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Research Article

Antifungal activity and phytochemical analysis of selected fruit peels

Abstract

Antifungal activity and phytochemical analysis of selected fruit peels were investigated in this work. The selected peels, obtained from orange, pineapple cashew and banana fruits were air dried at 28±2^{0}C for four weeks and later grounded into powders. Extracts were prepared from each powder using water and ethanol as solvents. The antimicrobial activity of each of the aqueous extracts (100mg/ml) against *Aspergillus niger* and *Alternaria alternata* (test isolates) was assayed using agar well diffusion method. Agar well without any extract served as control. Phytochemical screening of each extract was equally carried out. Results obtained on day three of incubation showed that *Aspergillus niger* had its respective inhibition zones with orange, cashew, pineapple and banana peel extracts as 0.33±0.33, 0.40±0.30, 0.60±0.20 and 0.87±0.33cm while inhibition zones of *Alternaria alternata* with the peels in the same order were 0.50±0.50, 0.60±0.35, 0.87±0.43 and 1.37±0.67cm. In each case, inhibition zone with banana peel was significantly different (P≥0.05) from others. However, the inhibition zone of the control could not be determined. Hence, the order of antifungal activity of the peel extracts against *Aspergillus niger* and *Alternaria alternata* was banana> pineapple> cashew> orange. The antifungal activity of these peel extracts could be due to different classes of compounds such as alkaloid, flavonoid, tannin, cardiac glycoside, saponin and phlobatannin found in them.

Introduction

Fungi are the most common cause of plants diseases and they are widespread and very destructive to both plants and humans [1]. This fungal pathogen enters the harvested fruits and vegetables through cracks, bruises and wounds during the harvesting process [2]. In fact, during storage, fungi can make food crops unfit for consumption, by changing the nutritional value of the seeds or producing mycotoxins that are harmful for human and animal health [2]. The two types of fungi that are important in food spoilage are yeasts and moulds. Moulds are multi-cellular fungi that reproduce by the formation of spores (single cells that can grow into a mature fungus). Spores arc formed in large numbers and are easily dispersed through the air. Once these spores land on a food substrate, they can grow and reproduce if conditions are favorable. Yeasts are unicellular fungi that are much larger than bacterial cells. They reproduce by cell division (binary fission) or budding. Fungi affect different types of fruits such as tropical (pawpaw, mango), subtropical (*Citrus species*, avocado) and temperate fruits (Apples, strawberries and grapes).

Post-harvest diseases which play a major role in reducing the quantity and quality of fruits include anthracnose and powdery mildew of various tropical fruits caused by *Fusarium*, *Collectotrichum gloeosporioides*, *Aspergillus*, *Lasiodiplodia*, *Penicillium* [3]. For example *Pencillium digitatum* causes green rot while *Penicillium italicum* causes blue rot on sweet orange fruits. Fungi like *Altenaria alternata* causes soft rot in mangoes. The fungus invades the fruit much more rapidly and predominates in mixed infections, causing approximately 60-80% of decay [3-6]. *Fusarium, solani* causes dry rot in potatoes, *Collectotrichum gloeosporioides* causes anthracnose in mangoes and avocado, *Botrytis cinerea* causes blue mould in grapes and *Rhizopus nigricans* causes soft rot on pawpaw fruits [7]. *Penicillum expansum* [8] and *Botrytis cinerea* [9] are pathogens of apples, pears, and a number of other pectin-rich fruits.

The use of chemical pesticides is the most common method for the control of various fungal diseases of fruit and vegetables [10]. The growers normally use chemical fungicides to solve the problem of fruit spoilage [11]. Commonly used fungicides include mancozeb, benomyl, captan and basic copper sulphate. However, health conscious consumers now prefer fruits that are not treated with synthetic fungicides due to their adverse effect. Restrictions on the use of these compounds are also being imposed because of their carcinogenicity [12]. Besides, environmental issues such as pollution which can lead to the death of living organisms inside the water bodies pose another risk to the use of chemical pesticides. Furthermore, synthetic fungicides are expensive and inaccessible to indigenous farmers who are the bulk producers of fruits in Nigeria [13]. Hence, there is need for non-chemical control alternative.

