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## Antifungal Effects of some Plant Extracts on Fungi Isolated from Post-Harvest Cocoyam

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### ABSTRACT

Cocoyam is one of the world's oldest edible crops, it is essentially a perennial plant. The majority of the shoot's components are leaves, which emerge in a whorl from the corm's apex. Farmers now face a very significant threat as a result of the post-harvest degradation of their cocoyam crop brought on by fungus illnesses. In this work, pathogenic fungi isolated from spoiled cocoyam sold in 15 markets in Awka, Anambra State, were tested for their in-vitro antifungal activity against *Pipiper guineense* (uziza), *Gongronema latifolium* (utazi), and *Allium sativum* (garlic). Approximately 25g of the plants were combined with 100ml of sterile distilled water to create three (3) distinct extracts. Using accepted techniques, phytochemical screening of the extracts was carried out. Alkaloids at 0.48 0.20, flavonoids at 12.00 0.057a, phenol at 3.12 0.15b, saponin at 0.31 0.15c, tannin at 0.41 0.20b, glycoside at 15.70 1.30a, steroids at 12.00 1.00a, and terpenoids at 3.12 0.50b were all detected in utazi extracts. Alkaloids were present in garlic extract at 1.56 0.10 a and flavonoids at 4.86 0.52 c. Phenol 0.480.02, saponin 0.660.32b, tannanin 0.550.50b, glycoside 7.550.50b, steroids 11.50 0.50a, terpenoids 3.75 0.50a, and uziza exhibited presences of alkaloids at 1.430.28b, flavonoids 5.97 0.051b, phenol 15.77 0.20a, saponin 3.06 0.10a, tannanin Five distinct species of fungus were tested for presence using the pure culture and isolation method; the findings indicated that *Aspergillus niger*, *Penicillium citrinum*, *Fusarium solani*, *Rhizooous stolonifera*, and *Mucor piriformis* were present. Since ethanolic extracts were more potent than aqueous extracts, *Pipiper guineense* (uziza) ethanolic extract proved to be the most effective. *P. guineense* depicted an effective/high rate of inhibition on the growth of all the test fungal, whereas extract of *Gongronema latifolium* and *Allium satitum* showed a lower inhibition. The most fungitoxic substance, *P. guineense*, at 100% concentration, significantly inhibited the test fungi pathogens in cells (p 0.05). As a result, the extracts demonstrated good fungal infection-inhibiting action. To lessen post-harvest losses of cocoyam produce and further minimise post-harvest losses, it is advised that they be included in the chemical composition of fungicides and further investigations should be done on the chemical nature of the active principles of the plants.

**Keywords:** Cocoyam, post-harvest, antifungi and fungi.

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## INTRODUCTION

Postharvest diseases caused by fungi constitute a worldwide public health problem and preventing them is a major goal of societies. Microbiological foodborne diseases are typically caused by fungi or their metabolites, parasites, virus or toxins [1]. Some fungi produce poisons in food without signs of spoilage and consumers may therefore be unaware of the contamination. Tuber crops are moist, low acidic foods that can support the growth of food poisoning fungi. Tubers are staples found in many parts of the tropic regions of the world and they belong to the class of food that basically provides energy in the human diet. The main components of tubers are water (60-90%), starch and fiber, with lesser amounts of proteins, fat, sugars, vitamins and minerals [2]. Tubers are cheap but nutritionally rich staple foods which are associated with high transportation cost, short shelf life, and limited market margin in developing countries [3].

The term "cocoyam" refers to a variety of tropical root and vegetable crops from the Arum family (Aroids). The genus *Colocasia* or the genus *Xanthosoma* may be the home of the herbaceous perennial plant known as the cocoyam. There are several varieties of cocoyam, some of which are used as decorative plants (e.g., especially *Xanthosoma roseum* and *Colocasia antiquorum*). As noted by [4], several others are found in the wild, including *Xanthosoma weeksii*, *Xanthosoma eggersii*, and *Xanthosoma brasiliense*, as well as *Colocasia affinis*, *Colocasia fallax*, *Colocasia heterochroma*, and *Colocasia gigantea*. The species of cocoyam that are typically grown for food include: Old cocoyams are taro (*Colocasia esculenta*) while modern cocoyams are malanga (*Xanthosoma* species). The main reason these two types are developed is for their tasty roots, Despite the fact that the entire plant may be eaten. According to [5] in terms of overall output, land area under cultivation, and consumption, it comes in third place behind cassava and yam. The corms can be cooked, baked, crushed, roasted over a fire, or made into a porridge. They can also be used as soup thickeners. The cocoyam's flour may also be used to make puddings, breads, biscuits, and soups [6].

According to reports, cocoyam corms provide easily digested starch and are known to include high amounts of protein, vitamin C, thiamine, riboflavin, niacin, and dietary fiber. They also contain significant amounts of protein and vitamin C. Depending on the types and regional cultural customs, other parts of the cocoyam plant, including as the leaves, blossoms, and stems, are often eaten, notably in sauces, purees, stews, and soups [7] [8] reported on the therapeutic effects of the plant's different parts in 2014. It's South Central Asian in origin. It is a significant crop around the world. The crop is an important component of many people's life since it provides food security and has significant sociocultural and economic significance [9] Products made from cocoyam have demonstrated significant potential in several sectors. Recent research has shown that cocoyam starch is fine and has tiny granules, which is a quality needed in many sectors [10] Furthermore, there is a general perception that cocoyam processing and production in the nation lag behind other significant root and tuber crops. This is said to be due to the low cocoyam storage ability and infections that harm stored cocoyam. The main variables impacting the number and quality of corms for eating and planting in storage are significant losses from rotting of the corms and cormels [11]. According to studies, the main factor causing root and tuber loss during storage is fungal rot [12]. *Aspergillus niger*, *Botryodiplodia theobromae*, *Sclerotia rolfsii*, *Fusarium solani*, and *Erwinia carotovora* are the main microbial species linked to cocoyam rot in Nigeria. These fungi were reported to be pathogenic to four cultivars of *Colocasia esculenta*, causing rot of cocoyam in several areas of southern Nigeria [13]. The surrounding tissues of the infected tubers become locally discoloured as a result of these fungi, which worsens the texture and flavour of the tubers. Rot fungi results in post-harvest losses, a decline in market value, and farmers' bad luck [14] Therefore, there are major ramifications, especially with regard to the availability of enough planting materials to support yearly crops.

However, certain plants have historically been employed as medicines to cure illnesses. The potential of botanicals (plant extracts) as alternatives or complements to synthetic chemicals is now the focus of significant research efforts. In addition to being easily accessible, inexpensive, and environment-friendly, botanicals also serve as supplies of non-phytotoxic, rapidly biodegradable fungicides and antibiotics [15]. In addition, a variety of herbs are widely utilised locally in traditional medicine to cure and manage disease. In several laboratory experiments, these plants have proven to have potent, broad-spectrum antifungal properties. Modern scientific research have verified their efficacy. In order to combat the issue of antibiotic resistance by pathogenic organisms, medicinal plants have undergone substantial research as a complementary therapy for illnesses. There are many plants, and their phytochemical components which have been studied to have medicinal value of which Garlic (*Allium sativum*), utazi (*Gongronema latifolium*) and Uziza (*Piper guineense*) are among.

Asclepiadaceae family of the plant *Gongronema latifolium* (Utazi). It is a plant that is both edible and nutritious that is mostly found in rain forest regions in Nigeria and other tropical African nations. It has previously been documented that several ethnic groups used *G. latifolium* leaves as folk medicine to cure illnesses including malaria, motion sickness, coughing, anorexia, diabetes, hypertension, constipation, diarrhoea, etc [16] For its nutritional and therapeutic advantages, *Gongronema latifolium* leaves are also consumed fresh, with pourage or roasted yam and/or

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plantain, seasoned in soups, as gravy, etc [17]. The plant may be reproduced by stem cuttings or seeds, and it has yellow blooms and white latex. *G. latifolium* is known as Kurutu Nsurogya in Akan-Asantes of Ghana, Gasub to the Serers of Senegal, and Ndodo-Polole to the Kissis of Sierra Leone. The Ikaes of Ondo State of Nigeria name the plant Iteji, the Igbos call it Utazi, the Efik/Ibibio call it Utasi, and the Yoruba [18] The bitter-sweet flavour of *G. latifolium*'s leaves is added to soups when they are prepared as vegetables. Additionally, the leaves are also used to flavour locally manufactured beer. According to [16], *G. latifolium* is used in several West African cultures to cure malaria, diarrhoea, intestinal worms, dyspepsia, and cough. He stated that in Ghana and Senegal, *G. latifolium* leaves are applied to children's joints to aid in walking, and the cooked fruits of this plant are used as a laxative. In Sierra Leone, an infusion or decoction of the stems with lime juice is given to cure colic and stomach problems. According to [19], the leaves *G. latifolium* are used as a stomach vermifuge (medicine). They stated that some local people also utilise the leaves to cure dyspepsia. According to [20], the leaves of *G. latifolium* is used to cure chicken cough in Nigeria.

The spice plant *Piper guineense* (Uziza) belongs to the Piperaceae family and genus. It is a spice plant from West Africa known as Ashanti pepper. In Igbo, it is referred as Uziza, and in Yoruba, as Iyere. Benin pepper, Guinea pepper, and fake cube are some additional common names [21]. Spices are often harvested from a variety of plants for their fragrant, pungent, or other desired properties. They are made up of leaves, rhizomes, bulbs, flower buds, fruit, and seeds. Typically, they are divided into green vegetables, nuts, herbs, and small wild fruits. The tiny, clustered fruits of *P. guineense* are reddish or reddish brown in colour when ripe and black when dried [21]. The fruit is an oval, meaty drupe called a mesocarp. The leaves have aperitif, carminative, and eupeptic properties. The leaves are used to cure syphilis, rheumatism, and respiratory diseases [22]. The leaves' antimicrobial properties have been demonstrated in Nigeria. Aseptic in nature and able to cure flatulence are *P. guineense* leaves. Male low sperm count and female infertility are both treated with the leaves, and the fruits are used as an artificial sweetener [23]. After giving birth, women in certain parts of Nigeria swallow seeds to help the uterus contract more forcefully, allowing the placenta and other remnants to be expelled from the womb. During the postpartum period, it is added to the meals of breastfeeding women because it is thought to stimulate or promote uterine contractions, assisting in the rapid recovery of uterine muscle to its previous shape [24] It is also employed as an abortifacient in the eastern region of Nigeria. It is also used as an aphrodisiac, an anti-asthmatic, an adjuvant in the treatment of rheumatic symptoms, and as a weight-control supplement [25].

A member of the Lillaceae family botanically, Garlic (*Allium sativum* L.) has gained a reputation in various cultures as a preventative as well as curative medicinal herb, playing a significant part in the treatment of various ailments. Garlic was suggested by [6] as a helpful ingredient in treating infectious disorders, gynaecological problems, arthritis, toothaches, chronic cough, hypertension, constipation, parasite infestation, and snake and bug stings (as antibiotic). Several clinical and experimental studies point to numerous beneficial benefits of garlic and its derivatives. The majority of these effects has been linked to the following causes: I) decreased risk factors for cardiovascular disease, ii) decreased risk of cancer, iii) antioxidant impact, iv) antibacterial action, and v) increased hepatic protection and foreign substance detoxification [26]. It is frequently used in cooking as a flavouring. Due of its strong flavour, it is frequently used as a condiment. Most plants make use of its bulb. Garlic is often separated into fleshy portions called cloves, with the exception of the varieties with a single clove. You may eat it either raw or cooked. Organosulfur chemicals, such as allicin found in fresh garlic cloves and ajoene produced when they are crushed or diced, are mostly responsible for the characteristic scent. It is well known that garlic causes halitosis, or foul breath, as well as body odour. The metabolite Allyl Methyl Sulphide (AMS), which causes bad breath, is what gives garlic its characteristic flavour [27]. The skin covering each clove and the root cluster are among the inedible or infrequently consumed components of the garlic plant. The different plant sections' papery protective coverings are typically removed before preparation for most culinary purposes [28]. Garlic is frequently referred to as "stinking rose" because to its pungent odour. When consumed in large quantities, garlic can be so potent that it can be seen in the diner's perspiration and breath the next day. Since the AMS is indigestible, it enters the circulation. It is transported to the skin and lungs, where it is expelled. Garlic may have a long-lasting impact since digestion takes many hours and the release of AMS takes another few hours. Some people are allergic to allium species, including garlic. The signs and symptoms might include breathing issues, diarrhoea, mouth and throat ulcers, and irritable bowel syndrome [29]. This work is aimed to evaluate the antifungal activity of utazi, uziza and garlic extracts against some pathogenic fungi isolated from spoilt cocoyam sold within Anambra metropolies.

**MATERIALS AND METHODS****Culture Media**

In this study, two commercially accessible mediums will be used. These were Sabouraud Dextrose Agar (SDA), a variation of Dextrose Agar, and Potato Dextrose Agar (PDA), a general-purpose culture medium. For PDA, about

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39g of the medium were suspended in one litre of distilled water, heated over a Bunsen flame while being stirred frequently, and allowed to boil for one minute to thoroughly dissolve the medium/contents. The solution was autoclaved for 15 minutes at a temperature of 121°C and one atmosphere of pressure (15 PSI). Allow to cool for ten minutes after withdrawing from the autoclave. To act as antibiotics, 500 mg of streptomycin sulphate was added to the molten solution. For SDA media preparation, about 65g of the medium were suspended and dissolved in 1 litre of distilled water by heating to boiling and stirring frequently. It was heated for one minute to dissolve the solution, and then sterilised for 15 minutes at 121°C in an autoclave. After that, while the solution was still molten, 500mg of the antibiotic streptomycin was added.

**Methodology for isolation and identification of post-harvest spoilt cocoyam**

In this work, the isolation method from [30] was used. The surfaces were sterilised by dipping completely in a concentration of 40% hypochlorite solution for 60 seconds; the sterilised sections to be inoculated were then removed and rinsed with three changes of sterile distilled water. A small section of infected *Colocasia esculenta* tissues containing the advancing margin of rot and adjoining healthy tissue was cut using a sterilised scalpel and cork borer. The tuber pieces were blotted dry in a laminar airflow cabinet using sterile filter paper. Ten pieces of each cut sample were individually infected (90° apart) on solidified Potato Dextrose Agar (PDA) and Sabouard Dextrose Agar (SDA) plates with the help of sterile forceps. The paper tapes were used to secure the inoculation plates, and they were then incubated at 28°–30°C for 72 hours. The inoculation plates were examined for fungi linked to the rotting of the tubers. In order to purify the materials, a sterile inoculating needle was washed in selecting mycelia threads from the samples and then transferred to freshly prepared PDA and SDA plates. The isolates were then subjected to several subcultures for further purification. All of the samples were plated using the pour plate technique [31]; [32]. Using a sterile pipette, 1 ml of dilution (10<sup>-1</sup>) (the dilution that is neither too murky nor too light) was added to a 9 ml Petri plate. Each dish received a pour of molten potato dextrose agar (10 ml). To make it simple for the sample and the medium to mix together, the plates were turned clockwise. All plates were left on the bench to set up. The plates were all copied. The entire collection of hardened plates was placed in an incubator set at 25 oC for 3-5 days. Mycelia colonies were counted daily based on observation.

