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Efficacy of extracts of water yam (*Dioscorea alata*) and aerial yam (*Dioscorea bulbifera)* peels in the control of white yam (*Dioscorea rotundata*) rot

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The efficacy of ethanol and aqueous extracts of the peels of *Dioscorea alata* and *Dioscorea bulbifera* on the growth of pathogenic fungi of rotten white yam (*Dioscorea rotundata)* were investigated. Pathogenicity test revealed that *Sclerotia rolfsii, Botryodiplodia theobromae and Fusarium oxysporum* induced rot in healthy white yams after seven days of inoculation with *Botryodiplodia theobromae* being the most virulent. All the extracts showed varying degrees of antifungal effect, ethanol extract proved to be more potent. *Dioscorea alata* extracts had slightly, moderate and effective inhibition on mycelial growth of all the test fungi ranging from 13.04% to 94.44% while the extracts of *Dioscorea bulbifera* showed slightly, moderate and effective inhibition on the mycelia of the test fungi ranging from 6.66% to 88.88%. The most fungitoxic of all the extracts was observed with the 10% ethanol extract of *Dioscorea alata* which showed significant ($P<0.01$) inhibition on all the test fungal pathogens. Phytochemical screening of the extracts revealed the presence of saponins, alkaloids, tannins, phenols and flavonoids in both extracts but at different concentrations. The fungitoxic potentials of these extracts on white yam rots can proffer the need for genetically modification of the white yam which is more prone to rot and can also provide an alternative to synthetic fungicides since it is less expensive, environmental friendly and easy to prepare.

Key Words: Phytochemical compound, yam fungal pathogen

Introduction

Yams are monocotyledonous plants belonging to the genus *Dioscorea* of the family *Dioscoreaceae* which constitute a multi-species of crops that are important for food, socio-cultural activities and income. *Dioscorea* species are important food crops in West Africa, and other tropical countries including East Africa, Central Africa, The Carribean, South America, South East Asia and India (Coursey, 1967).

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The most important areas for the cultivation and usage of yam stretches from Ivory Coast through Ghana, Nigeria, Togo, Cameroon, Garbon, Central African Republic and the Western part of the Democratic Republic of Congo. According to F.A.O.(2000) these regions produce about 93% of the World's annual yam production, estimated at 38.5 million metric tons and Nigeria alone accounts for about 26.4 million tons (70%) in the year 2000.

There are about 600 species of *Dioscorea* with more than 10 species cultivated for food and 6 species for pharmaceutical use (Coursey, 1967). Only six species are economically important namely; white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata),* aerialyam(*Dioscorea bulbifera),* yellowyam (*Dioscorea cayenensis),* trifoliate yam (*Dioscorea dumentorum)* andChineseyam (*Dioscorea esculenta). (*Onwueme,1978), *Dioscorea rotundata* and *Dioscorea cayenensis* are indigenous to West Africa while *Dioscorea alata* and *Dioscorea esculenta* are native of Asia. According to Onwueme (1978) *Dioscorea rotundata* is the most important specie of yam in West Africa. Besides the cultivated yam species, there are a number of wild types which are also harvested as food (Wilson, 1982).

The yam tuber is the only economical part of the crop, consumed roasted, fried, boiled, pounded or as flour for baking and steaming for swallowing with soup. In addition to its nutritional value, yam has considerable social and cultural significance, especially among the people of South-Eastern Nigeria (Sangoyomi, 2004). According to Coursey (1967); Okigbo and Ogbonnaya (2006) yams are of a very high nutritional value where it is a major source of carbohydrate, minerals, phosphorus, calcium, iron and vitamins such as thiamine, riboflavin and vitamins B and C. Some species of yam have been used medically to treat diseases like *Diabetes melitus*, to increase coronary flow and prevent high hyperchosteromia (Undie and Akubue, 1986).

In spite of the importance of yams as major staple food and its socio-cultural value in the lives of the people of the Central Africa and West Africa sub-region, research and documentation of this important staple food crop is still limited (Sangoyomi, 2004).

Constraints in yam production include; disease and pest attack, cost of planting materials, cost of labour and other technical challenges in breeding and selection which include; the long breeding cycle, very low multiplication ratio of propagules and the existence of a juvenile phase during the seminal and early clonal stages of development (Sangoyomi, 2004). Considerable losses occur during prolonged storage of yam, losses up to 10-20% may be observed in the first 3 months and 30-60% after 6months in the barns (Coursey, 1967). Storage losses are normally caused by rotting, pests' activities and sprouting. However rotting causes the greatest amount of losses and it is mostly due to the effects of

fungi, bacteria and nematodes. Fungi are the most important and have been reported to be responsible for 80% of all storage rots of yam tubers in West Indies and 57 – 77% in Nigeria (Ikotun, 1983). The principal species of microorganisms associated with yam rot in Nigeria include: *Aspergillus niger, Rhizopus nodosus, Sclerotia rolfsii, Fusarium oxysporum* and *Botryodiplodia theobromae,* these fungi were reported to be pathogenic to yam tubers, causing severe loss in several parts of Southern Nigeria (Onuegbu, 1999; Okigbo *et al.* 2015).

