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# Phytochemical Composition and Antifungal Actions of Aqueous and Ethanolic Extracts of the Peels of two Yam Varieties

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## Abstract

The phenolic composition and antifungal actions of aqueous and ethanolic extracts of the peels of *Dioscorea bulbifera* and *Dioscorea alata* were investigated using standard techniques. The aqueous extract of the peel of *Dioscorea bulbifera*, contained 1.80 ± 0.14% flavonoids, 2.21 ± 0.16% tannins, 0.38 ± 0.04% alkaloids and 3.72 ± 0.03% saponins, while the ethanolic extract contained 1.70 ± 0.14% flavonoids, 1.47 ± 0.03% tannins, 0.68 ± 0.04% alkaloids and 6.07 ± 0.10% saponins. The aqueous extract of *Dioscorea alata* peel contained 1.95 ± 0.05% flavonoids, 2.18 ± 0.03% tannins, 0.25 ± 0.00% alkaloids and 3.96 ± 0.06% saponins, while the ethanolic extract contained 1.85 ± 0.14% flavonoids, 1.33 ± 0.04% tannins, 0.60 ± 0.00% alkaloids and 5.36 ± 0.01% saponins. There were no significant differences ($P>0.05$) in the flavonoid contents of the aqueous and ethanolic extracts of peels of the yam varieties, the aqueous extracts of the peels of *Dioscorea bulbifera* and *Dioscorea alata* contained significantly higher ($P<0.05$) amounts of tannins than their ethanolic extracts, while the ethanolic extracts of the peels of *Dioscorea bulbifera* and *D.alata* contained significantly higher ($P<0.05$) amounts of alkaloids and saponins than their aqueous extracts. The extracts of the yam peels exhibited various antifungal actions in a dose dependent manner and at 40% concentration exhibited higher inhibitory action on *Botryodiploidia theobromae* than standard Griseofulvin. Results show antifungal actions and medicinal potentials of the aqueous and ethanolic extracts of the peels of *Dioscorea alata* and *Dioscorea bulbfera*.

**Keywords:** Yam; Extracts; Peels; Varieties *Dioscorea alata; Dioscorea rotundata*

## Introduction

Yams belong to the family *Dioscoreceae* [1], and the most widely cultivated species in Nigeria are *Dioscorea rotundata* Poir (white yam), *Dioscorea cayenensis* Lam (yellow or guinea yam), *Dioscorea alata* L. (Water yam), *Dioscorea dumetorum* (Cluster, or bitter yam), *D. esculenta* (Loir) bark (Chinese yam) and *Dioscorea bulbifera* L (aeria yam) [2,3].

Out of the world production of over 30 million tones per annum, Nigeria alone produces 22 million tones [4]. In spite of this, the demand for yam tubers in Nigeria has always exceeded its supply. Report has it that over 25% of the yield is lost annually to diseases and pests [4,5], and more than 50% of the yam tubers produced and harvested in Nigeria is lost in storage [2].

The fungi pathogens that have been reported to be responsible for the storage rot of yams include: *Rhizopus nodosus, Fusarium solani*, *Aspergillus flavus, Rhizopus stolonifer, Sclerotium rolfsii* and *Fusarium oxysporum* [2,6,7].

One of the measures that are being applied to check the postharvest loss of yam from fungi attack is the use of fungicides. However, some of these fungicides have been reported to be harmful to human [2]. Other control methods involving the use of microorganisms [8], also have their own limitations as farmers in developing countries such as Nigeria have hardly adopted these measures, because of the cost implication.

Biological control is generally favored as a method of plant disease management because it does not have the disadvantage of chemicals [9]. According to Kuhn and Hargreaves [10], bioactive substances that were found fungicidal *in vitro,* in most cases, kill the fungus *in vivo*.

It is for this reason that we decided to investigate the antifungal actions of aqueous and ethanolic extracts of the peels of *Dioscorea alata* and *Dioscorea rotundata* on selected species of fungi.

## Materials and Methods

### Collection of yams

Post harvest spoilt and healthy yams (*Dioscorea alata* and *Dioscorea bulbifera*), were collected from the yam barn of the National Root Crops Research Institute, Umudike (NRCRI). Some samples of the healthy yams were taken to the Biochemistry Laboratory for phytochemical assays, some were taken to the Department of Biology, Michael Okpara University of Agriculture, Umudike, Nigeria for identification and authentication, while the rest of the yams (healthy and spoilt) were neatly packed in 2 different nylon bags and transported to the Pathology Laboratory.

### Processing of plant materials

The yams were washed, peeled, and their peels were sun dried for two days under aseptic conditions. The peels were later milled to flour, using a hand milling machine.