**Fungi identification and characterization**

To create a pure culture, isolated fungus were further sub-cultured. According to [33]; [34] identification was then conducted based on colony characteristics, morphology, and microscopic traits. Using morphological traits and matching the results to established keys as given by [35]. A colony and a microscope were used to study each isolate, and their morphological traits were examined and documented. Morphological features were investigated using growth patterns, mycelia colour, and microscopic analyses of vegetative and reproductive components. A little piece of mycelia was taken from the area between the colony's centre and edge utilising a clean inoculating device needle, and it was then put on a spotless microscopic slide with lactophenol in cotton blue. Using the sterile needle and a cover slip that was put carefully and slightly pressed to remove air bubbles, the mycelia were evenly disseminated throughout the slide. To further preserve the fungal forms on the slide, it was heated by steam from some boiling water. The excess lactophenol from the borders of the cover slip was removed with sterile blotting paper. Using the microscope's 10 and 40 objective lenses, the slide was examined. To help with the identification of the organisms, the Cultural Characteristics, growth pattern, pigmentation, and size of colonies were noted during the incubation period.

**Total Viable Count**

On a sensitive weighing scale, 9 ml of sterile, deionized water was added to around 1 g of the sample. The mixture was vigorously agitated, and it was let to stand for 5 minutes.

**Counting of the Colonies**

Using a hand tally counter, a counting tool that resembles a stopwatch, the number of colonies in each plate was counted after the plates had been incubated (Dhawale and La Master, 2003). The count's mean was calculated, multiplied by the proper dilution factor.

**Spore Staining**

The heat-fixed slide containing the isolate's smear was placed over a steaming water bath, and blotting sheets were placed over the region of the smear without sticking out over the corners of the slide as part of the staining step for spore identification. After being soaked with a 5.6% solution of malachite green, the blotting paper was steamed for 5 minutes. The slide was then carefully cleaned with tap water after being cooled to room temperature. After one minute of application, Safari was rinsed completely yet quickly before being blotted dry. The slide was then inspected under a microscope.

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### Motility Test

Fungal motility was measured by replacing the growth of the organism on a clean slide with a wire loop after putting a small drop of live isolates to the centre of a slip on a depression slide using petroleum jelly or 2-3 drops of peptone water. The slide wore a cover slip covering it., left alone for a while, and then inspected under a high power microscope. Swimming about were movable creatures. Biochemical Test Carbohydrate Assimilation Test: The pH of the medium was raised to 5.4 by adding NaOH or HCl, and filtered and sterilised carbohydrates were added at a concentration of 1%. A 10 ml test tube was filled with 2 ml of the medium. Additionally, isolates and carbohydrates were injected through the tubes. All tubes underwent a 14-day incubation period at 20° C. A shift in the medium's orange and yellow tint was viewed favourably. Pink or purple changes were seen as undesirable outcomes.

### Amino-acid Assimilation Test:

For the carbohydrate absorption test, the medium preparation and indication were as stated. The isolate and control tubes for each fungus and amino acid were inoculated into 10 mm test tubes containing 2 ml of the medium. Additionally, tubes were kept at 20° C for 14 days. A move to pink or purple was regarded as a favourable outcome, but a change to orange was viewed as a failure.

### Hydrolysis Test

With the addition of 0.05 mg milk and 1.2 mg agar, the base medium was comparable to that of the test for amino acid absorption. The medium was put into a petri dish after an autoclave at 110o C for 30 minutes. At 20°C for 14 days, isolates were incubated after being inoculation in the plate's centre. It was deemed successful when a clean zone appeared around the fungus colony.

### Lipase Activity Test

The 0.5% peptin, 0.3% yeast extract, and 1.0% agar medium was autoclaved for 10 minutes at 121° C. It was filtered and then put into test tubes that had been sterilised. Incubation was place at 20° C for 7 days after the isolates were injected onto the medium's surface. Clearance occurring in the middle column was viewed as a successful outcome.

### Carbohydrate assimilation test

On a tidy microscopic slide, lactophenol (LP) was dropped upon it. A little amount of the isolate was added to the lactophenol (LP) drop and suspended. The suspension was draped with a spotless cover glass, then under a microscope was looked at.

### Pathogenicity test

[36] methodology was used. After being carefully cleaned using tap water and rinsed thoroughly using distilled water, fifteen healthy cocoyam corms were used. Next, 75% ethanol was used to clean the corms' exteriors. The amount of the infection and the rate at which the illness spreads define the degree of rot-causing fungus become pathogenic. The combined final weight of each cocoyam tuber was measured after the entire tubers' bad parts were cut off. Controls who had not been immunised were put in fresh plastic bags. Here is how the percentage of rot severity (Sr%) was determined:

$$Sr \% = \frac{FW - wx}{w} \times 100$$

where,

FW = Final weight of a cocoyam tuber with an infection,  
w = weight of rotted tuber portion.

### Preparation of plant extracts

The samples were thinly cut and sun dried for 7 days to achieve consistent weight. For extraction, this will be ground into a fine powder and kept in an airtight plastic container.

### Ethanol extract

The 1000g of plant samples that were gathered will be macerated with ethanol. After standing for 48 hours, it was filtered. The filtrate was dried in a rotary evaporator at 55°C while being evaporated under decreased pressure.

### Determination of extraction yield (% yield)

The yield (% , w/w) from all the dried extracts was calculated as:

$$\text{Yield (\%)} = (W1 * 100) / W2$$

where W1 is weight of extract after lyophilization of solvent, and W2 is weight of plant powder.

### In vitro antifungal tests with plant extracts

The ethanolic extract was subjected to the disc diffusion technique, which was adapted from [3]. Using a sterile cotton swab, 100 L of prepared inoculum (10<sup>4</sup> CFU/mL) was evenly applied to PDA. The sterile paper disc was placed on top of the agar media and impregnated with 10 L of 1, 2, and 10 mg/mL extract. In this investigation, 10 mg/mL of miconazole solution and 10% Dimethyl sulfoxide (DMSO) will be utilised as the positive and negative controls, respectively. After that, the plate was incubated standing up for 48 hours at 30°C. Then, millimetre

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measurements of the distinct inhibition zones around the disc were taken. For each treatment, three duplicates of five plates were kept, and the infected plates were incubated for seven days at room temperature (28°C).

At the end of the incubation time, the diameter of the radial growth of the fungus was measured, and the fungitoxicity level of the powders and extracts was calculated using the following formula:

$$\text{Percentage growth inhibition (\%)} = \frac{dc - dt}{dc} \times 100$$

Where

dc = average diameter of fungal colony in control treatment

dt = average diameter of fungal colony with powder or extract.

**Determination of minimum inhibitory concentration (MIC)**

Plant extracts' minimum inhibitory concentrations (MICs) were calculated using a modified microplate approach. Plant extracts were serially diluted, with dilutions ranging from 1/2 to 1/100 of the original amount. Each extract dilution was combined with 100 mL of fungal spore suspension ( $2 \times 10^6$  spores mL<sup>-1</sup> in fresh PDB) in each well. The microplates were incubated at 27 °C for 2-3 days while being checked every day. Three copies of each experiment were performed. A microplate reader set to 595 nm was used to spectrophotometrically read the MIC values. By comparing the growth in control wells and the extract blank, which consisted of uninoculated plates, MIC values were determined. The lowest concentration of plant extract that resulted in growth inhibition of more than 90% after 48 hours when compared to the control was known as the MIC of the extracts [37].

Using MFC, *in vitro* fungicidal activity [37]. 20 L were subcultured onto PDA plates after 72 hours from each well that had no discernible growth (growth inhibition of > 98%), from the last positive well (growth comparable to the growth control well), and from the growth control (extract-free medium). The plates were incubated at 27 °C until the growth control subculture showed signs of growth. The lowest extract concentration that did not result in any fungus growth on the solid was considered to be the minimum fungicidal concentration used.

**Qualitative phytochemical screening**

To identify the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, and reducing sugars in the selected plant extracts, a procedure based on earlier reports by [38]; [39] will be used to perform phytochemical screening of the extract.

**Test for tannins**

Each powdered sample was individually cooked in a water bath for five minutes with 20 cc of distilled water, then it was hot-filtered. A few drops (2–3) of 10% ferric chloride were added to 1 ml of cold filtrate after it had been distilled to 5 ml with distilled water, and the mixture was then checked for precipitate development and colour changes. To establish the presence of tannins, the reaction mixture was examined for a brownish green or blue-black colouring.

**Test for saponins**

Each powdered sample weighed around 1g, and it was individually cooked in a bottle bath for 10 minutes with 10 ml of distilled water. While still heated, the mixture was filtered, then let to cool. Then, the further tests were run.

(a) An example of frothing: After shaking vigorously for two minutes, 2.5 ml of filtrate was diluted to 10 ml with distilled water. The creation of a froth that remained stable for a few minutes indicated the presence of saponin in the filtrate.

(b) Evidence of emulsifying properties: After adding around 2 drops of olive oil to the solution made by dilution of 2.5 ml filtrate to 10 ml with distilled water (above), which was then rapidly agitated for a few minutes, saponins were found to be present.

**Test for steroids**

(a) Two millilitres of chloroform were used to dissolve about 0.2 grammes of each component of the powdered material. To create a layer, 0.2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully applied. The deoxy-sugar properties of cadenolides are visible at the layer interface as a reddish-brown colour, which denotes the presence of steroids.

(b) 0.5 g of the sample's ethanolic extract was combined with 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> and around 2 ml of acetic anhydride. In certain samples, the transition from violet to blue or green indicates the presence of steroids.

**Test for alkaloids**

Each powdered sample weighed about 1 g, which was then individually boiled in water and acidified in a steam bath with 5 ml of 1% HCL. The resulting solution was filtered, and 2 ml of the filtrate was treated individually in various test tubes with a few drops of each of the following chemicals before being viewed.

(a) Mayer's Test: Filtrates were subjected to a Mayer's reagent treatment (potassium mercuric iodide). Alkaloids were present in the extract because a creamy white precipitate formed.

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(b) Wagner's Test: Filtrates underwent Wagner's reagent treatment (Iodine in potassium iodide). Alkaloids were thought to be present in the extract if a brown or reddish-brown precipitate formed.

(c) Dragendorff's Test: Filtrates were treated with the dragendorff's reagent (potassium bismuth iodide solution), and the formation of an orange-brown precipitate was taken as proof that the extract contained alkaloids.

(d) Hager's Test: Filtrates were treated with Hager's Reagent (saturated picric acid solution), and the appearance of a yellow precipitate was taken as proof that the extract contained alkaloids.

**Test for cardiac glycosides**

a) Two millilitres of glacial acetic acid containing one drop of ferric chloride solution were added to 5 millilitres of each extract. With 1 cc of concentrated sulfuric acid, this was underplayed. Cardenolides' deoxysugar properties were visible at the contact as a brown ring. Below the ring, a violet ring could show up, and in the acetic acid layer, a greenish ring might develop.

b) In separate test tubes, 1 ml of the filtrate was mixed with about 10 ml of 50% H<sub>2</sub>SO<sub>4</sub> and heated for 15 minutes. Thereafter, 10 ml of Fehling's solution was added, and the mixtures were cooked. The presence of glycosides was revealed by a brick-red precipitate.

**Test for free anthraquinones**

Each sample's dry powdered seeds were combined with 0.5 g of chloroform, which contained around 5 ml. After shaking for five minutes, the resultant liquid was filtered. Following that, an equal amount of 10% ammonia solution was shaken with the filter. The presence of free anthraquinones was suggested by the appearance of a brilliant pink colour in the aqueous layer.

**Test for flavonoids**

(a) Each sample's 1 g of powder was independently cooked in 20 ml of water before being filtered. A portion of the filtrate was mixed with 5 ml of diluted ammonia solution and then with concentrated H<sub>2</sub>SO<sub>4</sub>. The presence of flavonoids was indicated by a yellow hue.

(b) Each sample's powdered dried seeds were cooked in 10 ml of distilled water about 5 minutes before being filtered while still hot. To 1 ml of the cooled filtrate, a few drops of a 20% sodium hydroxide solution was added. When acid was added, the yellow colour changed to a colourless solution, signifying the presence of flavonoids.

**Test for terpenoids**

Each extract was diluted in 2 ml of chloroform with around 5 ml. Then, to create a layer, 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Terpenoids were detected by a reddish-brown precipitate coloration that developed at the contact.

**Test for phlobatannins**

The presence of phlobatannins was determined by the formation of a red precipitate after boiling an aqueous extract of each plant sample with 1% aqueous hydrochloric acid.

**Test for carotenoids**

Each extract was made using 10 ml of chloroform forceful shaking in a test tube. After filtering the mixture, 85% sulfuric acid was added. The presence of carotenoids was shown at the interface by a blue colour.

**Phenolics**

Each sample's powdered dry seeds were combined with 10 ml of distilled water and cooked for 5 minutes before being filtered while still hot. A solution of ferric chloride in 1 ml was then added. The appearance of brown or blue-black colouring suggested the presence of phenol.

**Test for reducing sugars**

About 10 ml of distilled water were added to each sample, which weighed about 1 g, in the test tube. The mixture was boiled for 5 minutes. The combination was heated, filtered, and then allowed to cool. In a test tube, 5 ml of the Fehling's solution (A and B) mixture was added to 2 ml of the filtrate, and the resulting liquid was then heated for 2 minutes. Brick red precipitate at the test tube's bottom revealed the presence of reducing sugar.

**Quantitative phytochemical determination****Determination of flavonoids**

This was accomplished using the technique outlined by [40]. 100 ml of 80% aqueous methanol was added at room temperature after five grammes of ground spice were weighed in a 250 ml titration flask. This was shook with an electric shaker for four hours. Twice, Whatman filter paper No. 1 was used to filter the solution (125 mm). Next, the filtrate was put into a crucible, dried out over a water bath, and weighed.

**Phenol determination**

The test was conducted using [41] methodology. Using a Soxhlet device, two grammes of the ground materials were defatted for two hours with 100 ml of diethyl ether. The samples without fat were boiled in 50 ml of ether for 14 min. A 50 ml flask was filled with 5 ml of the extract, followed by 10 ml of distilled water. Then, 5 ml of

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**Publications**

concentrated ethyl alcohol and 2 ml of  $\text{NH}_4(\text{OH})_2$  solution were added. The samples were then prepared as directed and given 30 minutes to react in order to acquire their colour. At a wavelength of 505 nm, a visible spectrophotometer was used to measure the solutions' absorbance.

**Determination of Glycosides**

Using 50 ml of distilled water, the extracts (1 g each) were macerated before being filtered. 4 ml of the pirate solution was added to the filtrates (1 ml), and the mixture were heated for 5 minutes before cooling. At 490 nm, absorbance was measured. Digitoxin was used to create a standard that was prepared using the same process at various concentrations. An absorbance vs. concentration standard curve was then constructed, and the concentration of glycosides in the extracts was extrapolated from the standard curve.

**Alkaloid determination**

As determined by [42]. A 250 ml beaker will be filled with about 5 g of the sample, 200 ml of 10% acetic acid in methanol, and then the beaker will be sealed. The combination will be let to stand for one hour, following which it will be filtered through paper and concentrated to (1/4) one-fourth of its original volume in boiling water. As the precipitate forms, concentrated ammonium hydroxide will be added drop by drop. After being collected and filtered, the precipitate will be washed with weak ammonium hydroxide. The alkaloid that is left over will be dried and weighed. Using the formula, the amount of alkaloids will be calculated as a percentage.

$$\% \text{ weight of alkaloid} = \frac{W_1 - W_2}{W_3} \times 100$$

Where  $W_1$  = Weight of filter paper with residue

$W_2$  = Weight of filter paper

$W_3$  = Weight of sample analyzed

**Test for Saponin**

As determined according [43]. The pulverised material was weighed out into a filter paper and placed into a Soxhlet extractor chamber with a condenser and flask, totaling twenty (20g). The flask contained 250 ml of methanol. By warming the flask on a heating mantle for three hours, the saponin was completely removed. The methanol was recovered after the extraction, leaving the saponin and a small amount of methanol in the flask. The remaining methanol was then evaporated by placing it on a heating mantle in a slanting position at 70°C temperature. The flask and its contents were weighed, and the amount of saponin extracted was determined by comparing the weights of the flask plus saponin with the flask alone.

