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Antifungal properties of *Musa paradisiaca* (Plantain) peel and stalk extracts

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ABSTRACT

Effect of plantain (*Musa paradisiaca* (L) AAB genomic group) peel and stalk extracts were investigated using percentage inhibition test. Complete inhibition of growth (100%) was observed for *Aspergillusniger, Aspergillus oryzae* and *Rhizopus stolonifer* at 1.0 mg/ml concentration of stalk extract. Peel extract inhibited *A. niger* 100%, *A. oryzae* 76.67% and *R. stolonifer* 56.67% at the same concentration. As concentration reduces, growth inhibition reduces also up to the minimum inhibitory concentration. The results of this work justify that the plant extracts were able to inhibit and kill the growth of spoilage fungi and this implies that the extract in appropriate doses can be used in food preservation and to treat infections caused by this spoilage fungi. The results further justify the claim that *Musa paradisiaca* (L) stalk and peel extract demonstrated antifungal action in which methanol was seen to be a better solvent for extracting active ingredients from medicinal plants considering the high susceptibility of test organisms to methanol extract than ethanol extract used in this study. Phytochemical screening showed the presence of hydrogen cyanide, tannin, alkaloid, steroid, saponin and flavonoid. The growth inhibition of *A. niger, A. oryzae* and *R. stolonifer* by *Musa paradisiaca* peel and stalk methanol and ethanol extract in this study suggest the presence of antifungal substance in the plant tissue and the possibility of using the extract to control plant pathogens especially where the spoilage fungi used in this study are involved.

Keywords: *Musa paradisiaca*, peel, stalk, extract, growth inhibition, spoilage fungi.

INTRODUCTION

Plantain (*Musa* spp., AAB genomic group), Banana (*Musa* spp., AAA genomic group) and cooking banana (*Musa* spp., ABB genomic group) are important food crop in tropical countries (Robinson, 1996) and are important staple food for rural and urban consumers in the humid forest and mid altitude of sub-Sahara Africa (Vuylsteke et al., 1993a). Pests and diseases are the major constrains in plantain and banana production worldwide (Gowen, 1995). Tetraploid hybrid resistant to major biotic constrains (eg Sigatoka leaf spot and Panama disease) have been selected through conventional breeding (Ortiz et al., 1995). In Nigeria, herbal medical practitioners use the leaf decoction of Plantain and Banana for treatment of typhoid fever, diarrhea, malaria, stomach ache or ulcers (Apata, 1979; Okigbo and Omodamiro, 2006). Plant extracts have been used successfully to control disease in plants and tuber crops (Amadioha and Obi, 1999; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005). Some antibiotic have become obsolete because of the problem of drug resistance

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(Okigbo and Omodamiro, 2006). New drugs both synthetic and natural must be sought to treat diseases. This implies that local medicinal plants need to be screened for antimicrobial properties of their extracts against known organisms. It has been widely accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veermuthu et al., 2006; Okorondu et al., 2010b; Okorondu, 2011).

The fungi used as test organisms (*Aspergillus niger, Aspergillus oryzae and Rhizopus stolonifer*) were frequently isolated from the surface of spoiling melon and groundnut seeds and is assumed to be responsible for most spoilage of stored seeds and grains. The aim of this research therefore is to investigate the antifungal properties of *Musa paradisiaca* stalk and peel extracts with a view to ascertain the concentration at which the fungi can be killed or the growth inhibited.

MATERIALS AND METHODS

Collection of sample and preparation

Samples of *Musa paradisiaca* L (Plantain), was collected from National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Abia State, Nigeria. *Aspergillus oryzae*, *Aspergillus niger* and *Rhizopus stolonifer* were isolated from agricultural soil sample from Obinze, Owerri West L.G.A. Imo State. They were sub cultured onto Sabouraud dextrose agar and reconfirmed to be pure isolates before being used. Mature but unripe plantain stalk and peel were carefully diced. The diced materials were separately dried in a stainless tray using carbolite moisture extraction oven at 60 °C for 24 h. The dried samples were separately ground in a laboratory mill (Arthur Thomas) and screened through 1 mm sieve. The resulting powder sample was used for extraction.

Extraction

The organic solvents used were methanol (BDH chemicals Ltd Poole England) boiling point 64.6 °C and ethanol (BDH chemicals Ltd Poole England) boiling point 78 to 78.5 ^{o}C.