The use of chemicals have helped in control of rots but due to the identifiable problems which include; biodegradation, pollution, chemical residues, phytotoxicity, high cost, development of resistance in target organism, atimes non availability and them being hazardous to man and his environment renders them either slow to adopt or farmers have totally failed to adopt them for one cultural reason or the other (Okigbo and Odurukwe, 2009; Okigbo *et al*., 2015). Recently considerable efforts are directed at exploring the potentials of plant extracts as alternative to synthetic chemicals. These plant extracts are readily available, affordable, non phytotoxic and are biodegradable thus being friendly to man and his environment (Akueshi *et al*., 2002; Okigbo and Nmeka, 2005; Okigbo and Omodamiro, 2006).

According to Okwu (2004) phytochemicals are natural bioactive compounds present in plants dietary fiber to protect against diseases. Research works have shown the phytochemicals working in conjunction with nutrients found in food plants may help to slow down the aging process and reduce the risk of many diseases including heart disease, stroke, urinary tract infections, cataracts and osteoporosis in humans. They can have a complete and over lapping mechanism of actions, including; antioxidant effects, antibacterial effects, antiviral effects, antifungal effect and modulation of hormone metabolism (Okwu, 2004). Phytochemicals like Tannins are known to inhibit pathogenic fungi. Alkaloids play some metabolic roles and control development in living systems (Burkil, 1985). According to Okwu (2004) saponins prevent disease invasion of plants by parasitic fungi thus, possessing antifungal properties.

Apart from food, yams are mainly used for medicinal purposes for the sapogenins, aglycons of yam saponins are important mainly because of their steroid structure (Okwu and Ndu, 2006). According to Crabbe (1979) yams are precursors for the hemisynthesis of birth control pills (with estrogen and progesterone) as well as similar hormones and corticosteroids.

Yams like higher plants have a complex phytochemical profile (Okwu and Ndu, 2006). The most predominant phytochemical characteristics of yams are the presence of dioscorine alkaloid and diosgenin saponin, although dioscorine

and diosgenin are naturally considered toxic, such toxicity is removed by washing, boiling and cooking (Eka, 1998). In some yam cultivars like water yam (*Dioscorea alata*) and the aerial yam (*Dioscorea bulbifera*) such toxicity cannot be removed because of the itchiness or acute toxicity or bitterness of the raw tuber. The bitterness or acute toxicity in yams may be due to its alkaloid content while the saponins and sapogenins may constitute the pharmaceutical agents. The pigments found in these yams may be due to the presence of flavonoids and carotanoids (Okwu and Ndu, 2006). It has been observed that some herbivores do not feed on the raw peels of some yam species like the water yam and the aerial yam which may be due to the chemical composition of the peels. However the natural attributes of water yam and aerial yam necessitated the need for undertaking this study to ascertain the efficacy of their peel extracts in the control of white yam (*Dioscorea rotundata*) rot.

Objectives

- To identify the organisms responsible for the rot of white yam.
- To evaluate the efficacy of the extracts from water yam and aerial yam in the control of white yam rot.
- To find out the best extraction medium for the extracts from water yam and aerial yam peels (between water and ethanol).

Materials and Methods

Source of Yam Species

White yam (*Dioscorea rotundata*) with symptoms of rot and the healthy yam tubers were gotten from National Root Crops Research Institute's yam barn Umudike, Umuahia. They were taken to the plant pathology laboratory of the same Institute, enclosed in a sterile polyethylene bags.

The two yam species, Water yam (*Dioscorea alata*) and Aerial yam (*Dioscorea bulbifera*) used in the experiment for the control were collected from a farmer in Achingala in Obowo Local Government, Imo Sate.

Sterilization of Laboratory Materials

The cork borers, scalpels and inoculation needles were sterilized by dipping them in 70% ethanol and flaming to red hot while the conical flasks and other glass wares were thoroughly washed with liquid soap and allowed to dry in the Lamina air flow.The Petri dishes were sterilized in a dry oven

(Gallen Kamp,hot box,USA). Sterilization of distilled water was done in 1 litre sterilized bottle, the beakers and conical flasks were wrapped with an aluminium foil and autoclaved at 121°C for 15minutes at 103KNM pressure. The laminar flow was sterilized by swiping them with cotton wool soaked in 70% ethanol.