### Plant extraction for phytochemical assays

A measured amount (40 g) of the flour sample of each variety of the healthy yams was dissolved in water and ethanol, respectively, and left overnight. The setup was filtered with Whatmann no 1 filter paper through vacuum pump (Model 2037 Gardner Denver) and the extracts were used for phytochemical assays.

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### Phenolic assays

The gravimetric method of Harbone (1973) was used in the determination of the percentage alkaloid contents of the extracts, while the AOAC methods (1990) was used in the analysis of the percentage flavonoid, saponin and tannin contents of the extracts.

### Extract preparation for antifungal assay

Varying amounts of the flours of the healthy yams (10 g, 20 g, 30 g and 40 g) were each dissolved in 100 ml of water and ethanol, respectively. The resulting solutions (10%, 20%, 30% and 40%) were filtered through Whatmann no 1 filter paper and the extracts were used for antifungal assays.

### Isolation of fungi from spoilt *Dioscorea rotundata*

The spoilt yams were rinsed in sterile distilled water, surface sterilized with 70% ethanol for 1 min [11,12]. This was done by cutting the spoilt yams into about 3-4 mm pieces, dropped into 70% ethanol in a beaker, and later washed twice in sterile distilled water. The washed pieces were blotted with sterile filter paper to remove water droplets. A flamed forceps was used in picking five pieces of sterilized yam and inoculated into the already prepared and solidified Potato Dextrose Agar media. The inoculated plates were incubated at 25°C for 3-5 days. Plates were observed daily for mycelia growth. All the plates were duplicated. Aseptic conditions were observed through out the experiment.

### Sub-culture and purification

Sub-culturing of different mycelia colonies from the inoculated plates were done to obtain a pure culture. Sterilized surgical blades were used to cut different mycelia growth, and transferred to a newly prepared Potato Dextrose Agar media plates. The plates were incubated at 25°C for 3-5 days. The purified plates were kept in slants and stored in the fridge for characterization.

### Characterization of the purified cultures

Macroscopic examination was done by physical and structural characteristics of the mycelia, as well as microscopic characteristics, using the morphology for septate, non-sepatate, presence special organs like rhizoids [12]. A wet-mount method [11] was used for viewing the isolates under an x40 compound microscope.

### Pathogenicity test

The healthy yams were washed with sterile distilled water twice. Thereafter, the yams were sterilized with 70% ethanol and washed with sterile water. The yams were allowed to air dry in a laminar air flow. A flamed 5 mm cork borer was used to bore holes into the healthy yams. Discs of five day old culture of isolates were used to plug the hole created in the yams, and the discs of the yams in the cork borer were replaced to cover the bored holes. Then blue seal vaseline was used to seal the surface of the inoculated portions [8]. The inoculated tubers were incubated for 7 days at 25°C. Regular observation was made for fungal growth. After 7 days, the inoculated yams were cut into two halves. The infected portions were measured with a transparent ruler and recorded, while the control was inoculated with agar plugs only.

### Confirmation of isolates used for pathogenicity test

Re-isolation of the microorganisms from the inoculated yams was carried out by cutting the yam into pieces (3-4 mm), after which they were washed, and their surfaces sterilized with sterile distilled water and dried on a sterile filter paper. The sterilized pieces of yams were later inoculated into a solidified potato dextrose agar media, sealed and incubated at 25°C for 3-5 days. The isolated microorganisms were purified and characterized. The re-isolated microorganisms were compared with the initial inoculated microorganisms.

### Antifungal actions of the extracts of *Dioscorea alata* and *Dioscorea bulbifera*

One ml from each concentration (10%, 20%, 30%,4 0%) was picked with the aid of a sterile pipette and dispersed into a sterile 9 cm petridish. A molten media of Potato Dextrose Agar was poured into the plates, swirled and allowed to solidify. After solidification, a sterile 3 mm cork borer was used to pick the fungi plugs from a pure, five day old culture plate. The plug culture was used to inoculate the potato dextrose agar plates by placing it at the centre of the plates, sealed and incubated at 25°C for 3-5 days. A transparent ruler was used in measuring the mycelia growth daily for 5 days.

The inhibition of fungi by the various concentrations of the extracts was calculated as [13]:

$$[(D_C-D_T)/D_C] \times 100)$$

Where $D_C$ = Average diameter of the fungal colony of the control; $D_T$ = Average diameter of the fungal colony treated with extract.

### Statistical analysis

Data was subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 15.0. Results were presented as the means ± standard deviations of triplicate experiments. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P<0.05$ using the Duncan Multiple Range Test.