Ethanol extract

Thirty grams (30 g) of samples (Plantain peel and stalk) were used. Using a soxhlet apparatus, the active ingredients of the ground particles were extracted. For extraction, 150 ml of ethanol was used. The extract was filtered using sterile filter paper. The filtrate was evaporated to dryness using the rotary vacuum evaporator at the boiling temperature range of the solvent (79 ^{o}C). The solvent was recovered in the recovery flask while the extracts remain in the sample holder, which was collected and stored in the refrigerator at 4 ^{o}C. The extract was sterilized with UV light before use.

Methanol extract

The procedure for the extraction of active ingredient from *Musa paradisiaca* (Plantain) peel and stalk using methanol as the solvent extractor was as described for ethanol extract. The filtrate was evaporated to dryness at 65 ^{o}C using rotary vacuum evaporator.

Fungal susceptibility testing

Extract-Sabouraud dextrose Agar (SDA) mixtures were prepared by mixing extract with molten SDA before the agar solidified (62 g SDA/ liter sterile water). The extract was sterilized before preparing mixtures by exposing to UV light (18). Discs of the fungus grown on SDA (3 mm in diameter) were cut with cork borer and placed in the Centre of Petri plates containing different concentrations of the extracts. Controls were Petri plates prepared with SDA but without the addition of extracts. Replicates of each treatment were incubated at 28 °C for 4days. Radial growth was measured with a metric rule in extract-treated and controls. Radial growth was monitored for both extract treated and control to quantify the effect of *Musa paradisiaca* peel and stalk extract on fungal development. Reduction in radial fungal growth as compared with control was expressed as percentage.

Minimum inhibitory concentration (MIC)

Aliquot of the extract (0.2 gm) was dissolved in 10 ml of sterile distilled water to obtain concentration of 20.0 mg/ml. This 20 mg/ml concentration was double diluted in sterile distilled water to obtain 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml. One milliliter (1 ml) of each concentration of extract was transferred to a sterile Petri plate and 10 ml of cooled Sabouraud dextrose agar were poured into the plates, swirled to mix and allowed to solidify. This gave a final concentration of 1.0 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml. This was incubated at 28 °C for 4 days and observed for fungal growth. The lowest concentration of the extract that inhibited the growth of fungi was recorded as the minimum inhibitory concentration. Plates without the test extracts were used as control.

Phytochemical tests

Hydrogen cyanide (qualitative)

The presence of hydrogen cyanide (HCN) was tested as an indicator of the presence of cyanogenic glycoside. The alkaline picrate paper method was used. One gram of each test sample was mixed with 50ml of distilled water in a conical flask. A picrate paper (filter paper soaked in alkaline picrate solution) was fitted to hang over the soaked sample in the flask, incubated for 18h at room temperature and examined for colour change. Orange colouration gave positive results (Balagopalan et al., 1988).

Tannin

Tannin was determined according to Trease and Evans (1983). Two grams of the samples were boiled in 20 ml of 45% ethanol for 5 minutes. The mixture was cooled and filtrate used for analysis.

a. Ferric chloride test: One ml of filtrate was diluted with 2.0 ml of distilled water and 2 drops of ferric chloride solution added and observed for transient greenish to black colour.

b. Lead acetate test: One milliliter (1 ml) of filtrate was added to 3 drop of 5% lead acetate solution and observed for gelatinous precipitate.

c. Bromine water test: One ml of filtrate was added to 0.5ml of bromine water and observed for pale brown precipitate.

Alkaloid

A quantity of 0.1g of the ground samples were boiled with 5ml of 2% hydrochloric acid on a steam bath. This was filtered and 1ml portion of the filtrate reacted with 2 drops of the following reagents (Trease and Evans, 1983).

a. Dragendorff's reagent (Bismuth potassium iodide solution) and observed for orange colour precipitate.

b. Wagner's reagent (Iodine in potassium iodide solution) and observed for reddish brown precipitate.

c. Meyer's reagent (Potassium mercuric iodide solution), and observed for creamy coloured precipitate.

d. Picric acid solution (1%) and observed for yellow precipitate.

Steroid

The test for steroid was done by the Liberman acid test. A portion of the organic extract was treated with three drops of acetic anhydride. Then concentrated H_2SO_4 was carefully added by the side of the test tube. The presence of a brown colour at the boundary of the mixture was taken as positive result (Trease and Evans, 1983).

Saponin

A quantity of 0.1 g of the powdered samples were boiled with 5 ml of distilled water for 5 minutes and decanted while still hot. The filtrate was used for frothing and emulsion tests (Trease and Evans, 1983).

Frothing test

One milliliter (1 ml) of the filtrate was diluted with 4 ml of distilled water and the mixture shaken vigorously and observed on standing for suitable froth.