Preparation of Culture Media

Potato dextrose agar (PDA) was used as the medium for the growth and maintenance of the fungal isolates. Twenty gramme of potato dextrose agar powder was weighed and dispensed into 1 litre of sterile distilled water in 1 litre conical flask according to the manufacturer's recommendations. The content was sterilized in an autoclave at 121°c for 15 minutes at 103KNM pressure. The medium was allowed to cool (47°C) and 1ml of lactic acid solution(10%) was added to a litre of the molten PDA to suppress bacterial growth and eliminate contaminants on the cultures (Green,1994).The medium was poured into sterile Petri-dishes and. allowed to cool and solidify and used as required.

Isolation of associated fungal pathogens from rotted white yam tubers

Samples of white yam tubers with rot symptoms were selected washed and air dried to remove soil debris. Each rotten yam was cut with the aid of flamed knife into small pieces (2cm-4cm) at the interphase between healthy and the infected portions of the tuber. The small rotten yam pieces were surface sterilized to remove surface contaminants in 70% ethanol for 1 minute, washed with sterile distilled water twice. After washing the pieces of cut yam were blotted dry in filter paper (removing droplets of water) placed in laminar flow cabinets to dry. Five pieces of the sterilized tubers were picked with sterile forceps and plated into the already prepared plates, each tuber was duplicated. The inoculated Petri dishes were sealed to prevent microbial or insert contamination and then incubated at the temperature of 28°C for fungal growth with aseptic conditions being applied throughout (Fawole and Oso 198; Agu *et al.* 2015).

Subculturing and purification

After the period of incubation, different growths of mycelia were subcultured with the aid of sterile surgical blade into newly prepared PDA plates. All the plates were transferred into the incubator at 25°C for 3-5 days.

Several subculturing was done, thus purifying the mixed culture plates. The purified fungi were transferred into Mcartney slant bottles of PDA and stored in a refrigerator for characterization (Fawole and Oso 1988, Okigbo *et al.* 2015).

Characterization

All the purified isolates were viewed under a compounded micro scope using the fungi identification manual of Barnett and hunter (1987). The following features were viewed and used for the identification; the possession of septate or non-septate mycelia, sporangiophores, conidiophores, possession of special organs like rhizoids etc.

Pathogenicity test of the isolated fungi

All the pure cultures obtained from the sub-cultured plates were used for pathogenicity test. Fresh healthy white yam tubers were washed thoroughly to remove soil and other debris on the tuber surface. The yam tubers were surface sterilized to remove surface contaminants in 70% ethanol for 1minute, washed with sterile distilled water twice and were placed on sterile paper towels in the lamina flow hood to dry for 30 minutes. Petri-dishes containing pure cultures were punctured with a sterile 4mm cork borer. Five millimeter cork borer was sterilized and used to make holes on the healthy clean tubers of white yam in an aseptic environment. An inoculation needle was used to pick a colony disc from the disc made in each plate and introduced into the holes made in the tubers. The cut off flesh from each of the yam tubers was returned into the hole thus using it to block the inoculated region on the yam. This was sealed with blue seal Vaseline to prevent invasion of other saprophytes. The inoculated white yams were kept in a sterile moisture chamber under an ambient condition. The incubation was done for 7 days.

After 7days incubation all isolates that caused rot on the inoculated yams were re-isolated, identified under microscope and compared with the original isolates. All the isolates that caused rots on the inoculated white yams were used for the interaction experiment

Extract preparation

Aqueous and ethanolic extracts were prepared from the peels of water yam (*Dioscorea alata*) and aerial yam (*Dioscorea bulbifera*). These yams were thoroughly washed with sterile water, peeled, sun dried for seven days and ground separately into fine powder using a hand milling machine. Each ground

product was weighed out into 10, 20, and 30 and 40g portions and soaked separately into 100ml of distilled water and ethanol in 250ml conical flasks. The flasks were allowed to stand for 6 hours at room temperature with intermittent hand shaking. The contents of the flasks were filtered separately into clean 250ml beakers using fourfold cheese cloth giving10, 20, 30 and 40% concentration respectively of each plant extract. Potato Dextrose Agar (PDA) with distilled water without plant extract served as negative control while 100mg of Griseofulvin tablet dissolved in 70% ethanol mixed with PDA served as positive control.