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Several studies have revealed plant extracts as source of natural pesticides that make excellent effort for new pesticide development. The damaging activities by plant pathogens could be reduced by the use of plant extracts. Plant extracts, offer potentially simple environmentally safe alternative for use as botanical fungicides, and could be exploited for the effective management of pre-harvest diseases of tropical fruits such as pawpaw, banana and mango etc. The added advantage includes that plant extracts are cheaper and non-toxic to man if the appropriate concentrations are used. Medicinal plant materials have been successfully used for the treatment of fungi and bacteria infections in humans [14]. The methanol leaf extracts of *Acacia nilotica* (gum arabic tree) *and Sida cordifolia* (flannel weed) showed significant antibacterial activity *Bacillus subitilis, Escherichia coil, Pseudomonas fluorescens, SlaphvIococcus aureus* and *Xanthomonas spp* and antifungal activity against *Aspergillius flavus* and *Fusarium verticillioides* [15]. Investigation into the antifungal properties of *Chomolena odorata* (siam weed), *Carica papaya* (pawpaw) *and Acalypha ciliata*, (copper leaf) showed that the crude extracts of these plants possessed some inhibitory components which cause significant reduction in mycelial growth of the fungi such as *Aspergillus niger* and *Fusarium solani* [16]. In fact, numerous scientific investigations point at consecutive rich sources of anti-microbes, especially among fruits and vegetables, but only few of these investigations involve waste parts of fruits such as seeds and peels. Hence, this study was carried out to investigate antifungal activity and phytochemical analysis of some selected fruit peels.

Materials and Methods

Preparation and sterilization of culture medium

The culture medium used for isolation of fungi from spoiled fruits and for preparation of pure cultures in the study was prepared by weighing 50g of malt extra agar (MEA) into a conical flask to which was added one liter of water. The mixture was shaken together and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, it was poured into oven-sterilized Petri-dishes and allowed to solidify.

Isolation from infected fruits

The test fungi were isolated from spoiled fruits by placing fruit rind affected by the fungi without surface sterilization on plates of solidified malt extract agar. The plates were then incubated at 28±2°C for 3 days. Sub culturing was then carried out by transferring agar cut with distinct mycelium to sterilized Petri dishes containing solidified MEA and then incubated at 28±2°C until pure cultures were obtained. The resulting pure culture was then used for morphological characterizations of each isolate.

Morphological identification of fungal isolates

Basic classification and identification tests on the fungi isolated from the spoilt fruits were carried out using the criteria outlined by [17]. A drop of lactophenol solution was put on a slide. The test fungal isolate was placed on the slide and stained with the lactophenol and was then covered with a cover slip. Excess liquid was drained off with a filter paper and examined under a binocular microscope at 40× objective magnification.

Collection and drying of selected plant peels

Four different fruits (orange, cashew, banana, pineapple) were harvested from Ibule Soro town (7.25°N and 5.195°E), Ondo state, Nigeria and brought to the department of Biology laboratory FUTA. The fruits were peeled with a knife and were air dried at 28±2°C for four weeks before they were grinded using a mechanized blender.

Preparation of peel extracts

Extracts were prepared from the powdered sample according to the method of [18], but with slight modification. Water and ethanol was used for the extraction. For aqueous extraction, exactly 100g of each powdered sample was soaked in 1000mls of cold water. Each solution was allowed to stand for 24 hours after which it was first sieved with a clean muslin cloth and filtered using the Whatman No. 1 filter paper. The filtrate was collected in a sterile clean beaker and concentrated in vacuo using rotary evaporator (Resona, Germany). This was also repeated for ethanol extraction.

Phytochemical screening of peel extracts

The phytochemical screening of the peel extract was done according to the method described by [19]. The phytochemicals screened for were tannin, saponin, phlobatinnin, flavoniod, alkaloid and cardiac glycosides.

Test for antifungal activity of peel extract

The peel extracts obtained suing aqueous extraction (water) was used for the antifungal assay. The antimicrobial activity of each of the extracts was assayed using agar well diffusion method described by [20], with slight modification. The concentration of the extract used was 100mg/ml. The isolate of the *Aspergillus niger* cut 6mm diameter cork borer was inoculated on the malt extract agar aseptically. This was done triplicate on the MEA plates. Wells of 6mm was bored on the agar with the sterile cork borer, 2cm away from the inoculums and the extracts were introduced into the wells on the agar plates. The plates were then incubated at 28±2°C and observed daily for clear zones which are indicative of the inhibition of the organism by the extract. Agar well without any peel extract served as control. This was also repeated for *Alternaria alternata*. Each set up was in triplicate.

Statistical analysis

The data obtained for antifungal activity of 100mg/ml of each fruit peel was subjected to one way ANOVA and where significant, the means were compared at 5% level of probability using New Duncan's Multiple Range Test (SPSS version 20.0).

Results

Effects of selected fruit peel extracts on mycelia growth of Aspergillus niger and Alternaria alternata

On day three of incubation, the mean zones of inhibition (cm) of *Aspergillus niger* with the selected peel extracts were

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0.33±0.33 (orange), 0.40±0.30 (cashew), 0.60±0.20 (pineapple) and 0.83±0.33 (cashew) (Table 1). Similarly, the mean zones of inhibition of *Alternaria alternata* with the selected peel extracts were 0.50±0.50 (orange), 0.60±0.30 (cashew), 0.87±0.43 (pineapple) and 1.37±0.67 (cashew) (Table 2). These values were not significantly different from one another except that of the banana peel extract that was significantly different (P≥0.05) from others. However, the mean zones of inhibition of *Aspergillus niger and Alternaria alternata* with the control could not be determined.

