# Fungicidal Potentials of *Piper Guineese* Seed, *Gongronema latifolium* Leaf and *Allium sativum* Rhizome Extract on Post-Harvest Rot of *Colocasia Esculenta*

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**ABSTRACT**

*This study investigated the fungicidal potential of ethanol extracts of Piper guineense seeds, Gongronema latifolium leaves, and Allium sativum rhizomes against postharvest rot of Colocasia esculenta (cocoyam) corms. Morphological and biochemical characterization of fungal isolates obtained from rotted cocoyam revealed the presence of seven pathogenic fungi: Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus stolonifer, Penicillium expansum, Lasiodiplodia theobromae, and Mucor circinelloides. Frequency of occurrence showed that A. flavus (30%) and A. niger (25%) were the most prevalent, while M. circinelloides (5%) was least frequent. Pathogenicity tests indicated that A. flavus and F. solani were highly virulent, producing lesion diameters greater than 50 mm, whereas other isolates showed moderate pathogenicity. Quantitative phytochemical analysis of plant extracts revealed significant levels of bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids, and saponins, with P. guineense showing the highest alkaloid content and G. latifolium exhibiting higher phenolic and flavonoid contents. GC–MS analysis identified several bioactive constituents including piperine, eugenol, limonene, phytol, squalene, and sulfur-containing compounds such as diallyl disulfide and allicin, known for their antifungal properties. Antifungal evaluation showed that G. latifolium exhibited the strongest inhibitory activity with the lowest minimum inhibitory concentration (MIC) values (12.5 mg/mL) against several isolates, followed by A. sativum while P. guineense the least required relatively higher concentrations. Minimum fungicidal concentration (MFC) results further confirmed the superior fungicidal efficacy of G. latifolium and A. sativum. Molecular identification through DNA sequencing and purification confirmed the identity of the fungal isolates and supported morphological classification, ensuring accuracy in pathogen identification. The study demonstrates that these plant extracts, particularly G. latifolium and Allium sativum, possess potent antifungal properties and can serve as eco-friendly alternatives to synthetic fungicides in controlling postharvest rot of cocoyam.*

**KEYWORDS**

*Antifungal, Biochemical compounds, Inhibitory, Plants extract*

## I. INTRODUCTION

Postharvest rot of *Colocasia esculenta is* primarily caused by fungal pathogens. These fungi invade harvested corms through wounds or natural openings, leading to soft rot, discoloration, foul odour, and tissue degradation. Conventional control methods rely heavily

on synthetic fungicides; however, their repeated use has led to several challenges, including environmental pollution, pathogen resistance, and potential toxicity to consumers. In response to these limitations, attention has shifted toward plant-derived antifungal agents as safer and more sustainable alternatives. Medicinal plants contain bioactive compounds such as alkaloids, flavonoids, terpenoids, and organosulfur compounds that exhibit antimicrobial properties. Among such plants, *Piper guineense* (West African black pepper), *Gongronema latifolium* (utazi leaf), and *Allium sativum* (garlic) have been traditionally used in ethnomedicine for treating infections and preserving food products.

Cocoyam (*Colocasia esculenta* L. Schott) is a perennial herbaceous monocot that belongs to the Araceae family and the Colocasia genus. It is thought to have originated in South and Central Asia, later spreading throughout tropical Asia and Oceania over 2,000 years ago, where it transformed into an important cultural and economic food source (Anusha et al., 2023). Currently, cocoyam is grown in various regions of Africa, Asia, and the Pacific Islands and remains vital for food security due to its high carbohydrate output and ability to thrive in a range of agro-ecological environments (El-Saber et al., 2024). In Nigeria, two main species of cocoyam are grown: *Colocasia esculenta,* known locally as ede-uli, and Xanthosoma sagittifolium, referred to as ede-oku. The species C. esculenta is more commonly found in the southeastern regions, especially in Anambra, where it serves as a dietary staple and is frequently used as a thickener for soups, whereas X. sagittifolium is less widely consumed but is eaten boiled or pounded (Carlile et al., 2019). Cocoyam constitutes a significant share of the dietary carbohydrates for rural households, but its cultivation is hampered by serious issues related to postharvest spoilage. Nigeria is the top producer of cocoyam globally, contributing between 35% and 40% of the worldwide output. However, recent research indicates that production is gradually declining, primarily due to insufficient prioritization, inadequate storage facilities, and the occurrence of storage rots. *Piper guineense* commonly known as West African black pepper or "Uziza" in Nigeria. It belongs to the family Piperaceae and is widely distributed across tropical West Africa. The plant is commonly used as a spice in food and as a traditional remedy for several ailments, including gastrointestinal disorders, inflammation, microbial infections, and reproductive issues (Ashokkumar et al., 2021). Phytochemical investigations have revealed that *Piper guineense* is rich in bioactive compounds such as alkaloids (notably piperine and piperidine), flavonoids, terpenoids, tannins, and saponins. These compounds are responsible for its broad pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, and antiparasitic effects (Alagbe et al., 2021). Gas chromatography–mass spectrometry (GC–MS) analyses have further identified secondary metabolites such as sesquiterpenes and aromatic compounds that contribute to its medicinal potency. Despite its widespread use, postharvest losses caused by fungal pathogens remain a major challenge in tropical root and tuber crops such as *Colocasia esculenta* (cocoyam).