Interaction of micro organisms with extracts

One millilitre of extract was transferred with the aid of a sterile pipette into an empty sterile Petri dish. A molten PDA (47°C) was poured into the plate (10ml) and swirled together for easy mix up. The plate was left on the beach for solidification of the medium (30 minutes). After solidifying, a sterile 4mm cork borer was used to punch a pure culture plate of the fungus to be used. An inoculating needle was used to pick each disc from the punched plate and put at the centre of the PDA plates. The plates were sealed with paper tapes and incubated at 25°C for 7days (Okigbo, 2002). A transparent ruler was used in measuring the length of the mycelia growth. The negative control plates had no extract mixed with the plates while the positive control plates were mixed with Griseofulvin (fungicide). All the plates were duplicated.

The percentage inhibition was calculated according to the method described by Whipps (1987)

Percentage inhibition $= R_1 - R_2 \times 100/1$

Where R_1, is the furthest radial distance of the pathogen in control plates and R_2 is the furthest radial distance of the pathogen in extracts incorporated agar plates.

The inhibition percentage was determined as a guide in selecting the minimum inhibitory concentration that will be effective in the control of rot-causing fungi. Extracts were rated for their inhibitory effects using the scale described by Sangoyomi (2004).

100% inhibition (highly effective)
50-99% inhibition (effective)
20-49% inhibition (moderately effective)
0-19% inhibition (slightly effective)
≤ 0% inhibition (not effective)

Phytochemical analysis

Some portions of the dried, ground peels of water yam (*Dioscorea alata*) and aerial yam (*Dioscorea bulbifera*) were subjected to phytochemical screening using standard methods (Harbone, 1998 and Okerulu and Ani, 2001) for the presence of saponins, tannins, alkaloid, flavonoid and phenols.

Determination of saponin

This was carried out by double solvent extraction gravimetric method (Harbone,1998). Five gramme of the processed samples were mixed with 50ml of 20% aqueous and ethanol solutions respectively and incubated for 12 hours at a temperature of 55°C with constant agitation. After that, the mixture was filtered separately through what man No 42 grade of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 minutes and the extracts weighed together respectively. The combined extracts were reduced to about (40mls) by evaporation and then transferred to a separating funnel. After mixing there was partition and the other layer was reserved. The aqueous layer was re-extracted with the other, after which its pH was reduced to 4.5with drop wise addition of dilute NaOH solution. The combined precipitate (extracts) were washed with 5% NaCl solution and evaporated in an oven at 60°C (to remove any residual solvent) the saponin content was calculated as shown below saponin =

$$\frac{W_2 - W_1}{W_3} \times \frac{100}{1}$$

Determination of tannins

The tannin content of the samples was determined using Folin-Denis spectrophotometer method (Harbone, 1998). Five gramme of processed sample was mixed with distilled water in the ratio of 1:10. The mixture was shaken vigorously for 30 minutes at room temperature and filtered through a whatman no 42 grades of filter paper to obtain the extract. A standard tannin acid solution was prepared, 2milliliters of the standard solution and equal volume of distilled water were dispensed separately into a 50 ml volumetric flask to serve as standard and reagent blank respectively. Then 2mls of each of the sample extracts were put in their respective labelled flasks. The content of each flask was mixed with 35mls of distilled water and 1milliliter of Denis reagent was added to each. This was followed by 2.5milliliters of saturated NA_2CO_3

solution. Thereafter each flask was diluted to the 50mls mark with distilled water and incubated for 90 minutes at room temperature. Their absorbencies were measured at 700nm in a spectrophotometer with the reagent blank at zero. Thus, the tannin content was calculated as shown:-

$$\textbf{\% T}\text{annin} = \frac{W_2 - W_1 \ X\ 100}{W_3 \qquad 1}$$

Determination of alkaloid

The alkaloid precipitation gravimetric method (Harbone, 1998) was used. Five gramme of the processed samples were dispensed separately in 100 millilitres of 10% acetic acid in ethanol solution. The mixtures were shaken thoroughly and were allowed to stand for 4 hours at room temperature respectively, and were shaken every 30 minutes, at the end of this period, the mixture were filtered separately through Whatman N0 42 grade of filter paper then the filtrate(extracts) were concentrated by evaporation to a quarter of its original volume, the extracts were treated with drop wise addition of concentrated NH_3 solution separately to precipitate the alkaloid.The alkaloid precipitates were removed by filtration using weighed Whatman No 42 filter paper,after washing with 1% NH_40H solution.The precipitates in the filter paper were dried at 60°C and cooled in a desicator 3 hours. The alkaloid content was then calculated as shown below:-

$$\%\ \text{Alkaloid} = \frac{W_2 - W_1 \ X\ 100}{W_3 \qquad 1}$$

Determination of flavonoids

Flavonoid was determined using the method described by Harbone (1998). Five gramme of the processed sample was boiled in 100 milliliters of HCl solution under reflux for 40 minutes.it was allowed to cool before being filtered. The flavonoid extract (contained in the ethyl acetate and the moisture was transferred to a separation funnel. The flavonoid extract (contained in the ethyl acetate portion) was received by filtration using weighed filter paper. The weight was obtained after drying in the oven and cooling in a desicator and the weight of the filter paper was expressed as a percentage of the weight analyzed. It was calculated as shown below.