Emulsion test

This was performed by adding 2 drops of olive oil to the frothing solution, and shaken vigorously.

Flavonoid estimation

Two grams (2 g) of samples were heated with 10ml of 5% ethyl acetate in a boiling water bath for 3 minutes. The mixture was filtered and 4.0 ml of filtrates were shaken with 1ml of 1% aluminum chloride and 1ml of 1% dilute ammonia solution. Yellow colouration of ammonia layer gave positive result (William et al., 1996).

RESULTS

The organisms used in this study demonstrated susceptibility to the organic extract of *Musa paradisiaca*. At 1.00 mg/ml concentration of *M. Paradisiaca* stalk methanol extract, the growth of *A. niger, A. oryzae* and *R. stolonifer* were inhibited 100%. At the same concentration, stalk ethanol extract inhibited these organisms 100%, 80% and 80% respectively (Table 1). At 0.5 mg/ml concentration of *M. paradisiaca* stalk methanol extract, inhibition were 56.67%, 56.67% and 75.33% on the organisms respectively while ethanol extract gave 50%, 50%, and 75% inhibition on the respective fungi (Table 1). At 0.25 mg/ml concentration of *Musa paradisiaca* stalk methanol extract, the fungi were inhibited 33.33%, 30.00%, and 50.00% respectively, ethanol extract inhibited the fungi 23.33%, 26.67%, and 26.67% respectively (Table 1). At 0.025 mg/ml concentration of *Musa paradisiaca* stalk methanol and ethanol extract, growth of *A. niger* and *A. oryzae* were not inhibited at all, *R. stolonifer* was inhibited by methanol extract 26.67% while ethanol extract did not inhibit the growth of *R. stolonifer* at this concentration. At 1.00 mg/ml concentration of *M. paradisiaca*peel methanol extract, *A. niger*, *A. oryzae* and *R. stolonifer* were inhibited 100%, 76.67% and 56.67% respectively while ethanol extract inhibited the fungi 80.00%, 70.00%, and 50.00% respectively (Table 2). At 0.50 mg/ml concentration of *M. paradisiaca* peel methanol extract, *A. niger, A. oryzae*, and *R. stolonifer* were inhibited 70.00%, 56.67%, and 26.67% respectively, ethanol extract inhibited the test fungi 60.00%, 50.00%, and 26.67% respectively (Table 2). At 0.25 mg/ml concentration of *M. paradisiaca* peel methanol extract, *A. niger* and *A. oryzae* were inhibited 53.33% and 26.67% respectively while ethanol extract inhibited the test fungi 50.00% and 26.67% respectively. At this concentration, the growth of *R. stolonifer* was not inhibited at all (Table 2). At 0.125 mg/ml concentration of *M. paradisiaca* peel methanol and ethanol extract, *A. niger* was inhibited 26.67%. Growth of *A.oryzae* was not inhibited at this concentration (Table 2). As the concentration of the extract reduces, the percentage inhibition reduces also up to minimum inhibitory concentration.

The minimum inhibitory concentrations of *Musa paradisiaca* peel methanol and ethanol extract were found to be 0.125 mg/ml, 0.25 mg/ml and 0.50 mg/ml on *A. niger, A. oryzae* and *R. stolonifer* respectively (Table 3). Minimum inhibitory concentration of *M. paradisiaca* stalk methanol extract was found to be 0.25 mg/ml, 0.25 mg/ml and 0.125 mg/ml on *A. niger, A. oryzae* and *R. stolonifer* respectively and MIC of *M. paradisiaca* stalk ethanol extract was found to be 0.25 mg/ml on all the test fungi (Table 4).

Phytochemicals screening revealed the presence of tannin, alkaloid, steroid, saponin and flavonoid, while hydrogen cyanide was absent (Table 5).

Table 1: Growth inhibition (%) of fungi by *Musa paradisiaca* (L) stalk methanol and ethanol extracts.

Isolates	Concentration of extract (mg/ml)				
	1.00 mg/ml	**0.50 mg/ml**	**0.25 mg/ml**	**0.125 mg/ml**	**0.0625 mg/m**
Control	0.00	0.00	0.00	0.00	
A. niger	100.00(100.00)	56.67(50.00)	33.33(23.33)	0.00(0.00)	
A. oryzae	100.00(80.00)	56.67(50.00)	30.00(26.67)	0.00(0.00)	
R. stolonifer	100.00(80.00)	75.33(75.00)	50.00(26.67)	26.67(0.00)	0.00(0.00)

Data represents mean of triplicate determinations; 0.00, no growth inhibition; Values in bracket are for ethanol extract.