It was measured and weighed for saponin content in percentage

**Calculation:**

$$\% \text{ Saponin} = \frac{(\text{Weight of beaker + sample}) - (\text{Weight of empty beaker}) \times 100}{\text{Weight of sample analyzed}}$$

**Tannin determination**

determined in light of [43]. A beaker will be filled with 100ml of N-hexane, 20g of the dried sample, and covered for two hours. After filtering the sample using filter paper, N-hexane will be allowed to evaporate for 15 minutes. By soaking it in 100ml of 10% acetic acid in ethanol for 10 minutes, it will be re-extracted. Filtered portions of the sample will be collected. To precipitate the alkaloids, 25ml of ammonium hydroxide will be added to the filtrate. To get rid of part of the ammonium hydroxide that is still in solution, the alkaloids will be heated on an electric hot plate. 33 ml of the remaining liquid will be measured. This will be diluted by 20ml of ethanol. Using phenolphthalein as an indicator, it will be titrated with 0.1M NaOH until the pink end point is achieved. Using the formula, the amount of tannin present will be calculated.

$$\% \text{ of tannin acid content} = \frac{C_1}{\text{Weight of sample analyzed}} \times 100$$

Where  $C_1$  = conc. of tannin Acid

$C_2$  = Conc. of Base

$V_1$  = Volume of Tannin acid

$V_2$  = Volume of Base

$$\text{Therefore } C_1 = \frac{C_2 - V_2}{V_1}$$

**Statistical analysis**

For each treatment, each outcome was repeated twice or three times using triplicates ( $n = 2 \ 3 / n = 3 \ 3$ ), and the data were presented as a mean standard deviation. Using Minitab® Version 16 for Windows (Minitab Inc., USA), a one-way analysis of variance (ANOVA) was carried out, followed by a posthoc Tukey's test for means separation ( $p < 0.05$ ).

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RESULTS

Mean Fungal Count in PDA and SDA for infected cocoyam

The fungi pathogens that were constantly isolated from the rot-infested tissues of the cocoyam cormels includes *Aspergillus niger*, *Fusarium solani*, *Rhizopus stolonifer* and *Penicillium digitatum*. The occurrence frequency varied with different fungi connected with the rotten cocoyam cormels. The most frequently occurred were *Aspergillus niger*. The results for TVC which was incubated and observed under temperature of 25°C for 3-5 days, were serially diluted, growth of colonies were observed and counted with a hand tally counter for each Locations. According to Table 1 below, Location 13 gave the highest colony count of 43 with PDA while Location 11 gave the highest colony count of 67 with SDA, followed by Location 3,4,15 which has no fungal counts with PDA while Location 15 has the lowest unit count with SDA of 15 (Table 1).

Table 1: Mean fungal count in PDA and SDA for infected cocoyam

| Sample    | Mean total fungal count in PDA (x 10 <sup>2</sup> cfu/g) | Mean total fungal count in SDA (x 10 <sup>2</sup> cfu/g) |
|-----------|--|--|
| SAMPLE 1  | 27.0000 ±1.0000 <sup>e</sup>                             | 22.6667 ±2.51661 <sup>h</sup>                            |
| SAMPL 2   | 33.0000 ±2.64575 <sup>d</sup>                            | 29.3333 ±3.51188 <sup>fg</sup>                           |
| SAMPLE 3  | 0.0000 ±0.0000 <sup>h</sup>                              | 17.3333 ±0.57735 <sup>i</sup>                            |
| SAMPLE 4  | 0.0000 ±0.0000 <sup>h</sup>                              | 19.6667 ±0.57735 <sup>hi</sup>                           |
| SAMPLE 5  | 45.6667 ±2.51661 <sup>a</sup>                            | 45.3333 ±0.57735 <sup>c</sup>                            |
| SAMPLE 6  | 27.0000 ±1.0000 <sup>e</sup>                             | 41.0000 ±1.73205 <sup>cd</sup>                           |
| SAMPLE 7  | 14.0000 ±1.0000 <sup>fg</sup>                            | 53.0000 ±2.0000 <sup>b</sup>                             |
| SAMPLE 8  | 35.0000 ±1.0000 <sup>cd</sup>                            | 28.3333 ±0.57735 <sup>g</sup>                            |
| SAMPLE 9  | 9.0000 ±1.0000 <sup>g</sup>                              | 16.6667 ±1.15470 <sup>i</sup>                            |
| SAMPLE 10 | 16.6667 ±0.57735 <sup>f</sup>                            | 30.0000 ±1.0000 <sup>fg</sup>                            |
| SAMPLE 11 | 33.3333 ±1.52753 <sup>cd</sup>                           | 66.3333 ±2.51661 <sup>a</sup>                            |
| SAMPLE 12 | 38.3333 ±1.52753 <sup>bc</sup>                           | 33.6667 ±1.15470 <sup>ef</sup>                           |
| SAMPLE 13 | 42.6667 ±2.51661 <sup>ab</sup>                           | 29.3333 ±0.57735 <sup>fg</sup>                           |
| SAMPLE 14 | 30.0000 ±4.0000 <sup>de</sup>                            | 36.6667 ±0.57735 <sup>de</sup>                           |
| SAMPLE 15 | 0.0000 ±0.0000 <sup>h</sup>                              | 15.3333 ±0.57735 <sup>i</sup>                            |

\* Values in the column with the same letters are not significantly different (p<0.05).

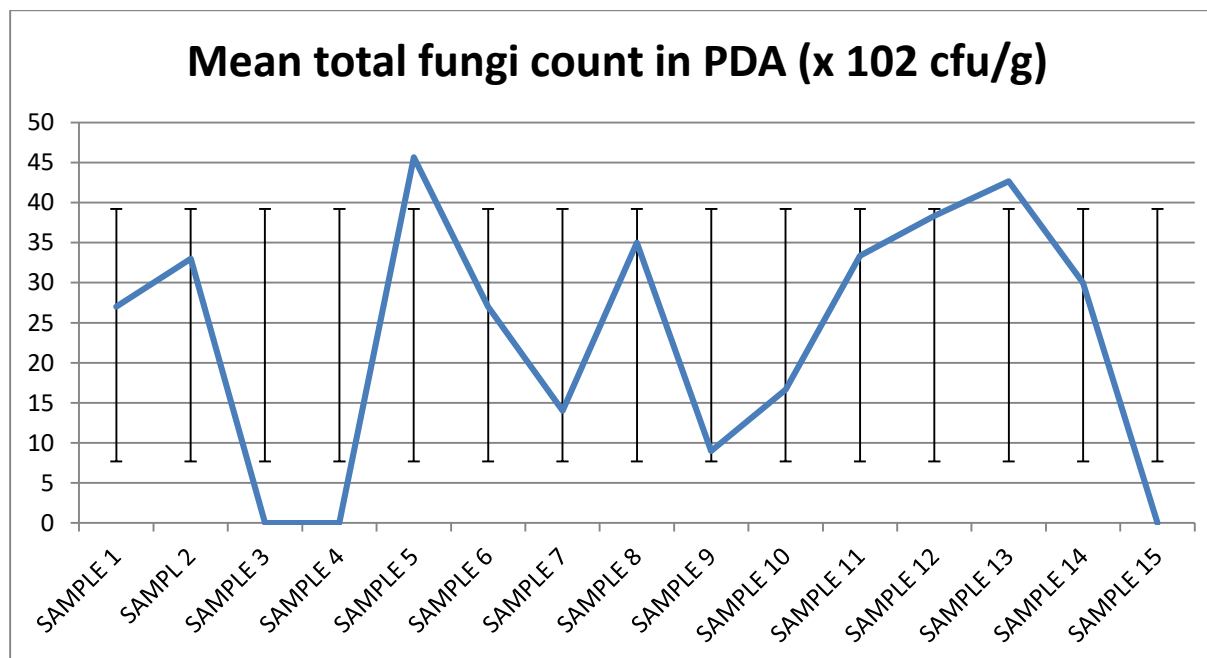
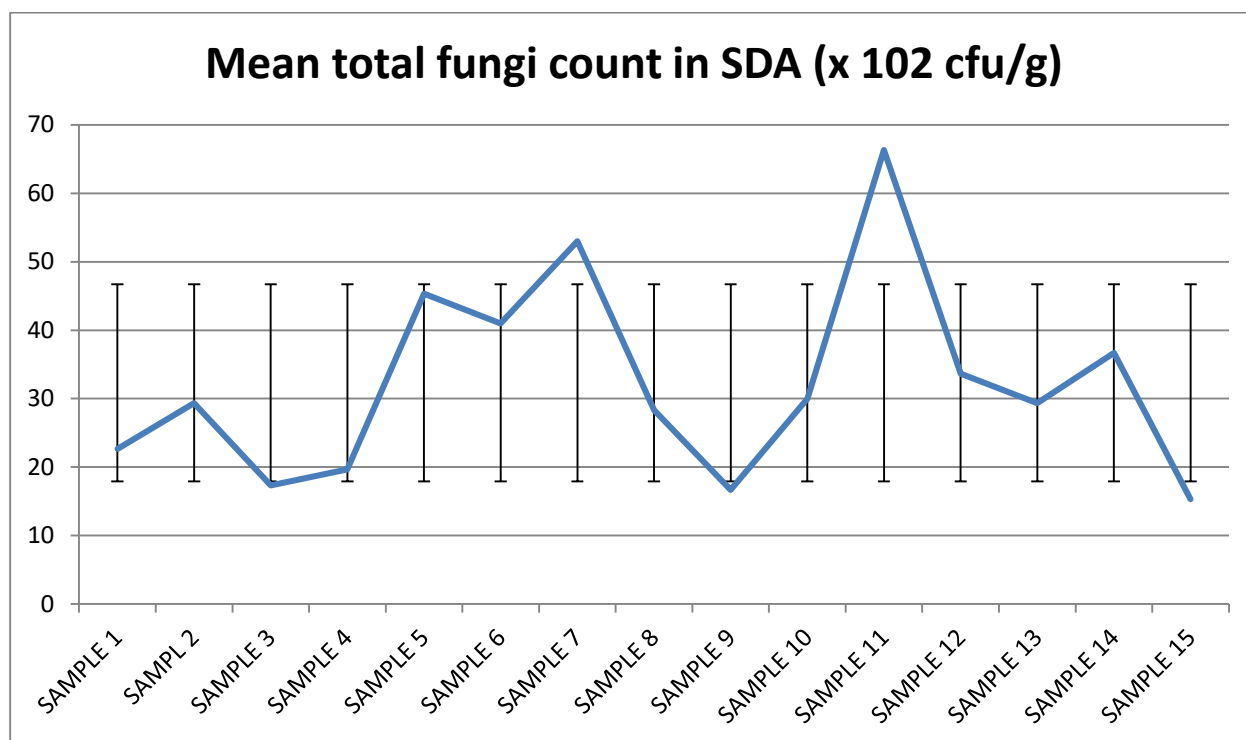


Figure 1: Representation of mean PDA count

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**Figure 2: Representation of mean SDA count  
Spore Formation, Motility and Biochemical Identification**

The biochemical characteristics of the isolated fungi were determined in terms Spore Formation, Motility and Biochemical Identification and the result shown in Table 2.

**Table 2: Spore Formation, Motility and Biochemical Identification**

| s/n | Isolate               | Carbohydrate assimilation | Spore formation | Amino acid assimilation | Motility | Hydrolysis | Lipase activity |
|-----|-----------------------|---------------------------|-----------------|-------------------------|----------|------------|-----------------|
| 1   | <i>Aspergillus</i> sp | +                         | -               | +                       | -        | -          | +               |
| 2   | <i>Penicillium</i> sp | +                         | -               | +                       | -        | -          | +               |
| 3   | <i>Fusarium</i> sp    | +                         | -               | +                       | -        | -          | +               |
| 4   | <i>Rhizopus</i> sp    | +                         | +               | +                       | -        | -          | -               |
| 5   | <i>Mucor</i> sp       | +                         | +               | -                       | -        | -          | -               |

### Frequency of Visible Colonies

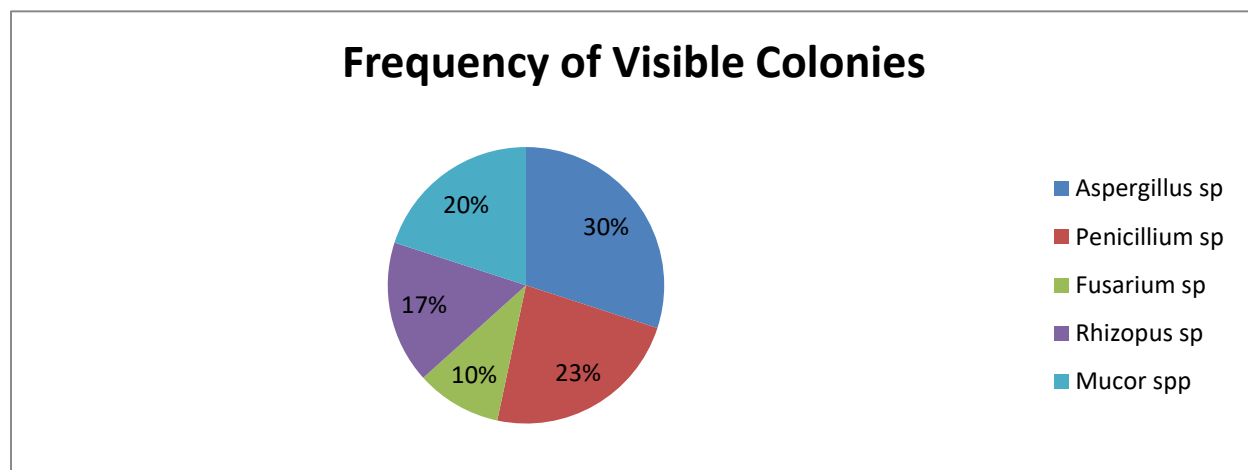
Results of the percentage occurrence of isolates from different Locations revealed that *Aspergillus spp* gave the highest percentage in 9 out of 15 locations with (30%) followed by *Penicillium spp* with 23.33% in 7 out of 15 locations while *Fusarium spp* gave the least occurrence in 3 out of 15 locations with just (10%) as seen in Table 6 shown below. In comparison the difference could be attributed to storage materials used or the soil condition where the samples were grown.

**Table 3: Frequency of Visible Colonies**

| s/n | Isolate               | (X) Frequency/Number of Occurrence From Samples | % Frequency |
|-----|-----------------------|---|-------------|
| 1   | <i>Aspergillus</i> sp | 9   | 30.00       |
| 2   | <i>Penicillium</i> sp | 7   | 23.33       |
| 3   | <i>Fusarium</i> sp    | 3   | 10.00       |
| 4   | <i>Rhizopus</i> sp    | 5   | 16.66       |
| 5   | <i>Mucor</i> spp      | 6   | 20.00       |
|     | <i>Total</i>          | 30  | 100.00      |

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**Figure 3: Representation of Frequency of Visible Colonies Pathogenicity Test**

The pathogenicity test showed that all the five fungi (*Rhizopus spp*, *Penicillium spp*, *Fusarium spp*, *Mucor spp* and *Aspergillus spp*) were all pathogenic, hence causes rot in healthy cocoyam cormels after three days of inoculation. The most virulent among the five spoilage fungi was *Aspergillus spp* which occurred very pathogenic in 6 out of 15 locations and mildly pathogenic in 5 locations but absent in the rest while the least virulent was *Mucor spp* with rot incidence very pathogenic occurring in just 2 locations and mildly pathogenic in 4 locations while absent in rest (Table 7).