$$\%\ \text{Flavonoid} = \frac{W_2 - W_1 \ X\ 100}{W_3 \qquad 1}$$

Determination of phenol

Phenol determination was done by the Folin-ciocattean spectro-photometer (AOAC, 1990) the total phenol was extracted in 200mg of the sample with 10millilitres concentrated methanol; the mixture was centrifuged for 15 minutes. One millilitre portion of the extract from each sample was treated with equal volume of folin-ciocactean reagent followed by the addition of 2millilitres 2% Na_2CO_3 solution. Before this, standard phenol solution was prepared and diluted to desired concentration.

One millilitre of the standard solution was also treated with the Folin-Denis reagent and Na_2CO_3 solution. The intensity of the resulting blue colouration was measured (absorbance) in a spectrophotometer at 560nm wave length and measurement was made with reagent blank at zero. The phenol content was calculated thus:-

$$\%\text{Phenol} = \frac{W_2 - W_1}{W_3} \times \frac{100}{1}$$

Results

Occurrence of fungal pathogens isolated from samples rotten white yams.

The fungal pathogens isolated from the rotted white yam tubers were; *Sclerotium rolfsii Sacc*, *Botryodiplodia theobromae* Pat, *Fusarium oxysporum* Schlect, *Aspergillus niger* Van Tieghem.

The frequency of occurrence varied with different fungi associated with the rotted white yam tubers. *Botryodiplodia theobrome* was the highest in occurrence (41.26%) followed by *Sclerotium rolfsii* which had 38.36%. *Fusarium oxysporum* had 33.80% occurrence while *Aspergillus niger* had 21.70% occurrence (Table 1).

Pathogenecity test

Sclerotium rolfsii isolated was very pathogenic to the healthy white yam tubers, causing rot of 42.4% of the total tissue surface within 7days (Table 2).*Fusarium oxysporum* isolates were also pathogenic with 36.2% of the rotted tissue surface area of the healthy white yam tubers after 7days of inoculation. While the healthy white yam tubers inoculated with *Botrydiplodia theobrome* gave 28.6% of rotted surface tissue (Table 2).

Interaction of the pathogenic microorganisms with the Aqueous and Ethanol extracts of the peels of water yam and aerial yam.

The inhibitory effects of aqueous and ethanolic extracts of water yam(*Dioscorea alata*) on the pathogenic fungi are shown in Table 3.For aqueous and ethanol extracts of *Dioscorea alata* there was general decrease in the colony growth of the pathogenic microorganisms with increase in extract concentration (Table 3). The lowest inhibition levels for the isolates were recorded at 10% aqueous and ethanol extract concentrations while the highest growth inhibition rate were recorded at 40% aqueous and ethanol extracts concentration. These were almost in the same growth level with the pathogenic microorganisms incorporated with Griseoflovin which served as the positive control (Table 3)

The percentage inhibitory effects of the aqueous and ethanolic extracts of *Dioscorea alata* on the mycelial growth of the isolated pathogens are shown in Table 4. The aqueous extract of *Dioscorea alata* gave the highest inhibitory effect of 35.48% (moderately effective)on *Sclerotium rolfsii*, followed by 26.60% inhibition on *Botryodiplodia theobromea* which also showed a moderately effective inhibition. While the least inhibitory effect was seen in *Fusarium oxysporum* where the aqueous extract was slightly effective at 16.66%.The ethanolic extract of *Dioscorea alata*, gave the highest inhibitory effect of 94.44% on *Sclerotium rolfsii* which is significant ($P<0.05$) and 38.46% for *Fusarium oxysporum* 13.04% for *Botryodiplodia theobromae*.

The inhibitory effects of aqueous and ethanolic extracts of aerial yam (*Dioscorea bulbifera*) are shown in Table 5.
Colony diameter decreased as the concentrations of both extracts increased with the highest inhibitory effect recorded for the 40% concentration.

The percentage inhibitory effects of the aqueous and ethanolic extracts of *Dioscorea bulbifera* on the mycelial growth of the pathogenic microorganisms are shown in Table 6. The aqueous extract of *Dioscorea bulbifera* gave the highest inhibitory effect on *Sclerotium rolfsii* 37.50% (moderately effective) and the least was on *Fusarium oxysporum* (6.66%).The ethanolic extract of *Dioscorea bulbifera* gave the highest inhibitory effect of 88.88% on *Sclerotium rolfsii* with the least 28.00% on *Fusarium oxysporum* (Table 6).