## Results and Discussion

Analysis of the phytochemical composition of the aqueous extracts of the peels of *Dioscorea bulbifera* indicated that it contained 1.80 ± 0.14% flavonoids, 2.21 ± 0.16% tannins, 0.38 ± 0.04% alkaloids and 3.72 ± 0.03% saponins, while the ethanolic extract contained 1.70 ± 0.14% flavonoids, 1.47 ± 0.03% tannins, 0.68 ± 0.04% alkaloids and 6.07 ± 0.10% saponins (Figure 1). The aqueous extracts of *Dioscorea alata* contained 1.95 ± 0.05% flavonoids, 2.18 ± 0.03% tannins, 0.25 ± 0.00% alkaloids and 3.96 ± 0.06% saponins, while the ethanolic extracts contained 1.85 ± 0.14% flavonoids, 1.33 ± 0.04% tannins, 0.60 ± 0.00% alkaloids and 5.36 ± 0.01% saponins (Figure 1). There were no significant differences ($P>0.05$) in the flavonoid contents of

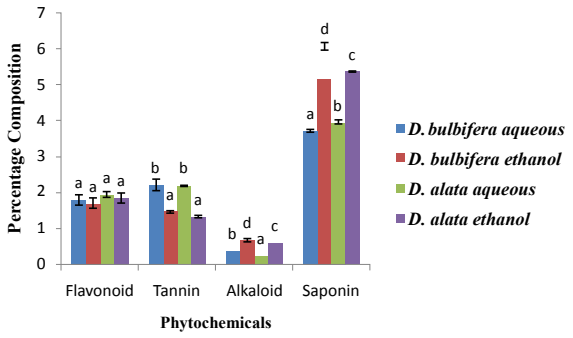


**Figure 1:** Phytochemical composition of peel extracts of 2 yam varieties. [a-d]Means with different superscripts for each extract, contain significantly different phytochemicals ($P<0.05$).

the aqueous and ethanolic extracts of the peels of the yam species, the aqueous extracts of the peels of *Dioscorea bulbifera* and *Dioscorea alata* contained significantly higher ($P<0.05$) amounts of tannins than their ethanolic extracts, while the ethanolic extracts of the peels of *Dioscorea bulbifera* and *Dioscorea alata* contained significantly higher ($P<0.05$) amounts of alkaloids and saponins than their aqueous extracts (Figure 1).

Phytochemicals, as compounds which occur naturally in plants, form part of plants defense mechanisms against diseases [14]. They are classified into primary and secondary, based on their activity in plant metabolism. The primary ones comprise of sugars, amino acids, proteins and chlorophyll [15], while secondary ones include the phenolic compounds such as tannins, flavonoids, flavonols, alkaloids, saponins, proanthocyanidins, etc. [16]. These phenolic compounds have been reported to possess considerable antimicrobial properties, which is attributed to their redox properties [17,18]. Thus the antimicrobial properties of plants have been attributed to the presence of these secondary metabolites [19].

Alkaloids are the largest group of secondary metabolites and are the most efficient therapeutic plant phytochemicals, comprising basically of nitrogen bases synthesized from amino acid building blocks. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and anti-bacterial properties [20]. The lower alkaloid contents of the aqueous extracts of the 2 varieties of yams investigated compared with their ethanolic extracts could be attributed to better extraction by ethanol, as alkaloids are more soluble in ethanol than in water [20]. Although the alkaloid contents of the aqueous and ethanolic extracts of the peels of the 2 yam varieties investigated were low, their presence could confer antimicrobial and therapeutic properties to the peels.

Flavonoids are important class of polyphenols in the plant kingdom. Structurally, they are made of more than one benzene ring [20]. Reports have it that they are potent water soluble antioxidants. In addition, flavonoids have also been reported to possess antimicrobial and anti-inflammatory properties [21]. Cordell et al. [22] reported that alkaloids and flavonoids are responsible for the antifungal activities in higher plants. Therefore, the considerable amount of flavonoids, that was observed in the aqueous and ethanolic extracts of the peels of the 2 varieties of yams investigated, could confer pharmacological properties to these peels.

Tannins are secondary metabolites of plants of high molecular weight that are responsible for the astringent taste of foods and drinks. They bind to proteins, carbohydrates, gelatins and alkaloids, and are classified as active antimicrobial compounds [19]. In terms of solubility, they are soluble in water and alchohol [20]. Tannin rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea, healing of wounds and diarrhea. The large amount of tannins in the extracts of the peels of the varieties of yams investigated implies they could quicken the healing of wounds and burns [21].