**Table 5: Result of Pathogen city Test**

| Sample    | <i>Aspergillus sp</i><br>(mm) | <i>Rhizopus sp</i><br>(mm) | <i>Penicillium sp</i><br>(mm) | <i>Fusarium sp</i><br>(mm) | <i>Mucor spp</i><br>(mm) |
|-----------|-------------------------------|----------------------------|-------------------------------|----------------------------|--------------------------|
| Sample 1  | 53.00                         | 0.00                       | 47.00                         | 45.00                      | 14.30                    |
| Sample 2  | 11.70                         | 0.00                       | 0.00                          | 0.00                       | 7.28                     |
| Sample 3  | 9.80                          | 10.50                      | 0.00                          | 0.00                       | 13.00                    |
| Sample 4  | 42.10                         | 29.00                      | 8.70                          | 0.00                       | 0.00                     |
| Sample 5  | 0.00                          | 0.00                       | 11.00                         | 0.00                       | 0.00                     |
| Sample 6  | 0.00                          | 0.00                       | 0.00                          | 0.00                       | 0.00                     |
| Sample 7  | 51.00                         | 5.70                       | 43.00                         | 39.00                      | 28.00                    |
| Sample 8  | 44.00                         | 0.00                       | 0.00                          | 0.00                       | 0.00                     |
| Sample 9  | 0.00                          | 0.00                       | 6.80                          | 0.00                       | 0.00                     |
| Sample 10 | 9.00                          | 27.60                      | 56.00                         | 0.00                       | 50.00                    |
| Sample 11 | 50.00                         | 55.00                      | 9.00                          | 0.00                       | 8.70                     |
| Sample 12 | 48.00                         | 0.00                       | 0.00                          | 0.00                       | 0.00                     |
| Sample 13 | 11.70                         | 0.00                       | 0.00                          | 46.00                      | 0.00                     |
| Sample 14 | 10.00                         | 8.00                       | 0.00                          | 0.00                       | 0.00                     |
| Sample 15 | 0.00                          | 0.00                       | 0.00                          | 0.00                       | 0.00                     |

Key:

+: mildly pathogenic (>10/50mm in diameter).

++: very pathogenic (>/50mm in diameter).

- = not detected

#### Antifungal tests with plant extracts

The effect of concentrations of extracts on the fungi was significant ( $P < 0.05$ ). Colony diameter of the inhibition increased as the concentration of the extract increased as follows (100%>50%>25%). The interaction of extraction medium and concentration of extract was also significant ( $P < 0.05$ ) on the inhibition of all the five test fungi (*Rhizopus spp*, *Penicillium spp*, *Fusarium spp*, *Mucor spp* and *Aspergillus spp*). Ethanol extracts of *Allium sativum* (Garlic) gave the highest inhibitory effect of most of the fungi using the three concentration followed by *Gongronema Latifoilium*

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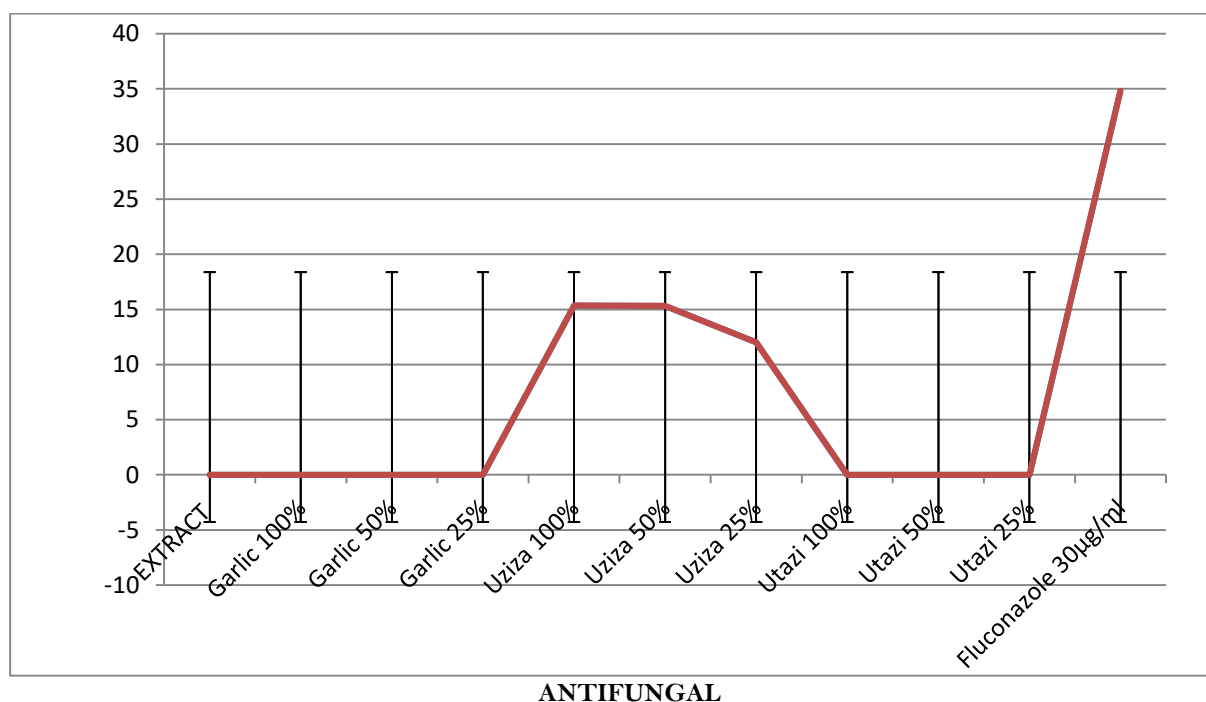
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(Utazi) while *Piper guineense* (Uziza) showed the least inhibition (Table 8). The inhibitory effect of *Allium sativum* (Garlic) was significantly ( $P < 0.05$ ) higher than that of other extracts.

Table 6: In-vitro antifungal activity of extract

| EXTRACT                | <i>Aspergillus sp</i> |           | <i>Penicillium sp</i> |         | <i>Fusarium sp</i> |            | <i>Rhizopus sp</i> |         | <i>Mucor spp</i> |         |
|------------------------|-----------------------|-----------|-----------------------|---------|--------------------|------------|--------------------|---------|------------------|---------|
| Garlic 100%            | 0.000±                | 0.00<br>0 | 9.000<br>±            | 0.86603 | 8.4000<br>±        | 0.793<br>7 | 28.1333<br>±       | 0.77675 | 0.000±           | 0.000   |
| Garlic 50%             | 0.000±                | 0.00<br>0 | 7.200<br>±            | 0.17321 | 7.5667<br>±        | 0.208<br>1 | 22.2000<br>±       | 0.26458 | 0.000±           | 0.000   |
| Garlic 25%             | 0.000±                | 0.00<br>0 | 0.00±                 | 0.00000 | 7.0000<br>±        | 0.000<br>0 | 20.8667<br>±       | 1.02632 | 0.000±           | 0.000   |
| Uziza 100%             | 15.36±                | 0.55<br>0 | 14.46<br>6±           | 0.75719 | 19.7333<br>±       | 0.832<br>6 | 28.1333<br>±       | 0.23094 | 0.000±           | 0.000   |
| Uziza 50%              | 15.333<br>±           | 0.28<br>8 | 14.10<br>0±           | 0.17321 | 18.0000<br>±       | 0.000<br>0 | 17.0000<br>±       | 0.00000 | 0.000±           | 0.000   |
| Uziza 25%              | 12.000<br>±           | 0.000     | 11.00<br>0±           | 0.00000 | 15.6667<br>±       | 0.152<br>7 | 13.8667<br>±       | 0.77675 | 0.000±           | 0.000   |
| Utazi 100%             | 0.000±                | 0.000     | 17.26<br>6±           | 0.25166 | 13.1333<br>±       | 0.416<br>3 | 0.0000±            | 0.000   | 0.000±           | 0.000   |
| Utazi 50%              | 0.000±                | 0.000     | 17.00<br>0±           | 0.00000 | 10.1333<br>±       | 0.230<br>9 | 0.0000±            | 0.000   | 0.000±           | 0.000   |
| Utazi 25%              | 0.000±                | 0.000     | 15.16<br>6±           | 0.28868 | 10.0000<br>±       | 0.000<br>0 | 0.0000±            | 0.000   | 0.000±           | 0.000   |
| Fluconazole<br>30µg/ml | 34.833<br>±           | 1.75<br>5 | 19.16<br>6±           | 0.28868 | 22.3333<br>±       | 0.577<br>3 | 41.6667<br>±       | 1.15470 | 23.6667<br>±     | 1.15470 |

\* Values in the column with the same letters are not significantly different ( $p < 0.05$ ).



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Figure 4: Means plot for In-vitro antifungal activity of extract against *Aspergillus spp*

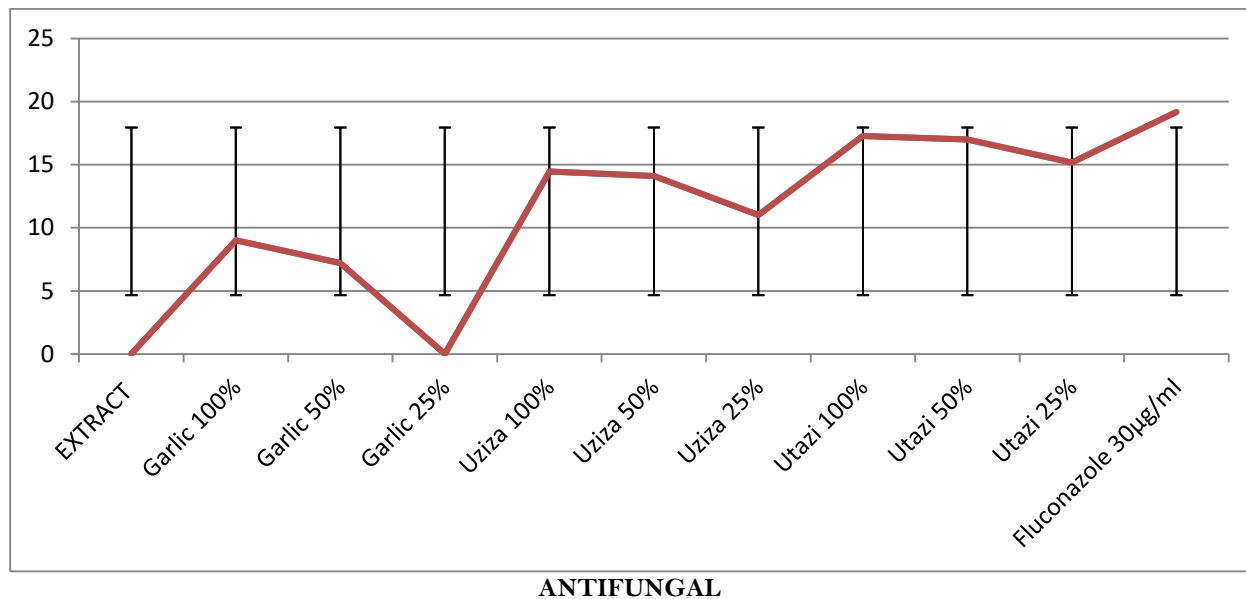


Figure 5: Means plot for In-vitro antifungal activity of extract against *Penicillium spp*

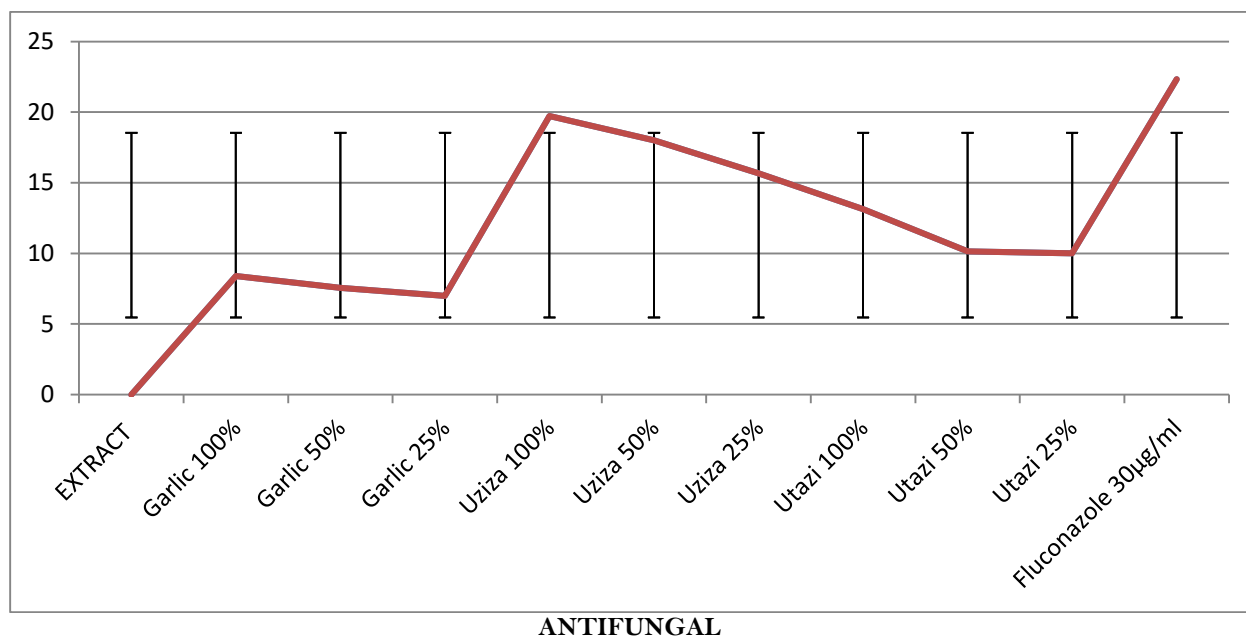
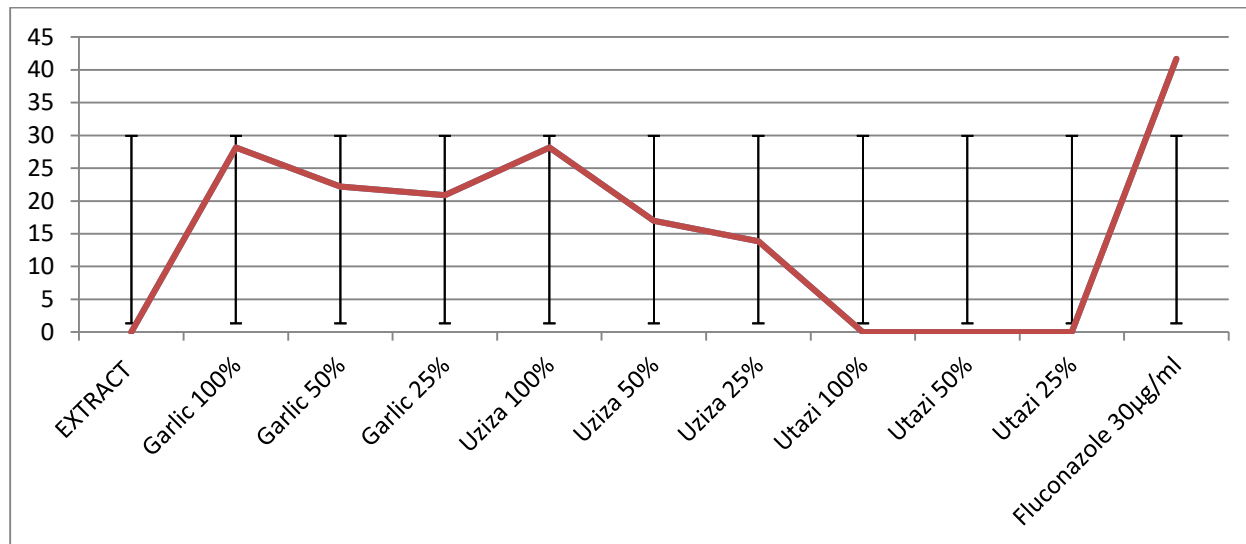