*Gongronema latifolium* is traditionally used for the management of several ailments including diabetes, malaria, cough, stomach disorders, hypertension, and liver-related diseases. Its leaves are commonly used as spices in soups and herbal preparations, indicating its dual role as both food and medicine (Eleyinmi, 2007). The plant has gained significant scientific attention due to its pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, hypoglycemic, and hepatoprotective activities (Ayodeji ET al., 2020). Phytochemical investigations have revealed that G. latifolium contains a wide range of secondary metabolites including alkaloids, flavonoids, saponins, tannins,

glycosides, and phenolic compounds, which are largely responsible for its medicinal properties (Anameze et al., 2023). These compounds contribute to its ability to inhibit microbial growth, reduce oxidative stress, and regulate metabolic processes in the body. In addition to its medicinal importance, the plant is also nutritionally valuable. *Allium sativum* is particularly valued for its rich phytochemical composition, especially sulfur-containing compounds such as allicin, alliin, diallyl disulfide, and ajoene. These compounds are responsible for its characteristic aroma and numerous biological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, antihypertensive, and antidiabetic properties. Allicin, in particular, is formed enzymatically when garlic cloves are crushed or damaged and is considered the major bioactive compound responsible for its antimicrobial activity. Recent pharmacological studies have demonstrated that garlic exhibits broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and protozoa, making it a promising natural alternative to synthetic antimicrobial agents. Additionally, its antioxidant properties help in scavenging free radicals, thereby reducing oxidative stress and preventing cellular damage associated with chronic diseases such as cancer and cardiovascular disorders.

Compared to synthetic fungicides, plant-based products are generally biodegradable, less toxic, more affordable, and readily available in local communities. These attributes make them particularly attractive for sustainable crop protection, especially in resource-limited settings. The increasing incidence of postharvest rot of *Colocasia esculenta* and the limitations of synthetic fungicides necessitate the exploration of plant-based fungicidal agents. This study therefore investigates the fungicidal potential of *Piper guineense* seed, *Gongronema latifolium* leaf, and *Allium sativum* rhizome extracts against postharvest fungal pathogens of *Colocasia esculenta.*

## II. MATERIALS AND METHODS

### *A. Sampling location*

The study was conducted in 15 selected locations across Anambra State, Nigeria, including Nkwelle-Ezunaka, Eke Awka, Ose market Onitsha, Nnewi, Ekwulobia, Igbariam, Obosi, Nkpor market, Amawbia, Awkuzu, Afo igwe Umudioka, Nwagu Agulu, Oyeagu Abagana, Uli and Nkwo Enugwu-Ukwu. These locations were chosen based on their high cocoyam production and market activity and also to capture variations in storage conditions, handling practices, and fungal diversity within Anambra State.

### *B. Identification, Collection, and Authentication of Plant Samples*

Plant samples used for this study included *Piper guineense* seed, *Gongronema latifolium* leaf, *Allium sativum* rhizome and *Colocasia esculenta* corm. These samples were collected from local farms and natural habitats within Anambra State using sterile collection bags to avoid external contamination. The collected plant specimens were taken to the Department of Botany Nnamdi Azikiwe University, Anambra State where they were identified and authenticated by Dr. Iroka Finian Chisom Voucher specimens were deposited in the Departmental herbarium for reference. Proper authentication ensured the botanical validity of the selected plants used for the phytochemical and antifungal analyses.

*C. Microbiological Analysis*

*1. Culture Media*

Two commercially available media were employed for the isolation of fungi: Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). PDA was used as a general-purpose medium suitable for the growth of a wide range of fungi, while SDA was selected for its ability to support the growth of dermatophytes and other pathogenic fungi (Watanabe, 2010). Both media were supplemented with streptomycin (500 mg/L) to suppress bacterial contamination. Thirty-nine grams (39 g) of PDA powder were suspended in one litre (1 L) of distilled water, heated to boiling while stirred, and autoclaved at 121 °C for 15 minutes under 15 psi. After sterilisation, 500 mg of streptomycin was added to the molten medium before dispensing into Petri dishes.

*2. Isolation of Fungi*

Distinct colonies were subculture onto fresh PDA plates. Repeated sub culturing was done to obtain pure isolates. Pure cultures were stored on PDA slants at 4°C. The isolation of fungi was carried out following the method of Roseline, (2023). Cocoyam tubers showing visible spoilage symptoms were first surface sterilised by immersion in 40% sodium hypochlorite solution for 60 seconds and rinsed three times in sterile distilled water. Sections (5 × 5 mm) containing both diseased tissue and advancing margins were excised aseptically and inoculated onto PDA plates. Inoculated plates were sealed with par film and incubated at 28 °C for 72 hours.

*3. Identification of Fungi*

Fungal isolates were characterised based on cultural, morphological, and microscopic features using standard identification keys,

*4. Pathogenicity Test of Isolated Fungi*

Pathogenicity tests were conducted to establish the causal relationship between the isolated fungi and cocoyam spoilage, following Ogbo and Agu (2015). Healthy cocoyam tubers were surface sterilised with 70% ethanol and punctured with a sterile 6 mm corn borer. Agar plugs containing 7-day-old fungal cultures were aseptically inserted into the wounds, which were sealed with petroleum jelly. Control tubers received sterile agar plugs. All inoculated tubers were incubated in sterile polyethylene bags at room temperature (28 ± 2 °C) for five days. Spoilage symptoms were recorded, and the diameters of rotted tissue were measured. The fungi were re-isolated from inoculated tissues and compared with the original isolates to confirm Koch’s postulates.

*5. Ethanol Extraction*

Ethanol extraction was carried out by cold maceration following the procedure of Ebana et al. (2016). A portion of 250 g of powdered plant material was weighed into a sterile conical flask and immersed in absolute ethanol until the material was fully submerged. The flask was tightly sealed with a glass stopper and intermittently agitated every four hours (except at night) for seventy-two (72) hours. At the end of the maceration period, the contents were filtered sequentially through sterile muslin cloth and Whatman No. 1 filter paper. The marc (residual plant material) was pressed to maximize recovery of the extract. The filtrate was concentrated under reduced pressure at 55°C using a rotary evaporator to obtain the crude ethanol extract. The dried extracts were weighed, stored in sterile airtight containers, and refrigerated at 4°C until further analysis.