Saponins are regarded as high molecular weight compounds, in which a sugar molecule is combined with triterpene or steroid aglycone. The two major types of saponin are the steroidal and triterpene saponins. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar solvents like benzene and n-hexane. Although saponins could cause hemolysis of blood when ingested in high concentrations, they also possess therapeutic potentials such as cholesterol lowering and anti-cancerous activities. As observed in this study, the aqueous extracts of the peels of the yam varieties extracted their saponins better than the ethanolic extracts. Some saponins like diosgenyl exert a large amount of biological functions, such as anti-fungal, anti-bacterial and anticancer activities. Thus, the considerable amounts of saponins that were observed in the aqueous and ethanolic extracts of the 2 varieties of yam peels (Figure 1), imply that they could possess some biological properties.

The species of fungi that were identified in this study as contributing to the postharvest deterioration of post harvest stored yams were *Sclerotium rolfsii* (*S. rolfsii*), *Fusarium oxysporum* (*F. oxysporum)* and *Botrydipodia theobromae* (*B. theobromae*) with percentage occurrences of 38.36%, 41.26% and 33.80% respectively calculated from 20 plates (Table 1).

Similar reports have been given by Okigbo and Nmeka [7], Amusa et al. [2], Ogaraku and Usman [6] respectively. These species of fungi not only lead to yam deterioration, but could also be toxic to man. For example, *Fusarium* species produce *Fusarium* toxins such as trichothecenes, diacetoxyscirpenol, nivalenol and zearalenone, which cause skin diseases, gastroenteritis, rectal hemorrhage and vomiting [6].

The different concentrations of the aqueous and methanolic extracts of the peels of the 2 varieties of yams investigated, exhibited various antifungal actions in a dose dependent manner against the species of fungi isolated in this study, when compared with the standard fungicide (Figure 2). At 40% concentration, the aqueous extract of *Dioscorea alata* showed higher inhibitory action on *Sclerotium rolfsii* than the ethanolic extract, while the ethanolic extract of *Dioscorea alata* at 40% concentration, showed higher inhibitory actions on *Botryodiploidia theobromae* and *F. oxysporum* than the aqueous extract. The aqueous extract of *Dioscorea bulbifera* at 40% concentration, showed higher inhibitory action on *Botryodiploidia theobromae and F. oxysporum* than the ethanolic extract, while the ethanolic extract of *Dioscorea bulbifera* at 40% concentration showed higher inhibitory action on *Sclerotium rolfsii* than the aqueous extract.

| Isolate | Percentage occurrence |
|---|---|
| *Botryodiploidia theobromae* | 33.80 |
| *Fusarium oxysporum* | 41.26 |
| *Sclerotium rolfsii* | 38.36 |

**Table 1:** Percentage occurrence of fungi isolates in spoilt species of *Dioscorea rotundata*.

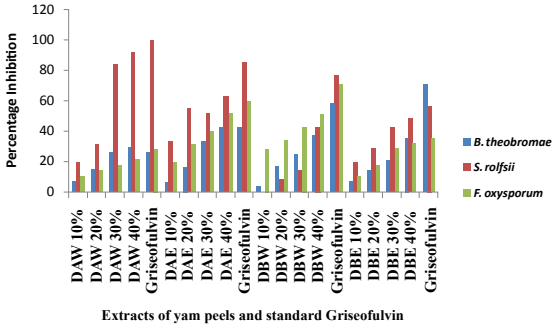


**Figure 2:** Percentage inhibition of extracts and Griseofulvin on fungi isolates in spoilt species of yam. DAW- *Dioscorea alata aqueous extract; DAE-Dioscorea alata ethanolic extract;* DBW-*Dioscorea bulbifera* aqueous extract; DBE-*Dioscorea bulbifera* ethanolic extract.

The higher scavenging activities of 40% aqueous and ethanolic extracts of the peels of *Dioscorea alata* on *Botryodiploidia theobromae*, compared with standard Griseofulvin, is a significant finding in this study. This is attributed to the amount of free or unbound phenolic compounds (alkaloids, flavonoids, saponins and tannins) in the extracts. The amphiphilicity of these bioactive compounds enables them to interact with the phospholipid bilayer of the membrane of the microorganisms, leading to altered membrane function, inhibition of protein synthesis and altered growth of the microorganisms [13,23]. Results indicate that the aqueous and ethanolic extracts of these varieties of yam peels contain bioactive compounds with fungicidal actions.

## Conclusion

This study shows the antifungal actions and medicinal potentials of the aqueous and ethanolic extracts of the peels of *Dioscorea alata* and *Dioscorea bulbfera*. This approach to the management of these fungi strains affecting *D. alata* and *D. bulbifera* could be economically viable if adopted. Finally, the study underscores the need for the purification and characterization of the phytochemical constituents of the aqueous and methanolic extracts of the peels of these yam varieties.

## Conflicts of Interest

The authors hereby declare that they do not have a direct financial relation with the commercial identities mentioned in the paper that might lead to a conflict of interest.

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