Phytochemical screening of the selected peel extracts

Results of the phytochemical constituents of ethanolic extracts of the selected peels are reported in Table 3. Flavonoid, alkaloid and cardiac glycosides were present in all the peel extracts while saponin and phlobatannin were absent in all the peel extracts. Tannin was present only in orange and cashew peel extracts (Table 3). Similarly, the results of the photochemical constituents of the aqueous peel extracts of the selected peels are shown in Table 4. Tannin, alkaloids and cardiac glycosides were present in all the peel extracts. Saponin was present only in orange and cashew peel extracts while flavonoid was absent only in cashew peel extract. Phlobatannin was absent in all the peel extracts (Table 4).

Discussion

Results obtained in this research work revealed the inhibitory potential of selected fruit peel extracts against *Aspergillus niger and Alternaria alternata* which are common storage moulds on fruits. Findings showed that mycelia growth of the two test isolates was most effectively inhibited by banana peel extract when compared with all the other peel extracts and consequently the banana peel aqueous extract had the highest antifungal activity against *Aspergillus niger* and *Alternaria alternata*, followed by pineapple peel and then cashew peel while orange peel extract had the least antifungal activity. This observation was supported by the work of [21] who reported that banana peel extract contained high antimicrobial activity against pathogenic fungi. It was also reported in the work of [22], though with methanolic and ethanolic extracts, that banana peel at different concentrations inhibited growth of *Aspergillus niger*, Aspergillus oryzae and Rhizopus stolonifer [22]. Banana peel ethnolic extract have equally been found to have a high antifungal activity against *Aspergillus flavus* using agar-well diffusion method [23].

Antimicrobial properties of plants extracts had been attributed to the presence of alkaloids and flavonoids [24, 25]. Phytochemicals with bitter taste such as alkaloids and flavonoids have been found to possess microbial properties and interestingly, results of the qualitative analysis of banana peel extracts used in this work showed the presence of cardiac glycosides, flavonoid and alkaloids and could therefore suggest that the possible antimicrobial activity was due to the presence of these phytochemicals in the peels. These bioactive groups of natural products have been reported for their inhibition roles against pathogens in ethnobotany, drug application and plant health management [26, 27].

Conclusion

The most effective peel extract against *Aspergillus niger* and *Alternaria alternata* among the selected peels as observed in this work was banana peel extract. Consequently, banana peel extracts may serve as potential antifungal alternatives for the treatment of fruits against storage moulds. This investigation has opened up the possibility of the use of this peel in the treatment of fruits against spoilage microorganisms. The peels

Table 1: *In vitro* antifungal activity of selected peel extracts against mycelia growth of *Aspergillus niger*.

Fruit peels (100mg/ml)	Day 3 of Incubation
	Mean zone of inhibition (cm)
Orange	0.33±0.33a
Cashew	0.40±0.30a
Pineapple	0.60±0.20a
Banana	0.83±0.33b
Control	ND

Mean ± SE of triplicate (n=3) followed by the same letter in a column are not ignificantly different (p > 0.05) by Duncan's New Multiple Range Test.
ND ---------Zone of inhibition not determined.

Table 2: *In vitro* antifungal activity of selected peel extracts against mycelia growth of *Alternaria alternata*.

Fruit peels (100mg/ml)	Day 3 of Incubation
	Mean zone of inhibition (cm)
Orange	0.50±0.50a
Cashew	0.60±0.30a
Pineapple	0.87±0.43a
Banana	1.37±0.67b
Control	ND

Mean ± SE of triplicate (n=3) followed by the same letter in a column are not significantly different (p > 0.05) by Duncan's New Multiple Range Test.
ND ---------Zone of inhibition not determined.

Table 3: Phytochemical constituents of ethanolic extracts of the selected peels.

Peels	Tannin	Cardiac glycosides	Flavonoid	Alkaloid	Saponin	Phlobatannin
Orange	+	+	+	+	-	-
Cashew	+	+	+	+	-	-
Pineapple	-	+	+	+	-	-
Banana	-	+	+	+	-	-

Key: + = present
- = Present

Table 4: Phytochemical constituents of aqueous extracts of the selected peels.

Plants	Tannin	Cardiac glycosides	Flavonoid	Alkaloid	Saponin	Phlobatannin
Orange	+	+	+	+	+	-
Cashew	+	+	-	+	+	-
Pineapple	+	+	-	+	-	-
Banana	+	+	+	+	-	-

Key: + = present
- = absent

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are novel, natural and economic sources of antimicrobics, which can be used in the control of post-harvest diseases.

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