*6. Determination of Extraction Yield*

The extraction yield was calculated gravimetrically as: Yield (%) = (W1x 100)/W2 where W1 is the weight of the extract (g) after lyophilisation of solvent, and W2 is the weight of the dried plant powder.

*7. Qualitative Phytochemical Screening*

Phytochemical screening of the plant extracts was conducted to detect the presence of bioactive secondary metabolites such as alkaloids, phenolics, terpenoids, cardiac glycosides, flavonoids, saponins, steroids, and tannins. The procedures described by Banso and Adeyemo (2019) were adopted with slight modifications.

*8. Test for Alkaloids*

The extracts were treated with 10% dilute hydrochloric acid and filtered. The filtrates were subjected to the following confirmatory tests:

Mayer's Test: A few drops of Mayer's reagent (potassium mercuric iodide) were added to the filtrate. The formation of a cream-colored precipitate indicated the presence of alkaloids.

Wagner's Test: The filtrate was treated with Wagner's reagent (iodine in potassium iodide). The appearance of a brownish precipitate confirmed the presence of alkaloids.

*9. Test for Phenolics*

Five grams (5 g) of the powdered plant sample was boiled with 10 ml of distilled water for 5 minutes and filtered. One millilitre (1 ml) of ferric chloride solution was added to the filtrate. The development of a blue-black or brown coloration indicated the presence of phenolic compounds.

*10. Test for Terpenoids*

Two millilitres (2 ml) of each extract was mixed with 2 ml of chloroform. Thereafter, 3 ml of concentrated $H_2SO_4$ was carefully added to form a separate layer. The appearance of a reddishbrown interface confirmed the presence of terpenoids.

*11. Test for Cardiac Glycosides*

A small quantity of the extract was dissolved in glacial acetic acid, followed by the addition of a few drops of ferric chloride solution and concentrated sulphuric acid. The formation of a reddishbrown ring at the interface between the two layers indicated the presence of cardiac glycosides.

*12. Test for Flavonoids*

Shinoda's Test: A portion of the extract was dissolved in ethanol. A small magnesium ribbon was added, followed by dropwise addition of concentrated hydrochloric acid. Development of a magenta or pink coloration indicated the presence of flavonoids.

Ferric Chloride Test: To the extract, a few drops of neutral ferric chloride were added. A blackish-red coloration further confirmed the presence of flavonoids.

*13. Test for Saponins*

Foam Test: The extracts were diluted to 20 ml with distilled water and shaken vigorously in a graduated cylinder for 15 minutes. Persistent foam formation on the surface indicated the presence of saponins.

Emulsification Test: Two drops of olive oil were added to the extract solution and shaken vigorously. The formation of a stable emulsion confirmed the presence of saponins.

*14. Test for Steroids*

Salkowski's Reaction: Two millilitres (2 ml) of extract were mixed with 2 ml chloroform and 2 ml concentrated $H_2SO_4$. The chloroform layer developed a red coloration, while the acid layer exhibited greenish-yellow fluorescence, confirming the presence of steroids.

Liebermann–Burchard Test: The extracts were treated with concentrated sulphuric acid, followed by glacial acetic acid and acetic anhydride. The formation of a green color indicated the presence of steroids.

*15. Test for Tannins*

Lead Acetate Test: A few drops of 10% lead acetate solution were added to the extract. The formation of a white precipitate indicated the presence of tannins.

Ferric Chloride Test: Addition of ferric chloride solution produced a greenish coloration, further confirming the presence of tannins.

*D. Quantitative Phytochemical Screening*

The quantitative estimation of secondary metabolites in the plant extracts was carried out to determine the concentration of steroids, saponins, alkaloids, terpenoids, tannins, flavonoids, phenols, and glycosides. Standard protocols as described by AOAC (2016), Joshi et al. (2015), and Harborne (2008) were followed with slight modifications.

*1. Steroids*

One gram (1 g) of each plant extract was macerated with 20 ml of ethanol. Two millilitres (2 ml) of chromogen solution were added to 2 ml of the filtrate and allowed to stand for 30 minutes. Absorbance was recorded at 550 nm using a UV spectrophotometer. A standard calibration curve was prepared using steroid hormone, and the concentration of steroids in each extract was extrapolated from the standard curve.

*2. Saponins*

Twenty grams (20 g) of pulverised plant material were extracted using a Soxhlet apparatus with 250 ml of methanol as solvent. Extraction was carried out for three hours on a heating

mantle until saponins were completely removed. The methanol was recovered, leaving behind saponins, which were further concentrated by evaporating the residual methanol at 70°C.

*3. Alkaloids*

Half a gram (0.5 g) of extract was dissolved in 96% ethanol and 20% sulphuric acid, then filtered. One millilitre (1 ml) of the filtrate was added to 5 ml of 60% sulphuric acid and allowed to stand for three hours. Absorbance was measured at 565 nm. A standard curve was prepared using caffeine, and the concentration of alkaloids was extrapolated accordingly (Joshi et al., 2015).

*4. Terpenoids*

Dried plant extract (100 mg) was soaked in 9 ml of ethanol for 24 hours. The mixture was filtered and re-extracted with 10 ml of petroleum ether using a separating funnel. The ether layer was collected in pre-weighed vials and allowed to evaporate completely. The percentage yield of terpenoids was calculated.

*5. Tannins*

Five grams (5 g) of dried sample were defatted in 100 ml of n-hexane for two hours. The residue was extracted in 100 ml of 10% acetic acid in ethanol and filtered. To the filtrate, 25 ml of ammonium hydroxide was added to precipitate tannins. The precipitate was dried, weighed, and reconstituted for titration with 0.1 M NaOH using phenolphthalein as indicator. Tannin content was expressed as percentage tannic acid equivalent (AOAC, 2016).

*6. Flavonoids*

One gram (1 g) of each extract was macerated with 20 ml of ethyl acetate for 5 minutes and filtered. To 5 ml of the filtrate, 5 ml of dilute ammonia solution was added, shaken vigorously, and the absorbance was measured at 490 nm. A standard curve was prepared using quercetin, and flavonoid concentration was extrapolated (Boham & Kocipai, 2014).