Figure 6: Means plot for In-vitro antifungal activity of extract against *Fusarium spp*

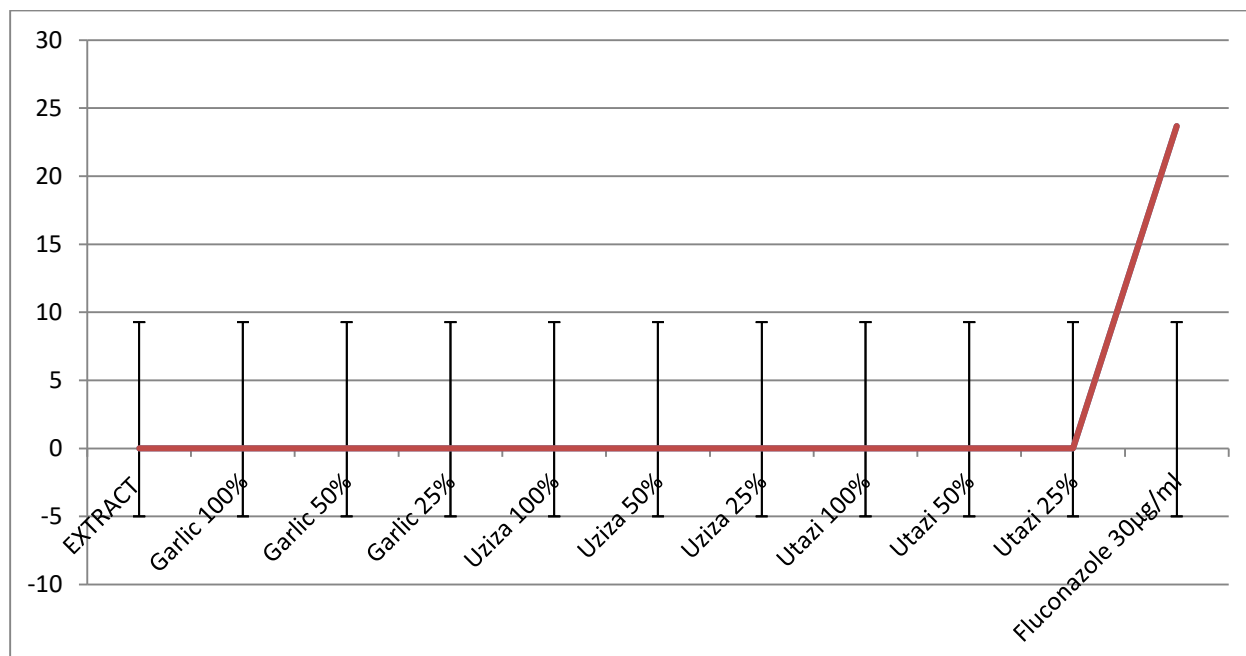
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Fig 7: Means plot for In-vitro antifungal activity of extract against *Rhizopus spp*



ANTIFUNGAL

Figure 8: Means plot for In-vitro antifungal activity of extract against *Muccor spp*  
Minimum Inhibitory Concentration

In table 9, it shows that the more each extracts efficiency is high it will have lower MIC, meaning that greater quantity of that extract will be added to the fungus before it will have inhibitory effect on it. Here the uziza seed will need higher concentration for it to inhibit fungal growth while grlic seed extracts and utazi leaf extracts need lower concentration (quantity) to kill the organisms. So among utazi leaf, uziza seed and garlic seed extracts, the extracts with more inhibitory potentials is Garlic seed followedby utazi leaf and the least is uziza seed extracts.

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Table 7: Minimum Inhibitory Concentration (Mg/MI)

| Microorganisms        | UTAZI | GARLIC | UZIZA | CONTROL |
|-----------------------|-------|--------|-------|---------|
| <i>Aspergillus sp</i> | 5.30  | 0.30   | 7.30  | 0.30    |
| <i>Penicillium sp</i> | 2.90  | 0.30   | 0.50  | 0.30    |
| <i>Fusarium sp</i>    | 0.30  | 0.50   | 0.50  | 1.00    |
| <i>Rhizopus sp</i>    | 0.30  | 0.30   | 5.30  | 0.30    |
| <i>Mucor spp</i>      | 10.20 | 10.60  | 12.50 | 0.30    |

**Quantitative and Qualitative Phytochemical**

Table 8 shows qualitative phytochemicals indicating if these parameters are present or not. Screening of all the test plants( *Allium sativum*, *Gongronema Latifoilium*, and *Piper guineense* ) for phytochemicals of interest (Saponin, Phytate, flavonoid, Alkaloid, Tannis, Oxalate and Phenol) identified that all the plants test positive to some of these phytochemicals while absent in some.

Table 8: Qualitative Phytochemical

| Parameters     | Utazi | Garlic | Uziza |
|----------------|-------|--------|-------|
| Saponin        | ++    | -      | +     |
| Flavonoid      | +++   | ++     | ++    |
| Alkaloid       | +++   | -      | -     |
| Tannin         | ++    | -      | ++    |
| Steroids       | -     | ++     | -     |
| Terpenoids     | ++    | -      | -     |
| Glycosides     | ++    | -      | +     |
| Anthracynin    | +     | -      | -     |
| Phlobotannins  | -     | -      | -     |
| Reducing Sugar | -     | -      | -     |
| Phenol         | +++   | +++    | ++    |
| Glycoside      | +++   | ++     | +     |

**Key**

- +++ = Present in high concentration
- ++ = Present in moderate concentration
- + = Slightly or sparingly present
- = Absent.

Table 9 shows the quantitative phytochemicals exact values of each of those constituents in qualitative phytochemicals present in each parameter and extracts. The quantitative test revealed that the quality of phytochemical in these plants ranged from 0.31% to 12.00% (Table 9). The highest quantitative yield of flavonoid and steroids were obtained in *G. latifolium* respectively (12.00%), steroids also yielded 12% in *P. guineense*, the least in flavonoid was *A. sativum* (4.86%). On the same hand, *A. sativum*, also recorded the highest quantitative yield of Alkaloid and saponin (1.56% and 0.66% respectively). For phenol, *P. guineense* yielded the highest with a quantitative yield of 15.77%, next to it was *G. latifolium* (3.12%) and the least in phenol was *A. sativum* with a yield of 0.4

TABLE 9: Quantitative Phytochemical

| Parameters          | Utazi | Garlic | Uziza |
|---------------------|-------|--------|-------|
| Alkaloids (%)       | 0.46  | 1.56   | 1.43  |
| Flavonoids (%)      | 12.00 | 4.86   | 5.97  |
| Phenol(Mg/G)        | 3.12  | 0.48   | 15.77 |
| Saponin (%)         | 0.31  | 0.66   | 3.06  |
| Tannin (Mg/100g)    | 0.41  | 0.55   | 11.30 |
| Glycoside (Mg/100g) | 15.70 | 7.55   | 2.65  |
| Steroids (%)        | 12.00 | 11.50  | 12.00 |
| Terpenoids (Mg/G)   | 3.12  | 3.75   | 3.81  |

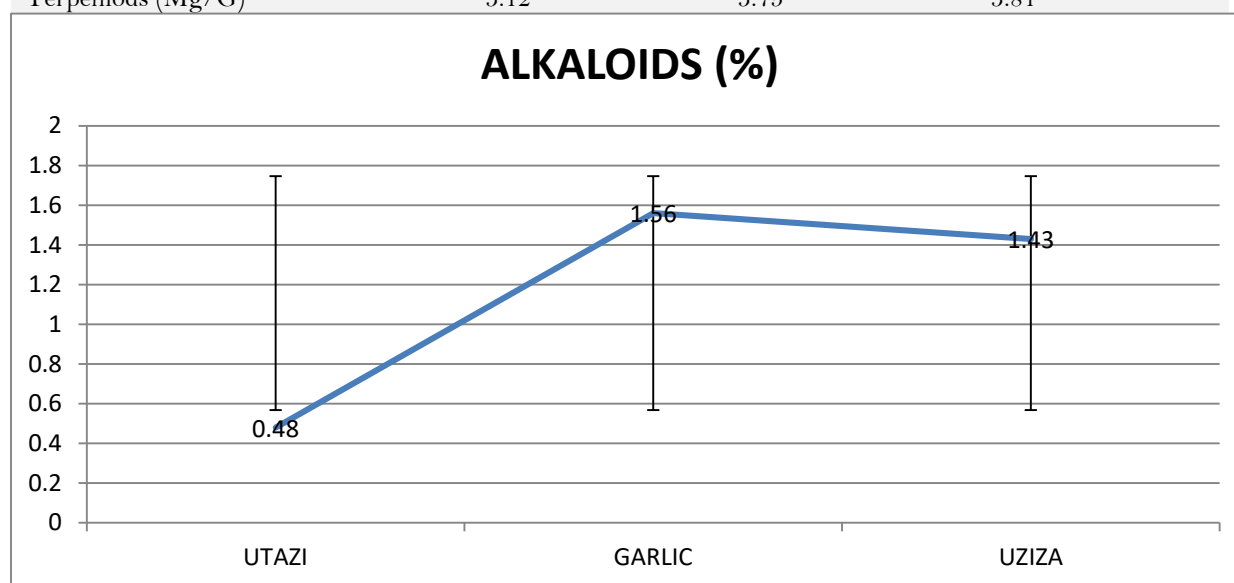


Figure 9: Means plot for alkaloid compositions of utazi, garlic and uziza.



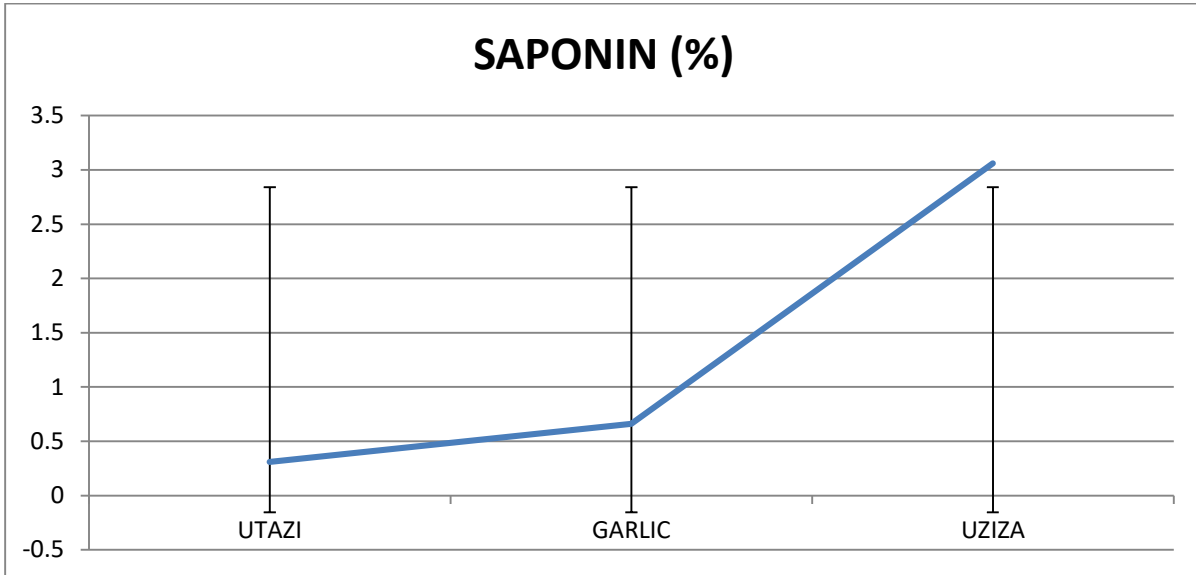


Figure 10: Means plot for saponin compositions of utazi, garlic and uziza

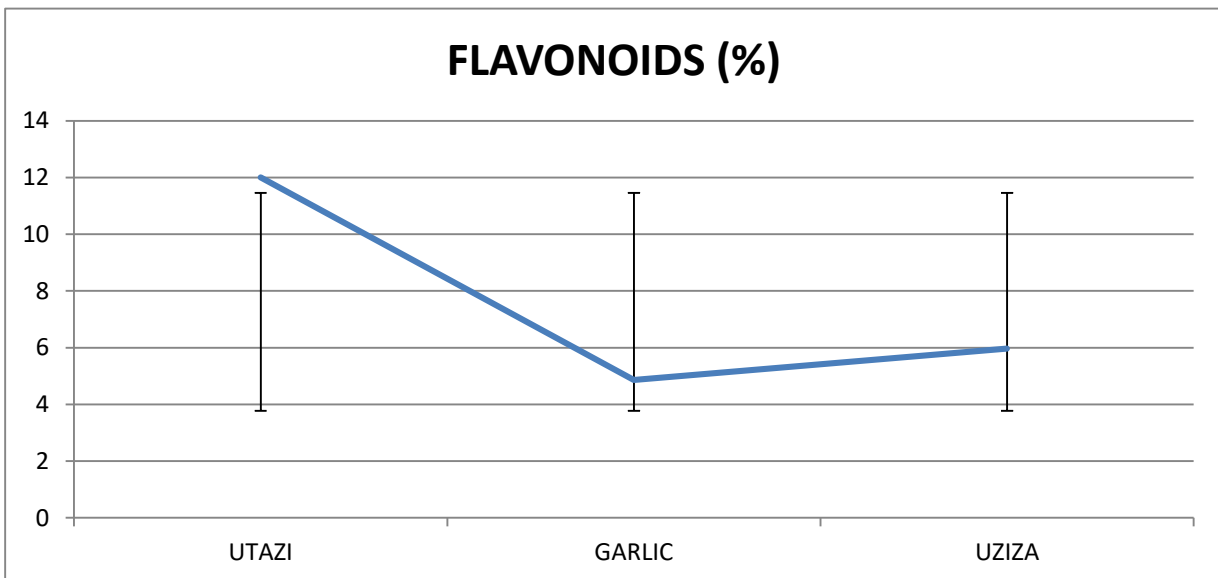


Figure 11 : Means plot for flavonoid compositions of utazi, garlic and uziza.