T-test Comparison between Aqueous and Ethanolic Extracts

Comparison between aqueous and ethanolic extracts of *Dioscorea alata* showed that ethanolic extract gave a mean percentage inhibition of $48.65\pm\pm2.377$ which is highly significant ($P<0.05$) compared to $26.27\pm\pm0.686$

of the aqueous extract.The mean inhibition of ethanolic extract of *Dioscorea bulbifera* (53.40±2.38) showed a highly significant difference (P<0.05) from the mean percentage inhibition recorded for the aqueous extract (24.90±0.68) (Table 7).

Phytochemical Screening of the Extracts

Qualitative analysis

The screening of the plants used as extracts (*D.alata* and *D.bulbifera*) for their phytochemical content reveal that both plants possess the listed phytochemicals; Saponin, Tannin, Alkaloid, Flavonoid and Phenol (Table 8)

Quantitative Analysis

The quantitative assay revealed that the phytochemicals in both plants ranged from 14.90±3.74% down to 0.004±0.001% (Table 9). Saponin was the most abundant phytochemical in both plants while the least was phenol. The contents of the other phytochemicals are shown in the table.

Table 1. Frequency of Occurrence (%) of Isolated Fungi from Rotten White Yam Samppples

Fungal Isolates	**% Occurrence**
Sclerotium rolfsii	38.36
Fusarium oxysporum	33.80
Botryodiplodia theobromae	41.70
Aspergillus niger	21.70

Table 2. Pathogenicity test and mean percentage of rot by Fungal Isolates on Healthy White Yams.

Isolates/Inoculated Fungi	**Percentage Rot**
Sclerotium rolfsii	42.4
Fusarium oxysporum	36.2
Botryodiplodia theobromae	28.6

Table 3. Quanlitative Phytochemical Analysis of *Dioscorea alata* and *Dioscorea bulbifera*

Phytochemicals	***Dioscorea alata***	***Dioscorea bulbifera***
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Phenol	+	+

Table 4. Quantitative Analysis of the Phytochemical constituents of *Dioscorea alata* and *Dioscorea bulbifera*

Phytochemicals	***Dioscorea alata***	***Dioscorea bulbifera***
Saponins	7.42±0.37	14.90±0.37
Tannins	0.05±0.02	0.09±0.02
Alkaloids	0.36±0.03	0.94±0.03
Flavonoids	2.90±0.35	9.94±0.35
Phenol	0.005±0.001	0.004±0.001

Table 5. Growth Inhibition (cm) of variousconcentrations of extracts of *Dioscorea alata*on the isolated pathogenic fungi.

Isolates	**Extracts**	**10%**	**20%**	**30%**	**40%**	**N**	**P**
Botryodiplodia theobromae	Aqueous	2.3±0.31	2.0±0.25	0.8±0.31	0.4±0.05	3.0±0.67	0.3±0.05
Sclerotium rolfsii	Aqueous	2.0±0.18	1.8±0.10	1.5±0.05	1.0±0.27	3.1±0.67	0.4±0.05
Fusarium oxysporum	Aqueous	2.0±0.31	1.2±0.05	1.0±0.13	0.8±0.22	2.4±0.50	0.6±0.31
Botryodiplodia theobromae	Ethanol	2.0±0.25	1.8±0.13	1.6±0.05	1.2±0.13	2.3±0.36	0.3±0.05
Sclerotium rolfsii	Ethanol	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	1.8±0.63	0.0±0.01
Fusarium oxysporum	Ethanol	1.6±0.10	1.5±0.05	1.2±0.10	0.8±0.27	2.6±0.54	0.6±0.36

Table 6. Growth Inhibition (cm) of variousconcentrations of extracts of *Dioscorea bulbifera* on the isolated pathogenic fungi.

Isolates	**Extracts**	**10%**	**20%**	**30%**	**40%**	**N**	**P**
Botryodiplodia theobromae	Aqueous	2.5±0.31	2.3±0.22	1.2±0.27	0.8±0.45	3.6±0.80	0.4±0.06
Sclerotium rolfsii	Aqueous	2.5±0.31	2.0±0.10	1.1±0.31	0.9±0.40	4.0±0.10	0.4±0.06
Fusarium oxysporum	Aqueous	2.8±0.36	2.5±0.22	2.0±0.00	1.0±0.45	3.0±0.45	0.8±0.54
Botryodiplodia theobromae	Ethanol	1.7±0.13	1.5±0.05	1.3±0.05	0.5±0.04	3.6±0.72	0.5±0.01
Sclerotium rolfsii	Ethanol	0.2±0.09	0.1±0.01	0.1±0.01	0.1±0.01	1.8±0.63	0.1±0.01
Fusarium oxysporum	Ethanol	1.8±0.13	1.6±0.05	1.3±0.09	1.1±0.01	2.5±0.45	0.9±0.27

Table 7. Percentage Inhibition of *Botryodiplodia theobromae, Sclerotium rolfsii* and *Fusarium oxysporum* in the culture of *Dioscorea alata*extracts.