*7. Phenols*

Two grams (2 g) of defatted sample were extracted with ether in a Soxhlet apparatus. The extract (5 ml) was treated with 10 ml of distilled water, 2 ml of 0.1 N ammonium hydroxide, and 5 ml of amyl alcohol. After 30 minutes, optical density was measured at 505 nm. A tannic acid standard curve (0.2–1.0 mg/ml) was used to quantify phenolic compounds.

*8. Glycosides*

One gram (1 g) of extract was macerated in 50 ml of distilled water and filtered. One millilitre (1 ml) of the filtrate was added to 4 ml of dilute ferric chloride solution and heated for 5 minutes, then cooled. Absorbance was measured at 490 nm. Digitoxin was used as standard for calibration, and concentrations of glycosides were extrapolated from the curve.

### *E. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out to determine the bioactive constituents present in the plant extracts. The analysis was performed at the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria, using an Agilent Technologies 5977 GC–MS system equipped with a capillary column. Helium gas was employed as the carrier at a constant flow rate of 1.0 mL/min. One microlitre (1 µL) of the plant extract was injected into the GC system in splitless mode. The oven temperature was initially programmed at 80 °C, held for 2 minutes, then increased to 200 °C at 15 °C/min, and finally raised to 280 °C at 5 °C/min with a 5-minute isothermal hold. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. Mass spectrometry was conducted in electron impact (EI) mode at an ionisation energy of 70 eV. The ion source temperature was maintained at 230 °C, and scanning was performed over a massto-charge ratio (m/z) range of 50–600. The chromatograms obtained were interpreted by comparing the mass spectra of individual components with reference spectra available in the National Institute of Standards and Technology (NIST) Library. The identified compounds were recorded along with their respective retention times, molecular weights, and peak area percentages.

### *1. Antifungal Activity*

The antifungal activity of the plant extracts against fungal isolates obtained from spoilt cocoyam was evaluated using the agar well diffusion method as described by CLSI (2012) and modified by Ohikhena.

### *F. Agar Well Diffusion Assay*

Fungal suspensions were standardised to a concentration of approximately $2.5 \times 10^4$ CFU/mL using sterile saline. A 100 µL aliquot of the fungal suspension was evenly spread onto the surface of freshly prepared SDA plates using sterile cotton swabs. The plates were allowed to stand for 15 minutes to ensure proper adherence of inocula. Sterile corn borers (6 mm diameter) were used to punch wells into the agar. Each well was filled with 50 µL of plant extract at concentrations of 25%, 50%, and 100%. Fluconazole (10 mg/mL) served as the positive control, while sterile distilled water was used as the negative control.

The inoculated plates were pre-incubated at 4 °C for 2 hours to allow proper diffusion of extracts, followed by incubation at 28 ± 2 °C for 5 days. Antifungal activity was determined by measuring the zone of inhibition (mm) around each well with a transparent ruler. All experiments were conducted in triplicate, and the mean ± SEM values were recorded.

### *G. Determination of Minimum Inhibitory Concentration (MIC)*

MIC values were determined using the broth microdilution method described by Blazic et al. (2019). Serial two-fold dilutions of the extracts were prepared in 96-well microtitre plates to yield concentrations ranging from 200 mg/mL to 0.39 mg/mL. Each well was inoculated with 10 µL of fungal suspension ($2.5 \times 10^4$ CFU/mL) and 100 µL of Sabouraud dextrose broth. Wells containing broth and inoculum without extracts served as growth controls, while wells with broth only served as sterility controls. Plates were incubated at 28 °C for 72 hours. After incubation, 10 µL of 0.01% resazurin dye was added to each well and incubated

for an additional 2 hours. A colour change from blue to pink indicated fungal growth. The MIC was defined as the lowest concentration of extract that prevented a colour change.

### *H. Determination of Minimum Fungicidal Concentration (MFC)*

MFC values were determined by sub-culturing 10 µL aliquots from wells showing no visible growth (at and above MIC concentrations) onto SDA plates. The plates were incubated at 28 °C for 72 hours. The lowest concentration of extract showing no visible fungal growth was recorded as the MFC (CLSI, 2012). All determinations were carried out in triplicate to ensure reproducibility.

### *I. Molecular Characterisation of Fungal Isolates*

#### *1. DNA extraction using Zr fungal/bacterial DNA miniprep (Manufactured by Zymo Research)*

- Add 2mLs of fungal cells broth to to a ZR BashingTM Lysis Tube. Add 750ul Lysis Solution to the tube.
- Secure in a bead fitted with 2 ml tube holder assembly and process at maximum speed for > 5 minutes.
- Centrifuge the ZR BashingBeadTM Lysis Tube in a microcentirifuge at > 10,000 x g for 1 minute.
- Transfer up to 400 ul supernatant to a Zymo-SpinTM IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute.
- Add 1,200 ul of Fungal/Bacterial DNA Binding Buffer to the filterate in the Collection Tube from Step 4.
- Transfer 800 ul of the mixture from Step 5 to a Zymo-SpinTM IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.
- Discard the flow through from the Collection Tube and repeat Step 6.
- Add 200 ul DNA Pre-Wash Buffer to the Zymo-Spin TM IIC Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute
- Add 500 ul Fungal/Bacterial DNA Wash Buffer to the Zymo-SpinTM IIC Column and centrifuge at 10,000 x g for 1 minute
- Transfer the Zymo-SpinTM IIC Column to a clean 1.5 ml microcentrifuge tube and add 100ul (35 ul minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

#### *2. Electrophoresis for DNA and PCR*

- Measure 1 g of agarose ( for DNA) ; 2g of agarose for PCR
- Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
- Microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel.
- Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.