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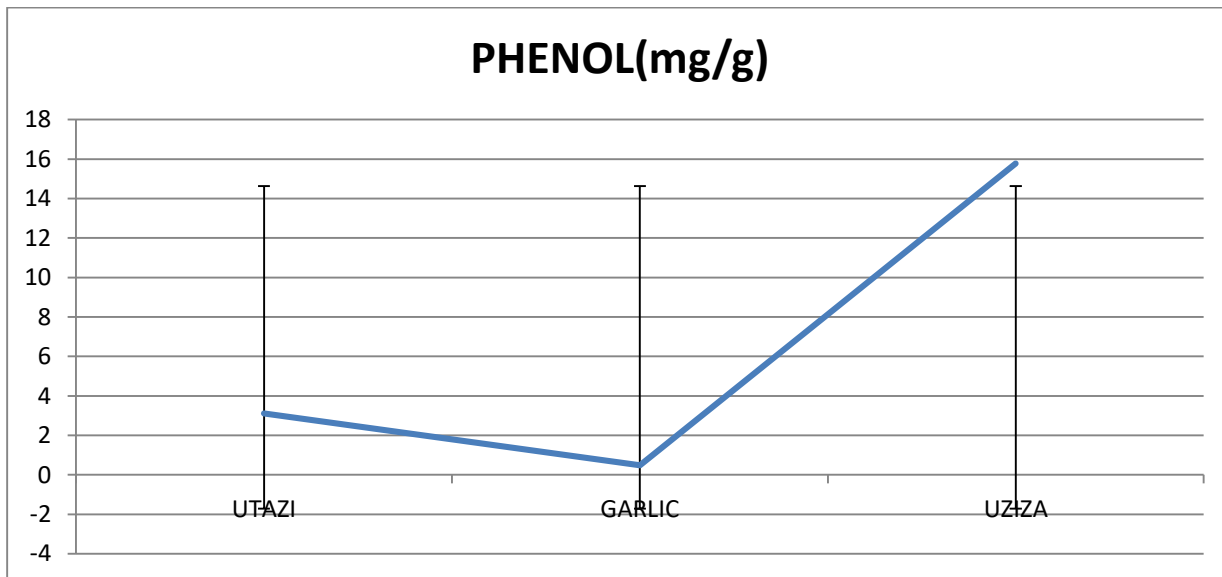


Figure 12: Means plot for phenol compositions of utazi, garlic and uziza.

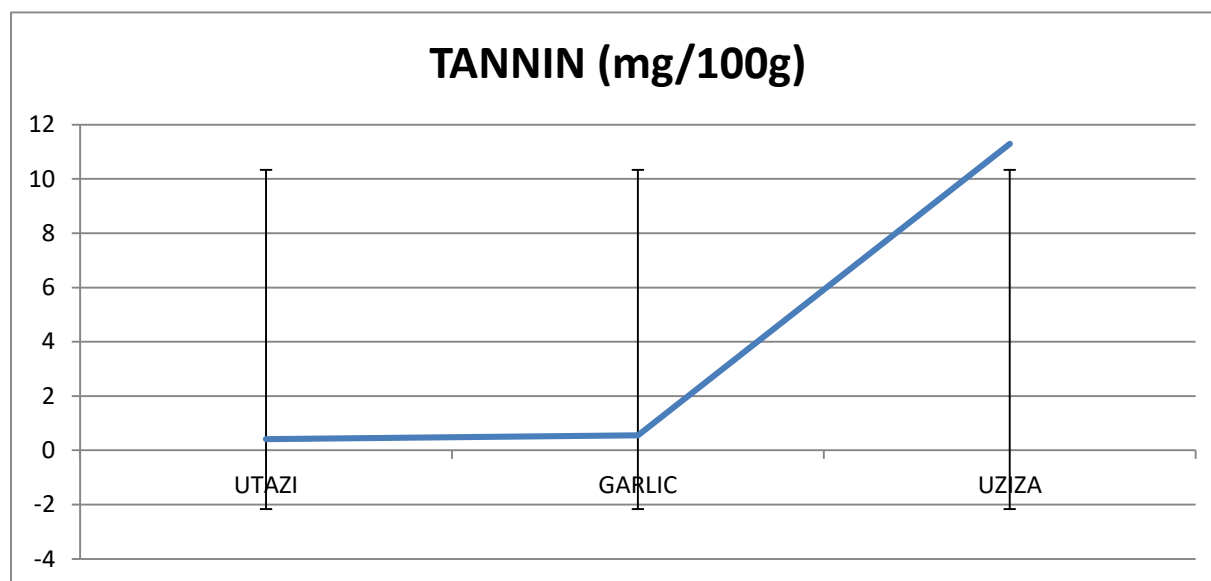


Figure 13: Means plot for tannin compositions of utazi, garlic and uziza.

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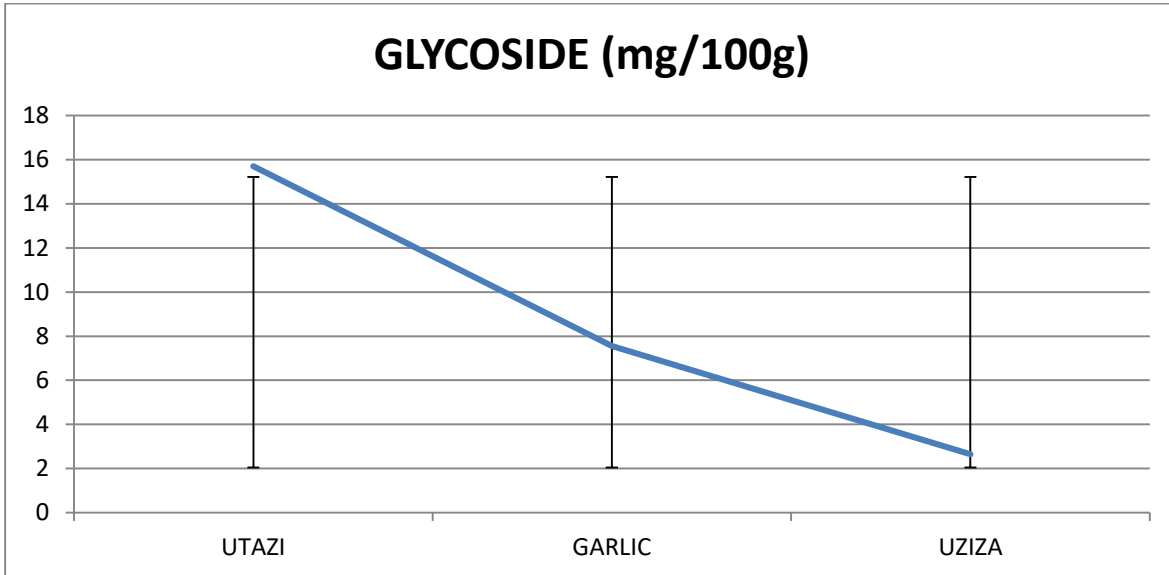


Figure 14: Means plot for glycoside compositions of utazi, garlic and uziza.

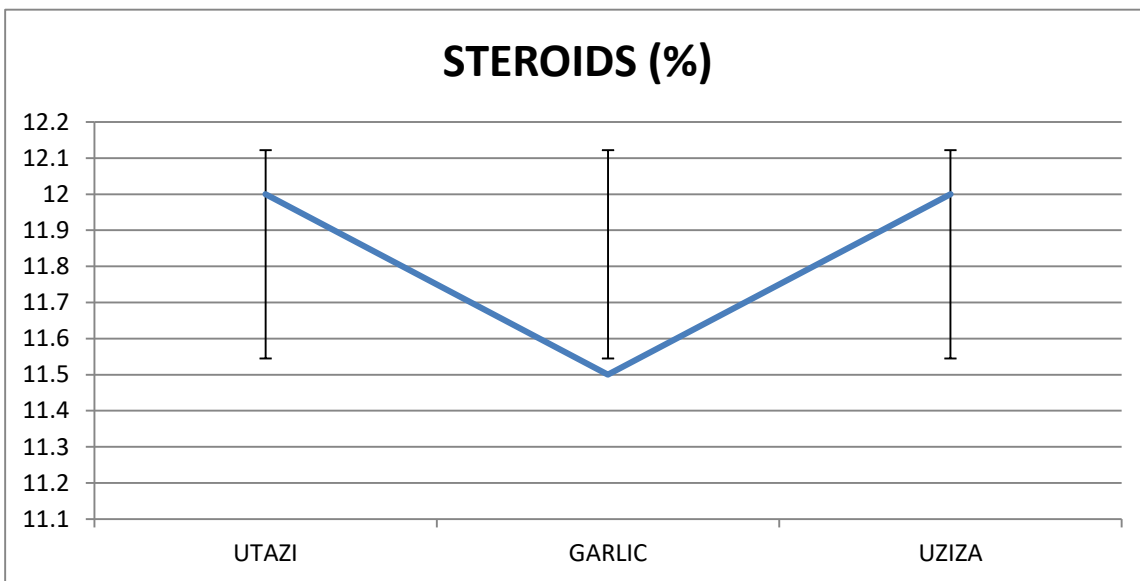


Figure 15: Means plot for steroid compositions of utazi, garlic and uziza.

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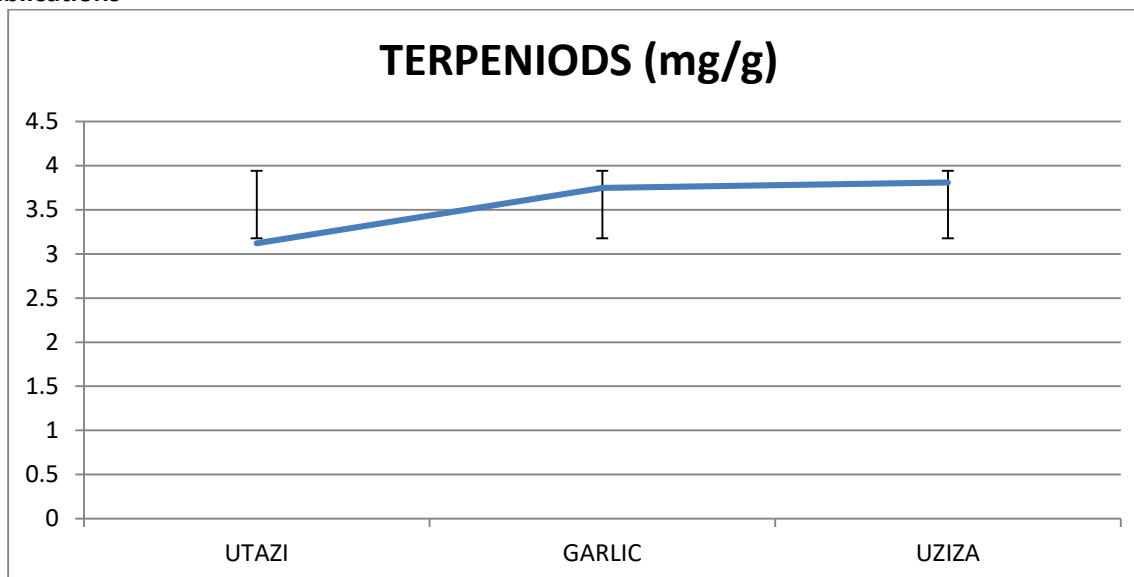


Figure 16: Means plot for terpenoid compositions of utazi, garlic and uziza.

## DISCUSSION

The organisms associated with post-harvest rot of cocoyam cormels obtained from different market within Anambra state in this study, the species responsible for the post-harvest rot of cocoyam corms were *Aspergillus niger*, *Fusarium solani*, *Penicillium citrinum*, *Rhizopus stolonifer* and *Mucor piriformis*. It has been found that these organisms significantly degrade cocoyam cormels when they are in storage [15]. Through, the pathogenicity test, it was determined that these fungi were the spoilage's main culprits. Given the danger that various fungal species, particularly in poor nations like Nigeria, might cause post-harvest rotting of roots and tubers (2016). According to [44]'s findings the spoiled cocoyam sold in certain chosen markets areas, *Aspergillus spp.* and *Rhizopus spp.* were present as agents of cocoyam spoiling. *Aspergillus niger* was identified by [33] as the most prevalent mycological flora linked to cocoyam deterioration. The results of [45] and [46], who identified *A. niger* and *R. stolonifer* from cocoyam in Nigeria, corroborated this conclusion. The results agreed with those of [47], who isolated fungal pathogens from cocoyam that had been kept and sold in the market. *A. niger* was also blamed by [48] for post-harvest deterioration in orange and lime at the field. *A. niger*, *Alternaria spp.*, *Botryodiplodia theobromae*, and *Colletotrichum gloeosporioides* were isolated from the damaged cocoyam, according to [49]. The prevalence of *A. niger*, which developed black mould on some fruits and generated strong mycotoxins called ochratoxins that can be dangerous to humans and animals, was measured instead. This outcome is consistent with many employees' reports on other root and tuber crops [50]. The probability of several infections whose combined influence may quickly destroy root and tuber crops is confirmed by the isolation of multiple pathogenic organisms from a single cormel. This is consistent with the yam reports from [51]. Most often, natural openings and wounds caused by harvesting, shipping, handling, and marketing allow fungus to enter cocoyam cormels. However, [52] reported that infections from diseased foliage, roots, or mother tubers/cormels may already be present in root and tuber crops at the time of harvest. The majority of the fungal species discovered in this investigation were quite important in contributing to food degradation. In food deterioration from a farm in Oyo state, Nigeria, [53] reported *Rhizopus nigricans*, *A. flavus*, *A. niger*, *Fusarium spp.*, and *Mucor spp.* This result agreed with the findings of [54], who claimed that *Aspergillus spp.*, *Mucor*, and *Rhizopus spp.* were to blame for the soft rots of cocoyam tubers in Nigeria. All the cocoyam samples were impacted by the fungal isolates, according to the pathogenicity tests, but further data is needed to compare how they affected the sugar and nutritional content of the crop. There is a need for proper training for the vendors on using hygienic ways of storing cocoyam because, according to the study's findings, some fungi were isolated and various literature revealed that the fungal species isolated were known to produce toxins that could cause severe food poisoning and other potential health hazards.

This study found that the plants ginger, uziza, and garlic contained fungitoxic compounds that were able to stop the growth of test fungi. This finding is consistent with earlier reports of numerous studies that focused on different fungi [55], and the plant extracts used may be used to protect mechanically damaged cocoyam corms/cormels from rot fungi. The effectiveness of the extracts varied depending on the plant material, concentration, extraction solvent,

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and individual test fungus, though. Since ethanol extracts were more effective than aqueous extracts, the water used in the extraction procedure presumably was unable to completely dissolve the active ingredients found in plants, present in ethanol extracts. According to [56] research on garlic, the higher yield from the ethanol extract was due to the fact that ethanol is an organic solvent and would dissolve organic molecules better than other solvents, freeing the active components (phytochemicals) needed for antifungal action. The varying susceptibilities of each test isolate to various quantities of the extracts may also contribute to the variation in the fungitoxic between the extraction medium; this also accords with some researchers' findings [57]. This contrasts with [50], who observed a moderately effective inhibition by *A. sativum*, but is comparable to the results obtained by [5] on Potato rot of post harvest rot of Potato, who reported a very effective suppression with *A. sativum* and utazi respectively. The radial mycelia growth of the studied fungus was significantly and effectively inhibited by commercial fungicides (flucozanol) (79.10–100%), whereas *A. niger* exhibited the highest level of inhibition by antifungal medication. Regarding concentration, the fungitoxic impact of each plant extract followed a similar pattern. On all test species, 100% extract concentration was shown to be the most fungitoxic, followed by 50% extract concentration, while 25% extract concentration had the least inhibitory impact. This supports [58] findings that there was a significant difference in the mycelia growth value recorded on different plant extract concentrations. It also suggests that there may be variations in the solvent-soluble antifungal component in the corresponding leaf extracts, according to [59] and [60]. This explains why the plant extracts used in this study demonstrated antifungal activity, as the presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi, and pests [60]. The antifungal properties of these plant extracts are therefore likely due to the presence of phytochemicals, which are anti microbial agents [61], that are inhibitory to the growth of these pathogens [61]. Plant phytochemical screening revealed that all of the phytochemicals tested were positive (Alkaloid, Flavonoid, Phytate, Saponin, Tannins, Oxalate and Phenols). All of these phytochemicals have been shown to have medicinal and pharmacological potential by the reports of several researchers [61].