Table 2: Growth inhibition (%) of fungi by *Musa. paradisiaca* (L) peel methanol and ethanol extracts.

Isolates	Concentration of extract (mg/ml)				
	1.00 mg/ml	**0.50 mg/ml**	**0.25 mg/ml**	**0.125 mg/ml**	**0.0625 mg/ml**
Control	0.00	0.00	0.00	0.00	0.00
A. niger	100.00(80.00)	70.00(60.00)	53.33(50.00)	26.67(26.67)	0.00(0.00)
A. oryzae	76.67(70.00)	56.67(50.00)	26.67(26.67)	0.00(0.00)	
R. stolonifer	56.67(50.00)	26.67(26.67)	0.00(0.00)		

Reduction in radial fungal growth as compared with control and expressed as percentage 0.00, no growth inhibition
Values in bracket are for ethanol extract.

Table 3: Minimum inhibitory concentration of *Musa paradisiaca* (L) peel methanol and ethanol extracts.

Isolates	Solvents extract (mg/ml)	
	Methanol	**Ethanol**
A. niger	0.125	0.125
A. oryzae	0.25	0.25
R. stolonifer	0.50	0.50

Table 4: Minimum inhibitory concentration of *Musa paradisiaca* stalk methanol and ethanol extract.

Isolates	Solvents extract (mg/ml)	
	Methanol	**Ethanol**
A. niger	0.25	0.25
A. oryzae	0.25	0.25
R. stolonifer	0.125	0.25

Table 5: Phytochemical screenings of *Musa paradisiaca* (L) stalk and peel methanolextracts.

Sample	HCN	Tannin	Alkaloid	Steroid	Saponin	Flavonoid
Musa paradisiaca						
Stalk	-	+	+	+	+	+
Peel	-	+	+	+	+	+

+, present; -, absent; HCN, hydrogen cyanide.

DISCUSSION

Musa paradisiaca (L) stalk and peel methanol and ethanol extracts inhibited the growth of *Aspergillus niger*, *Aspergillus oryzae* and *Rhizopus stolonifer*.The growth inhibition of test fungi reduces in percentage as the concentration of plant extract reduces up to the minimum inhibitory concentration (MIC).The growth inhibition of *A. niger*, *A. oryzae* and *R. stolonifer* by *Musa paradisiaca* peel and stalk methanol and ethanol extract in this study suggest the presence of antifungal substance in the plant tissue and the possibility of using the extract to control plant pathogens especially where the spoilage fungi used in this study are involved. *Musa paradisiaca* peel and stalk extract inhibited the growth of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Salmonella typhi* (Okorondu et al., 2010a, 2010b). Janovskyet al. (2003) reported that antimicrobials of plant origin are not usually associated with any side effects and have enormous therapeutic potential to heal many infectious diseases. Methanol extract of plants used in this study showed a better percentage inhibition than ethanol extract. This could be due to the fact that methanol being more volatile, dissolved more phytochemicals with antimicrobial properties than ethanol. *Aspergillus niger* was found to be more susceptible to the plant extracts. The use of plant extract in controlling plant diseases has advantage of being pathogenic specific, biodegradable, inexpensive, readily available and environmentally friendly (Fawceft and Spencer, 1970; Bayer, 1978). Chemical studies of local medicinal plants provide valuable clues for discovering and development of new drugs (Elizabetsky and Nunes, 1990; Rosoanairo, 1990). Antimicrobial properties of plants extract had been attributed to the presenece of alkaloids and flavonoids (Harborne, 1973; Tsuchiya et al., 1994; Hutchings et al., 2003; Okorondu et al., 2010b). Phytochemicals with bitter taste such as alkaloids and flavonoids has been found to possess antimicrobial properties in this study. The use of chemical pesticides on economic crops or plants may have adverse effect on soil, crop and not safe for consumption by man or animals. The presence of useful phytochemicals such as tannin, alkaloids, saponin and flavonoid contributed to antifungal properties exhibited by these plant extracts.

Conclusion

The results of this work justify that plant extracts were able to inhibit and kill the growth of these food spoilage fungi and this implies that the extract can be used in food preservation. The results obtained in this work further justify the claim that *Musaparadisiaca* (L) stalk and peel extract demonstrated antifungal action in which methanol was seen to be a good solvent for extraction considering the higher percentage growth inhibition of the extract on test fungi. This research will also provide current and useful documentary on medicinal plants.

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