- Add 10µL EZ vision DNA stain. EZ vision binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light.
- Pour the agarose into a gel tray with the well comb in place
- Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

*3. Loading Samples and Running an Agarose Gel*

- Add loading buffer to each of your DNA samples or PCR products
- Once solidified, place the agarose gel into the gel box (electrophoresis unit).
- Fill gel box with 1xTAE (or TBE) until the gel is covered.
- Carefully load a molecular weight ladder into the first lane of the gel.
- Carefully load your samples into the additional wells of the gel.
- Run the gel at 80-150 V for about 1-1.5 hours
- Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
- Visualize DNA fragments or PCR product under UV transilluminator.

*4. PCR Mix Components*

The PCR mix is made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward and reverse primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water.

Primer Sequences

- ITS 1: 5’ TCC GTA GGT GAA CCT GCG G 3’
- ITS4 5’ TCCTCCGCTTATTGACATGS 3’

*J. Cycling Conditions*

Initial denaturation at 94°C for 5mins, followed by 36 cycles of denaturation at 94°C for 30sec, annealing at 54°C for 30secs and elongation at 72°C for 45sec. Followed by a final elongation step at 72°C for 7 minutes and hold temperature at 10 °C forever.

*1. DNA Sequencing and Analysis*

PCR products were purified and sequenced. Sequences compared using BLAST (NCBI database)

Identification based on percentage similarity. Phylogenetic tree constructed using software such as MEGA

*2. Data Analysis*

The experimental data generated in this study were recorded and organised using Microsoft Excel 2016 and subsequently analysed with the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were employed to calculate means and standard error of the mean (SEM) for inhibition zone diameters and

phytochemical concentrations. One-way analysis of variance (ANOVA) was used to test for significant differences among group means. Statistical significance was considered at $p < 0.05$ for all analyses. The results were presented in the form of tables, charts, and graphs for clarity of interpretation.

## III. RESULTS

### *A. Morphological characteristics of fungal isolated from rotted cocoyam*

Based on these characteristics, the following fungal species were identified:

- *Aspergillus flavus*
- *Aspergillus niger*
- *Fusarium solani*
- *Rhizopus stolonifer*
- *Penicillium expansum*
- *Lasiodiplodia theobromae*
- *Mucor circinelloides*

These fungi have been widely implicated in the postharvest spoilage of root and tuber crops, including cocoya

Table: 1: Biochemical examination of fungal isolates from rotted cocoyam

| Isolate | Carbohydrate assimilation | Spore formation | Amino acid assimilation | Motility | Hydrolysis | Lipase activity |
|---|---|---|---|---|---|---|
| *Aspergillus flavus* | + | – | + | – | – | + |
| *Aspergillus niger* | + | – | + | – | – | + |
| *Fusarium solani* | + | – | + | – | – | + |
| *Rhizopus stolonifer* | + | + | + | – | – | – |
| *Penicillium expansum* | + | + | – | – | – | – |
| *Lasiodiplodia theobromae* | + | - | + | - | + | - |
| *Mucor circinelloides* | + | + | + | - | - | - |

### *B. Pathogenicity of Fungal Isolates on Healthy Cocoyam Tubers*

The pathogenicity test was carried out to determine the virulence of the fungal isolates on healthy cocoyam (*Colocasia esculenta*) tubers. All five fungi—Aspergillus flavus, A. niger, Fusarium solani, Rhizopus stolonifer, and Penicillium expansum—were found to be pathogenic, inducing rot within 21 days post-inoculation. The severity of infection varied significantly ($p < .05$) across fungal species and sampling locations. Highly pathogenic fungi (lesion diameter > 50 mm) were observed predominantly in Fusarium solani, Mucor circinelloides, Lasiodiplodia theobromae and A. flavus, while moderate pathogenicity (lesion

diameter 10–50 mm) was observed in A. niger, Rhizopus stolonifer, and Penicillium expansum.

Table 2: Pathogenicity of Fungal Isolates on Healthy Cocoyam Tubers

| **Fungal Species** | **Mean Lesion Diameter (mm)** | **Pathogenicity Level** |
|---|---|---|
| *Aspergillus flavus* | > 50 | +++ |
| *Fusarium solani* | > 50 | +++ |
| *Aspergillus niger* | 10–50 | ++ |
| *Rhizopus stolonifer* | 10–50 | ++ |
| *Penicillium expansum* | 10–50 | ++ |
| *Mucor circinelloides* | 10-50 | ++ |
| *Lasiodiplodia theobromae* | 10-50 | ++ |

Values represent mean ± standard deviation of three replicates.
Means within the same column followed by identical superscripts are not significantly different at $p < 0.05$.

Key:

- +++ = Highly pathogenic (> 50 mm lesion diameter)
- ++ = Moderately pathogenic (10–50 mm lesion diameter)

Note**.** +++ = highly pathogenic (> 50 mm); ++ = moderately pathogenic (10–50 mm); 0–9 scale of severity where 0 = least severe and 9 = most severe. Values represent means ± standard deviation of three replicates. Means in the same column with the same superscript are not significantly different ($p < .05$).