Minimal Inhibitory Concentrations (MIC) for ethanolic extracts of uziza, garlic, and ginger have been reported. The three samples that were examined all shown fungistatic and fungicidal action. The MIC values were in the range of 0.30 and 7.0 g mL<sup>-1</sup>. The uziza extract's lowest fungicidal concentration showed to have the strongest fungicidal activity against all five isolated fungi, as shown by the low value (Table 7). Our findings support [62], who hypothesised that each material solvent system exhibits a unique behaviour based on the chemical properties of the solvent, the extraction technique employed, and various structural and compositional elements of the natural products. Changes in the degree of activity are found to be caused by variations in the polarity of the solvents used to dissolve the active plant components. This result is consistent with the findings of [63], who also noted that antimicrobial drugs at higher doses exhibited greater growth inhibition. Additionally, the antibacterial properties of plant extracts may result from the synergistic effects of multiple minor chemicals found in plants rather than the action of a single active molecule [64]. These findings suggest that the type of plant employed and the extract's properties determine the proper extract concentration to exhibit a certain effect. This highlights the need of doing research to isolate, identify, and characterise the biomolecules in order to determine the compound(s) responsible for the inhibitory effect. To identify the chemical composition of the bioactive substances responsible for the reported antifungal action, more research is required. Natural fungicides generated from plants may provide novel, alternative active substances, particularly those with antifungal activity.

The results of this study also showed the presence of fungitoxic compounds in garlic, uziza, and utazi, which were able to inhibit the growth of the test fungi. This finding is consistent with earlier reports of numerous studies on various fungal organisms [65], and the plant extracts used may be used to protect mechanically damaged cocoyam corms/cormels from rot fungi. The effectiveness of the extracts varied depending on the plant material, concentration, extraction solvent, and individual test fungus, though. Since ethanol extracts were more effective than aqueous extracts, the water used in the extraction procedure presumably was unable to completely dissolve the active ingredients found in plants, which were seen in ethanol extracts. In line with reports by [66] on garlic, who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, liberating the active compounds (phytochemicals) necessary for antifungal activity, the ethanol extract produced higher yield in all of the plants.

The results of the phytochemical screening of garlic extract showed the absence of tannin, terpenoids, and glycosides but the presence of saponin, flavonoids, alkaloids, steroids, and phenol. This outcome is consistent with reports of the presence of alkaloids, saponin, flavonoids, steroids, and phenol from [67]. This study supports the findings of [68] study, which found that garlic extracts included alkaloids, steroids, flavonoids, saponins, and flavonoids. According to [69], the presence of saponin, flavonoid, and terpenoid, which were also discovered and identified in this study, gives garlic extract its antifungal properties. As a result of the phytochemical features of garlic extracts,

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which indicated the presence of saponins, flavonoids, and alkaloids, the results of this study also agree with the findings of [70]. [71], concur with this finding of alkaloids, saponins, and flavonoids in garlic extract. According to [72], garlic also contains saponin and flavonoids. [73], revealed a similar outcome and the presence of flavonoid and saponin in garlic, which also supports the findings of the current investigation. However, this presence result showed that tannin was lacking, which is in contrast to the research conducted by [73]. [74], on the other hand, disagree with this conclusion since tannin was present in their findings. Garlic doesn't contain any tannin, which might be a result of the experiment's location. This study also found that garlic extract contained phenol and alkaloids, contradicting findings from a study by [75]. Our research indicates that alkaloid and flavonoid are present in garlic extract, contrary to [75] assertion that they are missing. The findings of this study on the photochemical characteristics of garlic corroborated those of [76], who discovered that the presence of saponin, tannin, flavonoid, and terpenoid in garlic extract gave it action against both bacteria and fungi.

The result of photochemical properties of utazi extract showed the presence of Alkaloid at  $0.41 \pm 0.20$ , flavonoid  $12.00 \pm 0.57$ , phenol  $3.12 \pm 0.15$ , saponin  $0.31 \pm 0.15$  Tannin  $0.41 \pm 0.20$  glycoside  $15.70 \pm 1.30$  steroids  $12.00 \pm 1.00$ , Terperoids  $3.12 \pm 0.50$  which corresponds with the findings of Ojinnaka *et al.*, (2018) who reported the presence of Alkaloid, flavonoid, phenol, tannin, and saponin. This outcome is also consistent with [77] findings since utazi extract included flavonoid, alkaloids, phenol, tannin, and saponin. The results concur with those of [78], who reported that *G. latifolium* extract included flavonoids, alkaloids, phenol, tannin, and saponin. A research by [79] found that *G. latifolium* extract included the alkaloid saponin, flavonoid phenols, and glycosides, but it did not find the tannin that was found in this study. The variation can be brought on by the different locations where the findings were acquired. A research by [78] found that *G. latifolium* extract included saponin, tannins, phenol, flavonoids, and alkaloids, which is entirely consistent with the results of the current study. Contrary to the findings of this investigation, [79] analysis of *G. latifolium* failed to find any alkaloids or glycosides. This study also found tannin in *G. latifolium* extract, which is inconsistent with [80] findings, which claimed there was no tannin in *G. latifolium*. Analysis of the uziza extract similarly found the absence of alkaloids but the presence of flavonoids, saponin, tannin, steroids, glycoside, and phenol. This outcome is consistent with the study of [81], which found flavonoid, steroid, glycoside, and saponin presence. As opposed to [82], who reported the presence of alkaloid qualities in the *P. guineense* extract, our investigation revealed the absence of alkaloid. The presence of tannin is also demonstrated in this investigation, which contradicts the findings of [83]. This study's lack of alkaloid and terpenoid conflicts with that of [84], who found that alkaloid and terpenoid were present in *P. guineense* extract. As the *P. guineense* demonstrated the presence of tannin, flavonoids, glycosides, saponin, and phenol, this investigation is consistent with the study conducted by [85]. The findings of the research by [86], which corroborated Ogbuagu's assertion of the presence of alkaloid in *P. guineense*, This is comparable to [87] findings on yam rot and [88]'s findings on post-harvest yam rot, which both revealed a very efficient inhibition using *A. sativum* and *P. guineense* respectively, It contrasts with [89]'s findings, which showed a somewhat effective inhibition by *A. sativum*. The radial mycelia growth of the fungus studied was significantly inhibited by commercial fungicides (flucozanol) (79.10–100%), whereas *A. niger* showed the highest percentage of inhibition with antifungal medication. Regarding concentration, there was a consistent pattern in the fungitoxic activity of all the plant extracts. On all the test species, 100% extract concentration was the most fungitoxic, followed by 50% extract concentration, and 25% extract concentration had the least inhibitory impact. This supports [86] findings that there is a substantial variation in the mycelia growth values reported on the various plant extract concentrations. This shows that, as observed by Iwu, there are variations in the solvent soluble antifungal component in the corresponding leaf extracts (2017). This explains why the plant extracts used in this study demonstrated antifungal activity, as the presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi, and pests [88]. The antifungal properties of these plant extracts are therefore likely due to the presence of phytochemicals, which are anti-microbial agents that are inhibitory to the growth of these pathogens [90]. All of the phytochemicals examined (Alkaloid) were positive after the plants underwent phytochemical screening. Oxalate, Phenols, Tannins, Phytate, and Saponin). All of these phytochemicals have been shown to have medicinal and pharmacological potential by the reports of several researchers [88].

## CONCLUSION

This study have revealed the potentials of botanicals (*Utazi, uziza and garlic*) in the control of cocoyam rot in storage, with *uziza* exhibiting the most fungitoxic activity, this study also depicted that ethanol extracts demonstrated a higher antifungal activity over aqueous extract, indicating that ethanol extract of *uziza* could be an alternative or complimentary to synthetic chemicals in controlling cocoyam rot, where *uziza* leaves are not available, *utazi and garlic* can also be used as a second option because they exhibit a moderate fungitoxic activity on the test organisms.

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However, the result of this study has gone a long way in providing better alternative to the over dependence on synthetic fungicides, the use of plant extracts in controlling rot causing organisms and pests could reduce over reliance on one source of agricultural chemicals to the farmers, that are reported to predicate long term harmful consequences on environment, Man and wildlife, as well as reduce production cost, hence the antimicrobial activity of the extracts was comparable to those of the antibiotics, the demonstration of activity against the test fungi produces scientific bases for the local usage of these plants in controlling microbial rot, since these plants are locally available, less expensive, environment friendly with easy extraction method, it can be confidently exploited in the control of cocoyam rot.

## RECOMMENDATION

Therefore, the cogent data on the antimicrobial potentials of plant extracts deserve multi-institutional attention as early as possible. The prospects of relatively cheaper means of controlling rot inducing organisms could then be brighter, particularly for the numerous peasant farmers across the globe and in Nigeria in particulars. The challenges of cocoyam as a result of post harvest rot is readily obvious and as such requires urgent attention if cocoyam the third ranked root and tuber crops of economic importance after yam and cassava in Nigeria and the queen consort of yam in South-eastern Nigeria must not be pushed further down the ladder to the status of a minor vanishing crop. With respect to the plants used, further pharmacological evaluation, toxicological studies and possible isolation of the therapeutic antifungal from these plants are the future challenges, hence it is recommended that further investigations should be done on the chemical nature of the active principles of the plants, also further investigations can combine the plant extracts for possible synergistic effect, further research involving invivo assay would be needed to investigate the fungistatic effects of these botanicals on the fungal inducing rot of cocoyam corms and cormels that are not included among the test fungi in this research work. Also, very essential is the need to devise good storage facilities to prolong the shelf life of cocoyam after harvest. However, the future of cocoyam an age old crop in Nigeria is tied to the solutions to the above problems, to enlarge the potential uses of the crops as an incentive to increased production. This is the only path to greater expectations of substantial contribution of cocoyam to food supply and thereby to the economy of Nigeria. In short run and at the existing level of technology, cocoyam production/availability can be increased by merely containing the 40% or more preand post harvest spoilage (rot) losses.

## REFERENCES

- Oloruntoba, D. (2019). Production Efficiency in Yam Based Enterprises in Ekiti State, Nigeria. *Central European Agriculture Journal*; 7(4):627-636
- FAO, (2016). Root and Tuber Storage in: Prevention of Post-Harvest Food Losses: A Training Manual Italy: FAO, 93-95.
- Chandrasekara, D. and Kumar, T. (2016). Encyclopedia of Foods. Vol. 1., Humane Press, Washington, pp: 526
- Zhu, Beibei., Zou, Li., Qi, Lu., Zhong, Rong., and Miao, Xiaoping. (2014). *Allium* vegetables and garlic supplements do not reduce risk of colorectal cancer, base on meta-analysis of prospective studies. *Clinical Gastroenterology and Hepatology*. 12(12):1991-2001.
- Chukwuka, K. S., Okonko I. O. and Adekunle, A. A. (2020). Microbial ecology of organisms causing Pawpaw (*Carica papaya* L.) fruit decay in Oyo State, Nigeria. *Am.-Eurasian Journal of Toxicology Science*. 2: 43-50.
- FAO. (2021). Growing Taro in Asia and the Pacific; Food and Agricultural Organization of the United Nations (FAO): Rome, Italy, 2021 Available online: [http://ebooks.lib.ntu.edu.tw/1\\_file/FAO/67652/ac450e00.pdf](http://ebooks.lib.ntu.edu.tw/1_file/FAO/67652/ac450e00.pdf) (accessed on 15 February 2021).
- Memudu, A.E; Akinrinade, I.D; Ogundele, O.M and Dare, B.J (2015). Effects of crude extract of Dry fruits of *Piper guineense* on male fertility parameters of Adult Sprague Dawley rats. *European Journal of Medicinal plants*. 5 (3): 297 – 303.
- Obafemi, M. O., Alamu E. O. and Omitogun, L. A. (2020). Effect of processing on the nutritional contents of *Gongronema latifolium* *Journal of Applied Biosciences*. 46: 3086- 3092.
- Opata P.I and Ogbonna P.E. (2015). Storage profitability and effectiveness of storage methods in yield loss reduction in cocoyam in southeast Nigeria. *African Journal of Agricultural Research*. 10(49):496–504.
- Igbozulike, A. O. (2015). Evaluation of cocoyam corms, processing and storage in Nigeria. *Journal of Scientific and Engineering Research* 6 (7) 260 -263.
- Ijioma, J.C, Effiong J.B, Ogbonna, M.O and Onwuamaoka, E.A. (2014). Determinants of adoption of selected NRCRI cocoyam technologies among farmers in Umuahia South Local Government Area of Abia State, Nigeria. *American International Journal of Contemporary Research*.; 4:6-8.
- National Root Crops Research Institute (NRCRI) Umudike. (2015) Cocoyam Program. Accessed 13 Feb 2015.

