing solid wastes management and reducing environmental pollution. However, further research is needed to identify and Molecular biology study of phytochemicals responsible for antibacterial activity.

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