### *C. Phytochemical Composition*

Table 3: Qualitative Phytochemical Composition

| **Parameters** | ***Gongronema latifolium*** | ***Piper guineense*** | ***Allium sativum*** | ***Colocasia esculenta*** |
|---|---|---|---|---|
| Saponin | +++ | +++ | ++ | + |
| Flavonoid | +++ | ++ | ++ | + |
| Alkaloid | ++ | ++ | +++ | + |
| Tannin | ++ | +++ | + | + |
| Steroids | ++ | ++ | ++ | + |
| Tepernoid | ++ | +++ | ++ | + |
| Glycosides | ++ | ++ | + | + |
| Anthrocynin | +++ | - | - | + |
| Phenol | +++ | ++ | ++ | + |
| Oil and Resin | - | - | ++ | + |

Key

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

*D. Quantitative Phytochemical Composition*

Table 4: Quantitative Phytochemical Composition of Ethanol Extracts (mg/100 g)

| **Phytochemical** | ***Gongronema latifolium*** | **Piper guineese** | ***Allium sativum*** | ***Colocasia esculenta*** | **F-Value** |
|---|---|---|---|---|---|
| Alkaloids | 5.21±025 | 7.34±0.40 | 6.15±0.30 | 2.15±0.17 | 47.21 |
| Flavonoids | 6.57±0.30 | 5.44±0.23 | 4.15±0.20 | 1.98±0.15 | 50.65 |
| Phenols | 7.10±0.34 | 4.10±0.20 | 3.85±0.11 | 1.17±0.12 | 49.03 |
| Saponins | 6.45±0.27 | 3.25±0.12 | 2.10±0.11 | 1.42±0.10 | 37.89 |
| Tannins | 3.75±0.11 | 5.75±0.21 | 5.21±0.27 | 2.05±0.17 | 42.11 |
| Steroids | 3.10±0.16 | 2.74±0.36 | 3.55±0.17 | 1.60±0.11 | 29.45 |
| Terpenoids | 4.50±0.23 | 6.95±0.33 | 4.80±0.26 | 2.05±0.11 | 46.50 |
| Cardiac Glycosides | 4.35±0.21 | 3.47±017 | 2.33±0.13 | 1.45±0.09 | 31.42 |

Table 5: Minimum Inhibitory Concentration (MIC) of Plant Extracts

| **Fungal isolates** | **A. sativum** | **P. guineense** | **G. latifolium** |
|---|---|---|---|
| *Aspergillus flavus* | 25.0 ± 2.9 | 50.0 ± 3.2 | 12.5 ± 1.8 |
| *Aspergillus niger* | 25.0 ± 3.1 | 50.0 ± 2.7 | 12.5 ± 1.5 |
| *Fusarium solani* | 12.5.0 ± 2.5 | 100.0 ± 4.0 | 25.0 ± 2.2 |
| *Rhizopus stolonifer* | 50.0 ± 3.0 | 100.0 ± 3.6 | 25.0 ± 2.0 |
| *Lasiodiplodia theobromae* | 12.5.0 ± 2.2 | 50.0 ± 2.5 | 12.5 ± 1.6 |
| *Penicillium expansum* | 50.0 ± 3.4 | 100.0 ± 4.3 | 25.0 ± 2.4 |
| *Mucor circinelloides* | 50.0 ± 2.8 | 100.0 ± 3.9 | 12.5.0 ± 3.9 |

Table 6: Minimum Fungicidal Concentration

| **Fungal isolates** | **A. sativum** | **P.guineense** | **G. latifolium** |
|---|---|---|---|
| Aspergillus flavus | 50.0 ± 5.8 | 100.0 ± 6.4 | 25.0 ± 3.6 |

| | | | |
|---|---|---|---|
| Aspergillus niger | 50.0 ± 6.2 | 100.0 ± 5.4 | 25.0 ± 3.0 |
| Fusarium solani | 25.0 ± 5.0 | 200.0 ± 8.0 | 50.0 ± 4.4 |
| Rhizopus stolonifer | 100.0 ± 6.0 | 200.0 ± 7.2 | 50.0 ± 4.0 |
| Lasiodiplodia theobromae | 25.0 ± 4.4 | 100.0 ± 5.0 | 25.0 ± 3.2 |
| Penicillium expansum | 100.0 ± 6.8 | 200.0 ± 8.6 | 50.0 ± 4.8 |
| Mucor circinelloides | 100.0 ± 5.6 | 200.0 ± 7.8 | 25.0 ± 7.8 |

*E. UV-VIS Analysis*

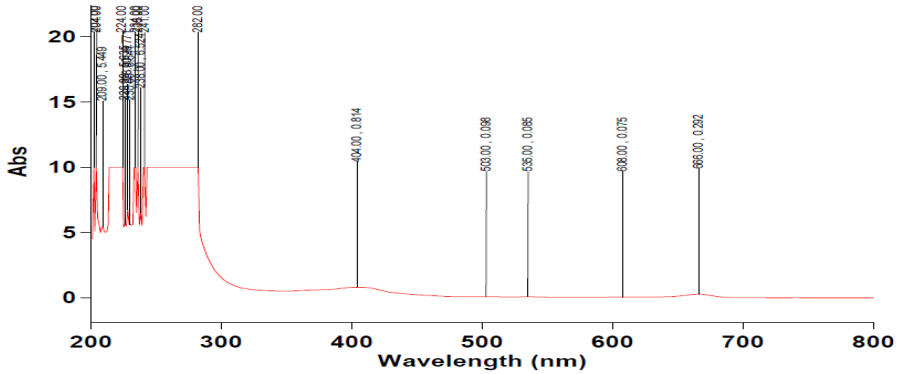


Figure 1: UV-VIS spectra of Extract of Gongronemalatifolium

Table 7: UV-VIS Peak Values of Extract of Gongronemalatifolium

| S/N | Wavelength (nm) | Absorbance |
|---|---|---|
| 1 | 202 | 10.000 |
| 2 | 204 | 10.000 |
| 3 | 209 | 5.449 |
| 4 | 224 | 10.000 |
| 5 | 226 | 5.635 |
| 6 | 228 | 6.771 |
| 7 | 230 | 5.671 |
| 8 | 234 | 10.000 |
| 9 | 236 | 10.000 |
| 10 | 238 | 6.524 |
| 11 | 241 | 10.000 |
| 12 | 282 | 10.000 |
| 13 | 404 | 0.814 |
| 14 | 503 | 0.098 |
| 15 | 535 | 0.085 |
| 16 | 608 | 0.075 |
| 17 | 666 | 0.292 |