Pathogens	**Extracts**	**% Inhibition**
Botryodiplodia theobromae	Aqueous	26.66
Sclerotium rolfsii	Aqueous	35.48
Fusarium oxysporum	Aqueous	16.66
Botryodiplodia theobromae	Ethanol	13.04
Sclerotium rolfsii	Ethanol	94.44
Fusarium oxysporum	Ethanol	38.46

Table 8. Percentage Inhibition of *Botryodiplodia theobromae, Sclerotium rolfsii* and *Fusarium oxysporum* in the culture of *Dioscorea bulbifera* extracts.

Pathogens	**Extracts**	**% Inhibition**
Botryodiplodia theobromae	Aqueous	30.35
Sclerotium rolfsii	Aqueous	37.50
Fusarium oxysporum	Aqueous	6.66
Botryodiplodia theobromae	Ethanol	43.33
Sclerotium rolfsii	Ethanol	88.88
Fusarium oxysporum	Ethanol	28.00

Table 9. T-Test Comparison between Aqueous and Ethanolic Extracts

Plant Extracts	**Aqueous**	**Ethanol**	**T-Statistics**
Dioscorea alata	26.27±0.69	48.65±2.37	-4.384
Dioscorea bulbifera	24.90±0.68	53.40±2.38	-6.427

Discussion

Fungal isolates associated with deteriorated white yam tubers in this study were *Sclerotium rolfsii* Sacc, *Botryodiplodia theobromae* Pat, *Fusarium oxysporum* Schlect, and *Aspergillus niger* Van Tieghem. These pathogens were frequently isolated from rotted white yam tubers and these organisms have been reported to cause rots of yams (Coursey, 1981; Arena *et al.*, 1985; Okigbo and Ikediugwu, 2000 and Okigbo and Ogbonnaya 2006). Some of these pathogens have also been isolated from Potato (Agu *et al*. 2015). The pathogenicity test revealed that *Sclerotium rolfsii*, *Botryodiplodia theobromae* and *Fusarium oxysporum* induced rot in white yam tuber with *Sclerotium rolfsii being* most virulent. This is in agreement with the reports of many researchers on yam tubers (Sangoyomi; 2004; Okigbo *et al.*, 2009b). The pathogenicity test also revealed that *Aspergillus niger* was a secondary pathogen to the rotted white yam. The isolation of more than one pathogenic microorganism from a

particular yam tuber sample shows the possibility of multiple infections whose joint effect may lead to severe rotting of tuber crops. These pathogenic fungi gain entry into yam tubers through natural openings and wounds created during harvesting, transportation, handling and marketing of the yam tubers. However, Okigbo and Nmeka (2005) reported that the root and tuber crops at the time of harvest may already be infected by pathogens derived from disease foliage, roots or mother tubers.

This study showed that fungal toxic compounds were present in *Dioscorea alata* and *Dioscorea bulbifera*, since they were able to inhibit the growth of the pathogenic fungi of the white yam tubers.This result is in agreement with the earlier report of several workers but they worked with different plant extracts (Okigbo *et al.*, 2009; Sangoyomi *et al.*, 2009; Duru, 2010; Suleman, 2010). However, the efficiency of the extracts differed with plant material, concentration, extraction medium and each test fungus.

Ethanolic extracts were observed to be more effective than the aqueous extracts, this suggests that water was not able to dissolve all the principal compounds present in the yam peels as did the ethanolic extracts. The ethanolic extracts were more effective in all the test fungi, this agrees with the report of Ekwenye and Elegalam (2005) on garlic who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds (phytochemicals) needed for antifungal activity. The difference in the fungal toxicity between the extraction medium may also be as a result of the different susceptibility of each of the test pathogenic fungi to different concentrations of the extract.This also agrees with the report of some workers (Amadioha, 2000; Onifade, 2002; Okigbo and Nmeka,2005; Okigbo and Ogbonnaya, 2006; Okigbo and Odurukwe 2009; Okigbo et al., 2009a). The present observations revealed that the extracts of *Dioscorea alata* and *Dioscorea bulbifera* have proved effective against the mycelial growth of all the test fungi. *Botryodiplodia theobromae* showed slightly effective and moderately effective inhibitions in all the extract concentrations tested. This is similar to the result obtained by Sangoyomi (2004) on post harvest rot of yam but with different plant extracts.She reported slightly effective inhibition of *Chromolaena odorata* on *Botryodiplodia theobromae*.