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## Publications

13. Anukworji, C.A, Ramesh, R.P; and Okigbo, R.N. (2012). Isolation of fungi causing rot of cocoyam (*Colocasia esculenta* (L.) Schott) and control with plant extracts: (*Allium sativum*, L., *Garcinia kola*, heckel., *Azadirachta indica*, L. and *Carica papaya*. *Global Advance Research Journal of Agricultural Science*. 1(1):202–17.
14. Tao, J.; Li, Y.; Li, S. and Li, H.B. (2018). Plant foods for prevention and management of colon cancer. *Journal of Functional Foods*. 42:95–110.
15. Okigbo, R. N. and Igwe, D. I. (2007). Antimicrobial effects of *P.guineense* "Uziza" and phylanthrusamarus on *Candida albican* and *Streptococcus faecalis*. *Acta microbiologica et immunologica Hungarica*. 54(4):353-366.
16. Onuh, J.O., Shiriki, D., Ubwa, S.T. and Shambe, T. (2015). Isolation of Six Microorganisms from Rotten *Dioscorea alata* (Water Yam), and Antimicrobial Sensitivity Test with Nine Plant Extracts. *Food and Nutrition Sciences*, 6: 1381-1394.
17. Okpala, B. (2015). Benefits of Gongronema latifolium (utazi). Global food book. Pp 51-62.
18. Nasir, B., Fatima, H., Ahmed, M., Haq, I.U., 2015. Recent trends and meth-ods in antimicrobial drug discovery from plant sources, *Journal of Microbiology*. 1:10-12.
19. Efiuwewwere, B.J. and Amadi, L.O. (2015). Effects of Preservatives on the Bacteriological, chemical and sensory qualities of mangrove oyster (*Crassostrea gasar*) harvested from the Niger Delta Region, Nigeria. *British Journal of Applied Science and Technology*, 5(1):76-84.
20. Ali, A., Hamiduddin, N and Zaigham, M. (2017). Shubb-E-Yamani (Alum) A unque drug and its utilization in Unani Medicine: A physicochemical and pharmacological review. *International Journal of Research in Pharmacy*. 8(2):17-22.
21. Katzer and Gernot (2015). Gernot Katzer Spice pages 70-112.
22. Okonkwo C and Ogu A (2014). Nutritional evaluation of some selected spices commonly used in South Eastern part of Nigeria. *Journal of Biology, Agriculture and healthcare*.4(15): 45 -51.
23. Chibuzor, O and Assumpta, O (2014). Nutritional Evaluation of some selected spices commonly used in the south-eastern part of Nigeria. *Journal of Biology and Agriculture*. 4 (5): 56-60.
24. Mailafia, S., Okoh, G. R. Hamza, O. Olabode K. and Osanupin, R. (2017). Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Vet. World*, 10: 393-397.
25. Sara,H.F; Jens, S; and Anna,K.J (2015). Medicinal plants used as excipients in the history in Ghanaian herbalmedicine. *Journal of Ethnopharmacy*. 174:56-58.
26. Zhu, Beibei., Zou, Li., Qi, Lu., Zhong, Rong., and Miao, Xiaoping. (2014). *Allium* vegetables and garlic supplements do not reduce risk of colorectal cancer, base on meta-analysis of prospective studies. *Clinical Gastroenterology and Hepatology*. 12(12):1991-2001.
27. Guercio, Valentine., Turati, Federica., La Vecchia, Carlo., Galeone, Carlotta., and Tavani, Alessandra. (2016). *Allium* Vegetables And Upper Asrodigestive Tract Cancers. A Meta- analysis of observational Studies. *Molecular Nutrition and Food Research* . 60(1):212-222.
28. Chinwendu, S., Ejike, E.N., Ejike, Bu., Oti, W. & Nwachukwu, I. (2016) phytochemical properties of uziza (*piper guineense*).
29. Akueshi, C. O., Kadir, C. O., Akueshi, E. U., Agina, S. E. and Ngurukwem, C. (2020). Antimicrobial potential of *Hytissau veolus poit (lam inaceac)* *Nigerian Journal of Botany* 15:37-41.
30. Onuh, J.O., Shiriki, D., Ubwa, S.T. and Shambe, T. (2015). Isolation of Six Microorganisms from Rotten *Dioscorea alata* (Water Yam), and Antimicrobial Sensitivity Test with Nine Plant Extracts. *Food and Nutrition Sciences*, 6: 1381-1394.
31. Dhawale, S. and LaMaster,A. (2003).*Microbiology laboratorymanual*. The mcHillcompany.inc.USA (5):209-229.
32. Chinwendu, S., Ejike, E.N., Ejike, Bu., Oti, W. & Nwachukwu, I. (2016) phytochemical properties of uziza (*piper guineense*).
33. Marthur S.B. and O. Kongsdal (2013). Common Laboratory Seed Health Testing Methods for Detecting Fungi, 2nd Edition. International Seed Testing Association. Switzerland
34. De Hoog, J. A., Hodge, N. C. Perez, Y. and Concelmo, D. (2020). Two different gram positive bacteria cause a decay of tomato that is similar to sour rot. *Pytopathology*, 85: 1123-1123.
35. Nwachukwu, E.O. and Osuji, J.O. (2018). Evaluation of plant extracts for antifungal activity against *Sclerotium rolfsii* causing cocoyam argel rot in storage. Department of Plant Science & Biotechnology, University of Port Harcourt. *Research Journal of Agriculture and Biological Sciences*, 4(6): 784-786.
36. Okigbo, R.N., and Mmeka, E.C. (2008). Antimicrobial effects of three tropical plant extracts on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *African Journal of Traditional Complement and Alternative Medicine*. 5:226-229.
37. Clinical and Laboratory Standards Institute (CLSI) Performance Standard for Antimicrobial Susceptibility Testing. (2018). 28<sup>th</sup> edition Clinical and Laboratory Standards Institute, Wayne, PA, USA. (CLSI) Supplement M100.

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## Publications

38. Harborne, J.B. (1995). *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis* (Third Edition). Chapman and Hall, New York. Pp 674-681.
39. Banso, A. and Adeyemo, S. (2019). Phytochemical screening and antimalarial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. *Biochemistri*, 18, 39-44.
40. Boham, A. B and Kocipai, A.C. (2014). Flavonoid and condensed tannins from Leaves of Hawaiian vaccinium vaticulum and vicalycinium. *Pacific Science* 48:458-463.
41. Harborne, J.B. (1995). *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis* (Third Edition). Chapman and Hall, New York. Pp 674-681.
42. Joshi, A., Bhohe, M. and Saatarkar, A. (2013). Phytochemical investigation of the roots of *Grewia microcos*. *Journal of Chemistry and Pharmacy Research*, 5, 80-87.
43. Association of Analytical Chemists (2010). *Official methods of analysis of AOAC International* 18<sup>th</sup> edition. Gaithersburg, MD., USA: AOAC International. ISBN-13:9780935584752.
44. Ewekeye, T.S., Oke, O. A., Quadri, A. I., Isikal, A. O., Umenwaniri M. O. and Durosinmi, M. L. (2017). Studies on post-harvest deterioration of some fruits and vegetables in selected markets in Lagos State, Nigeria. *American Journal of Research Community*. 1: 209-223.
45. Baiyewu, R. A., Amusa, N. A. Ayoola, O. A. and Babalola, O. O. (2020). Survey of the postharvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya* L.) in South Western Nigeria. *African Journal Biotechnology*, 6: 178-181.
46. Chayty, D. Amienyo, C. A. and Ataga, A. E (2017). Use of indigenous Plant extracts for the production of mechanically injured sweet potato (*Ipomoeabatatas* (L) Lam) tubers. *Academy Journal. Science Reserve Essay*.2:167-170.
47. Gadgile, D. P. and Chavan, A. M. (2019). Impact of temperature and relative humidity on development of *Aspergillus Flavus* rot of mango fruit. *Journal of Science and Technology*. 3: 48-49.
48. Bali, R. V., Bindu, M. G. Chenga, R. V. and Reddy, K. (2018). Post-harvest fungal spoilage in sweet orange (*Citrus sinensis*) and acid lime (*Citrus aurentifolia* Swingla) at different stages of marketing. *Agricultural Science Digest*. 28: 265-267.
49. Okereke, V. C, Godwin-Egein M. I. and Arinze, A. E. (2021) Assessment of Postharvest Rot of Mango at Different Stages of Market in Port Harcourt, Nigeria. *International Journal of Current Research*. 11: 6-10.
50. Okigbo, R.N. and Odurukwe, C.N. (2009). Occurance and control of fungal rot pathogens of yam (*Dioscorea* spp) with leaf extracts of *Chromolena odorata*, *Carica papaya* and *Aspilia Africana*. *Nigerian Journal of Mycology*. 2(1): 154-165
51. Okigbo, R. N., Anyaegbu, C. F and Dalafi, T. P. (2020). Pollution from Spent Engine Oil with their Bioremediation Potentials In Auto-Mobile Workshops in Awka, Anambra State, South Eastern Nigeria. *Nigerian Journal of Biomedical Engineering*. 13:39 - 44
52. Okigbo, R.N., and Mmeka, E.C. (2008). Antimicrobial effects of three tropical plant extracts on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *African Journal of Traditional Complement and Alternative Medicine*. 5:226-229.
53. Okigbo, R.N, Ezebo, R.O and Ugwu, S.C. (2018). Antifungal attributes of extracts of *Ocimum gratissimum*, *Zingiber officinale* and *Cymbopogon citrates* on rot fungi of soursop fruit. *Clinical Journal of Nutrition and Dietetics*. 1(1): 1-7.
54. Bukar, L. L., Agbor-Egbe, T. and Mbofung, C. M. F. (2019). Anti-nutritional factors changes occurring in trifoliate yam (*Discorea dumetorum*) tubers after harvest *Journal of Food Chemistry* 102:716 - 720.
55. Okigbo, R.N and Nwatu, C.M. (2015). Ethnostudy and Usage of Edible and Medicinal mushroom in some parts of Anambra state. *Nature Resources*. 6(1): 79-89
56. Okigbo, R.N and Ezebo, R.O. (2017). Effect of leaf extracts of *Cymbopogon citrates*, *Chromolaena odorata* and *Nerbouldia laevis* on *Dioscorea alata* rot. *Integrative food, Nutrition and Metabolism*. 5(1): 1-7.
57. Okigbo, R.N., Anuagasi, C.L., and Amadi, J.E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plants Research*, 3:86-95.
58. Suleman M.M (2010) Fungitoxic activity of neem and paw-paw leaves extracts on alternaria solani causal organism of yam rots. *Adu. Environ. Biol*. 4(2):159-161
59. Iwu, M. M. (2017). Dietary plants and masticatories as sources of biologically active substances. In, 4th OAU/ STRC INTERAFRICAN symposium on *traditional pharmacopoeia and African medicinal plants*. Abuja-Nigeria.;70 & 379
60. Owusu-Darko, P. G., Peterson, A. and Omenyo, L. E. (2014). Cocoyam (corms and cormels) an underexploited food reserve. *Journal of Agricultural Chemistry and Environment* 3 (1): 22-29.
61. Okigbo, R. N. and Igwe, D. I. (2007). Antimicrobial effects of *P.guineense* "Uziza" and phylanthrusamarus on *Candida albican* and *Streptococcus faecalis*. *Acta microbiologica et immunologica Hungarica*. 54(4):353-366.
62. Park, B.M.; Cha, S.A.; Kim, H.Y.; Kang, D.K.; Yuan, K.; Chun, H.; Chae, S.W. and Kim, S.H. (2016). Fermented garlic extract decreases blood pressure through nitrite and sGC-cGMP-PKG pathway in spontaneously hypertensive rats. *Journal of Food Science*. 22:156-165.

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63. Banso, A. and Adeyemo, S. (2019). Phytochemical screening and antimalarial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistri*, 18, 39–44.
64. Davicino R., Anesini, C. and Saatarkar, A. (2017): Antioxidant and Antimicrobial Activities of LarreaDivaricataCav. Aqueous Extract on Vitamin C from Natural Orange Juice, *Food and Nutrition Sciences*. 2.35-46.
65. Okigbo, R.N and Anyagebu, C.F. (2021). Underutilized plants of Africa. *Journalof Biology and Nature*. 13(2):34-49.
66. Okonkwo C and Ogu A (2014). Nutritional evaluation of some selected spices commonly used in South Eastern part of Nigeria. *Journal of Biololgy, Agriculture and healthcare*.4(15): 45 -51.
67. Akinmusire, O. O. (2021). Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. *Advance Environmental Biology*. 5: 157-161.
68. Ali, T., Awa, E. and Eleazu, C. (2019) Bioactive constituents and antioxidant activities of raw and processed cocoyam. *Journal of Nutra Foods*. 14: 133-140.
69. Tao, J.; Li, Y.; Li, S. and Li, H.B. (2018). Plant foods for prevention and management of colon cancer. *Journal of Functional Foods*. 42:95–110.
70. Droby, S., (2016). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horti.*, 709: 45-51.
71. Bnyan, I.A., Alta'ee, A.H., and Kadhum, N. H. (2018). Antibacterial Activity of AluminumPotassium Sulphate and Syzygium Aromaticum extract against Pathogenic microorganisms. *Journal Natural Sciences Research*. 4(15):11-14.
72. Ejimofor, C. F and Oledibe, O. J (2022). Fungi associated with spoilage of pineapple (*Ananas comosus L*) fruit sold in Anambra State, Nigeria. *Asian Journal of Advances in Research* 16(2): 17-22.
73. Ayoola, G. A., Coker, H. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C. and Atangbayila T. O. (2018). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Tropical Journal Pharmacology Research*. 7: 1019–1024.
74. Ali, T., Awa, E. and Eleazu, C. (2019) Bioactive constituents and antioxidant activities of raw and processed cocoyam. *Journal of Nutra Foods*. 14: 133-140.
75. Mailafia, S., Okoh, G. R. Hamza, O. Olabode K. and Osanupin, R. (2017). Isolation and identification of fungi associated with spoiled fruits vended in Gwagwalada market, Abuja, Nigeria. *Vet. World*, 10: 393-397.
76. Igbozulike, A. O. (2015). Evaluation of cocoyam corms, processing and storage in Nigeria. *Journal of Scientific and Engineering Research*6 (7) 260 -263.
77. Anyaegbu, C. F, Oledibe, O.J and Amadi, J.E. (2019). Proximate and phytochemical analysis of healthy and infected maize grains in Anambra State. *European Journal of Biology*. 4(1):15-40.
78. Ejimofor, C. F., Oledibe, O.J. and Nwakoby N.E (2021). Control of yam rot pot using leaf extracts of utazi and moringa oleifera. *Asian journal of research in Botany* 6(2):11-20
79. Obafemi, M. O., Alamu E. O. and Omitogun, L. A. (2020). Effect of processing on the nutritional contents of *Gongronema latifolium* *Journal of Applied Biosciences*. 46: 3086- 3092.
80. Birhanu, S., Akhtar, M.S. and Muleta, D. (2014). Management of post-harvest fruit spoilage fungi by some potential spice extracts. *Archives of Phytopathology and Plant Protection*, 47(17), 2124- 2140. <https://doi.org/10.1080/03235408.2013.869891>
81. Ejimofor, C.F, Nwakoby N.E and Ezenwata, I.S (2022). Antifungal screening of garlic clove extract against fungi causing spoilage in ogbono seed (*Irvingia gabonensis*). *Journal of Applied Chemical Science International*. 13(4): 14-24.
82. Ebana, R.U.B, Ekanemesang, U.M. Edet, U.O. and Omoruji E.F. (2016) phytochemical screening and antimicrobial activity of xylopia aethiopia and *gonogronema latifolium* on common pathogens. *Journal of advances in Biology& Bictchnology* 9(4):1-7.
83. Edim E. H., Egomi, U. G., Ekpo, U. F. and Archibong, E. U. (2016). A review on *Gongronema latifolium* (Utasi): A novel antibiotic against Staphylococcus aureus related infections. *International Journal of Biochemistry and Biotechnology*. 1(8):204-208.
84. Akueshi, C. O., Kadir, C. O., Akueshi, E. U., Agina, S. E. and Ngurukwem, C. (2020). Antimicrobial potential of *Hytissau veolus poit (lam inaceac)* *Nigerian Journal of Botany* 15:37-41.
85. Okigbo, R.N and Anyagebu, C.F. (2021). Underutilized plants of Africa. *Journalof Biology and Nature*. 13(2):34-49.
86. De Lange, W. J., Wingfield, B. D. and Wingfield, M. J. (2018). A rapid, apple-based test for virulence in *Cryphonectria cubensis* isolates. *Eur. J. For. Pathol.*,28: 409-412.
87. Srivanesan, E. H. (2017). *Gongronema latifolium* In diabetic rats, crude leaf extract stops weight loss and changes to haematological parameters. *J Pharmacol Toxicol*. 6(2):174-181.
88. Okigbo, R. N., Anyaegbu, C. F and Dalafi, T. P. (2020). Pollution from Spent Engine Oil with their Bioremediation Potentials In Auto-Mobile Workshops in Awka, Anambra State, South Eastern Nigeria. *Nigerian Journal of Biomedical Engineering*. 13:39 – 44.

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**Publications**

89. Anyaegbu, C.F; Oledibe, O.J and Amadi J.E. (2019). Proximate and phytochemical analysis of healthy and infected maize grains in Anambra State. *European Journal of Biology*. 4(1):15-40.
90. Onwueme, L. (2018). The tropical tuber crops: yams, cassava, sweet potato and cocoyams. John wiley and sons. Pp 199-225.

**Ejimofor, C.F<sup>1</sup> and Okigbo, R.N. (2023). Antifungal Effects of some Plant Extracts on Fungi Isolated from Post-Harvest Cocoyam. NEWPORT INTERNATIONAL JOURNAL OF SCIENTIFIC AND EXPERIMENTAL SCIENCES (NIJSES) 3(1):23-49.**