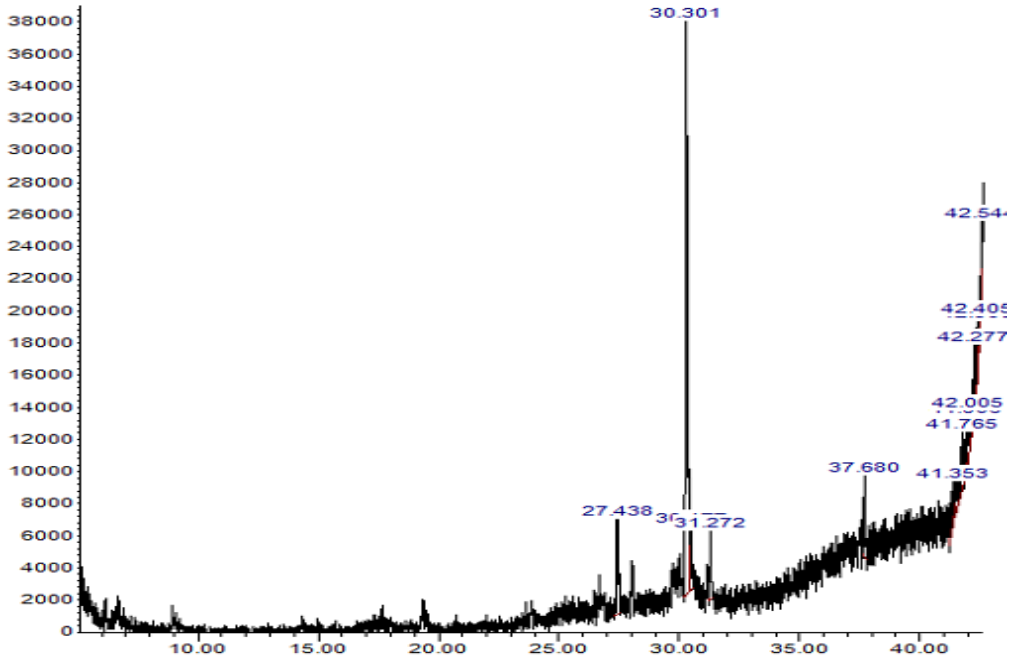


Figure 2: GC/MS Chromatogram of Gongronemalatifoliumplant extract

Table 8: UV-VIS Peak Values of Extract of Garlic

| S/N | Wavelength (nm) | Absorbance |
|---|---|---|
| 1 | 202 | 4.694 |
| 2 | 205 | 4.596 |
| 3 | 214 | 10.000 |
| 4 | 217 | 10.000 |
| 5 | 220 | 5.013 |
| 6 | 222 | 4.936 |
| 7 | 227 | 4.573 |
| 8 | 230 | 4.623 |
| 9 | 233 | 4.647 |
| 10 | 236 | 4.704 |
| 11 | 238 | 4.666 |
| 12 | 247 | 5.076 |
| 13 | 250 | 6.038 |
| 14 | 254 | 10.000 |
| 15 | 256 | 10.000 |
| 16 | 259 | 10.000 |
| 17 | 261 | 10.000 |
| 18 | 264 | 5.682 |
| 19 | 266 | 5.494 |
| 20 | 269 | 6.553 |
| 21 | 274 | 10.000 |
| 22 | 276 | 10.000 |
| 23 | 278 | 10.000 |
| 24 | 281 | 5.447 |