Aqueous extract concentrations of *D. alata* and *D. bulbifera* showed slightly effective inhibition of *Fusarium oxysporum.* While the ethanolic extracts were moderately effective on the test fungi.With respect to *Sclerotium rolfsii*, the aqueous extract concentrations of both yams showed moderately effective inhibition while the ethanolic extract concentration gave high effective inhibition. This is similar to the results obtained by Suleman (2010) on post harvest rot of yam (but with different plant extract), who reported a

highly effective inhibition with *Azadirachia indica* but differs with Okigbo *et al.*,(2009b) who reported a moderately effective inhibition with *Allium sativum.* The percentage inhibition showed by the ethanolic extracts on *Sclerotium rolfsii* were almost in the same level with commercial fungicide (Griseovulin) used for the positive control, which showed a very significant effective inhibition on the radial mycelia growth of the fungi tested although, *Sclerotium rolfsii* showed the highest percentage inhibition with Griseovulin.

The fungitoxic effects of both the extracts of *D. alata and D. bulbifera* with respect to concentration showed similar trend. Where 40% extract concentration proved to be the most fungitoxic on all the test fungi, and the least fungitoxic effect was observed at 10% extract concentration. This agrees with the observation of Suleman (2010) who observed a significant difference between the mycelial growths values recorded for various plant extract concentrations. This suggests that there is a difference in the solubility of the antifungal elements in the respective extracts as reported by Iwu (1993) and Sofowara (1997).

According to Srinivauson *et al.* (2001) the presence of bioactive substances has been reported to confer resistance to fungi, bacteria and pests, this therefore explains the demonstration of antifungal activities by the extracts used in this work. Thus, the antifungal properties of these yam extracts are probably due to the presence of phytochemicals which are antimicrobial agents (Okwu and Joshia, 2006), and inhibitory to the growth of these pathogens (Okigbo *et al.* 2009b). The phytochemical analysis of the yam peels used as extracts showed the presence of all the phytochemicals tested (Saponins, Tannins, Alkaloid, Flavonoids and Phenols). Pharmacological and medicinal potentials of all these phytochemicals were proved by the reports of several workers (Caragay, 1992; Okwu, 2004; Okigbo and Ajalie, 2005; Okigbo *et al.*, 2009a).

This study has revealed the potentials of the peel extracts of *Dioscorea alata* and *Dioscorea bulbifera* in the control of white yam rot. The study also indicated that ethanolic extracts demonstrated a higher antifungal activity than aqueous extracts, revealing that ethanolic extracts of *Dioscorea alata* and *Dioscorea bulbifera* could bealternatives or complimentary to synthetic chemicals in controlling white yam rot. The fungicidal effects of plant extracts in the inhibition of different pathogens of crop plants have been widely reported by several researchers (Onifade, 2002; Okigbo and Ogbonnaya 2006). However, the result of this study has gone a long way in providing another better alternative to the over dependence on synthetic fungicides which pose a serious threat to man, wildlife and the environment. The use of plant extracts provides an alternative way of reducing the cost of production and their

antimicrobial activities were comparable to those of the antibiotics. The demonstration of the activities of the yam extracts against the test fungi produces scientific bases for the local usage of these yam extracts in controlling microbial rots. Since these yam extracts are locally available, easy to prepare, non-phytotoxic and environmentally safe, hence the prospect of having cheaper means of controlling rot inducing organisms could be actualized, particularly for the peasant farmers across the world at large and particularly in Nigeria.

Conclusion and Recommendation

The results obtained in this study, obviously showed that the peels of *Dioscorea bulbifera* possess potential inhibitory effects against rot – inducing fungi to varying degrees.Thus the antifungal potential demonstrated by these yam extracts on white yam rot causing fungi, recommends their use as natural fungicide on white yam rot. This might be a proof for the fact that white yams are easily affected by rots than water yam and aerial yam in storage

The post harvest rot of white yam is an obvious challenge which requires urgent attention considering its economic importance in our country Nigeria. With respect to the yam extracts used, further toxicological studies, pharmacological evaluation and possible isolation of the therapeutic agents are recommended.

Antifungal agents from these yam extracts are the future challenges, thus it is recommended that further investigations should be carried out on the chemical nature of the active principles of the yam peels used as extracts. Also further research can combine the yam extracts for possible synergistic effects, further investigation involving in vivo assay would be recommended to investigate the fungistatic effects of these yam extracts on the fungi causing rot of white yam which were not included among the test organisms in this study. Also, very important is the need to adopt good storage patterns to prolong the storage of white yam after harvest.

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