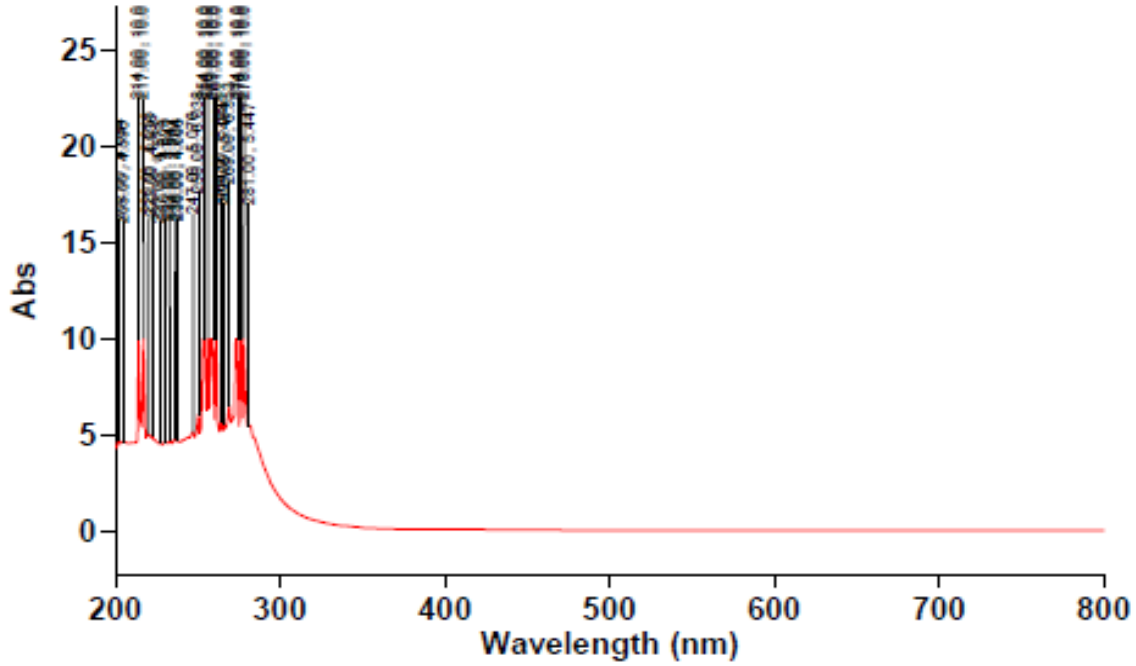


Figure 3: UV-VIS spectra of extract of *Allium sativum*

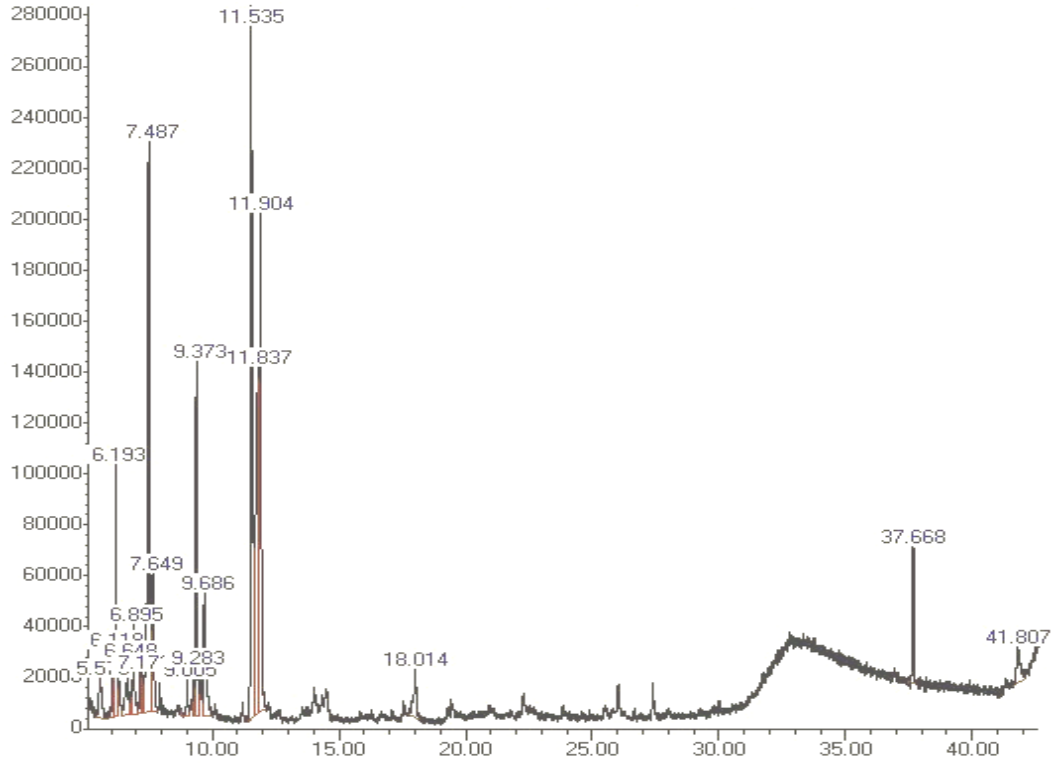


Figure 4: GC/MS Chromatogram of Garlicplant extract

## IV. DNA SEQUENCING

### *A. Sequence Results*

Isolate 1 has 97.29% pairwise identity with Rhizopus oryzae isolate RoKSA13-01 which has NCBI accession number KP764872.1. The e value is 0 while your isolate sequences are as shown below:

#### *1. Isolate 1 sequences*

GGGGTGGCGGCAAGGGGAGTTGGGGGGTAGTTGGAAGGTGAGGGATTGCTTCTACGCTGTTGAA
ATTTGGCTGAGAGACTCAGACTGGTCATGGGTAGACCTATCTGGGGTTTGATCGATGCCACTCCTG
GTTTCAGGAGCACCCTTCATAATAAACCTAGAAATTCAGTATTATAAAGTTTAATAAAAAACAACTTT
TAACAATGGATCTCTTGGATCTCGCATCGATGAAGAACGTAGCAAAGTGCGATAACTAGTGTGAAT
TGCATATTCAGTGAATCATCGAGTCTTTGAACGCAGCTTGCACTCTATGGTTTTTCTATAGAGTACG
CCTGCTTCAGTATCATCACAAACCCACACATAACATTTGTTTATGTATAATGGGTCGCATCGCTGTT
TTATTACAGTGAGCACCTAAGAGGTGTGTGATTTTCTGTCTGGCTTGCTAGGCAGGAATATTACGC
TGCGCTCAGGATCTTTTTCTTTGGTCGCCCAGGAAGTAAAGTACAAGAGTATAATCCAGCAACTTTC
AAACTATGATCTGAAGTCAGCTGGGATTACCCGCTGAACTTAAGCATGTCACAAGCGGAGGAA

Isolate 2 has 97.25% pairwise identity with Rhizopus oryzae isolate NDA02a which has NCBI accession number MK742815.1. The e value is 0 while your isolate sequences are as shown below:

#### *2. Isolate 2 sequences*

TTGGCTTAAGTCGGCAATACCTCTTAGGGTTCCTCTGAGGTGATGGATTATACTCTACGCACTTTAC
TTCCTGGGCGAACCACAGAAAAAGATCCTGAGACCAGCGATATATTCCTGCGAAGCAAGCCACAC
AGAAAATCACACACATTTTAGGTGCTCACTGTAATAAAACAGCGATGTAACCCATTACCACATAAAC
AAATGTTATGTGTGGCTTTGTGATGATACTGAAGCAGGCGTACTCTATAGAAAAACCATAGAGTGC
AAGCTGCGTTCAAAGACTCGATGATTCACTGAATATGCAATTCACACTAGTTATCGCACTTTGCTAC
GTTCTTCATCGATGCGAGAACCAAGAGATCCATTGTTAAAAGTTGTTTTTTATTAAACTTTATAATAC
TGAATTTCTAGGTTTATTATGAAGGGTGCTCCGGAAACCAGGAGTGGCATCGATCAAACCCCAGAT
AGGTCTACCCATGACCAGTCTGAGTCTCTCAGCCAAATTTTCACAGTGTAGAGGCAATCACTTACC
CCCGAGGAAACCCTAAGAGGTAAGGCGCTTTAACATAATTAATGAACCTTCCGCAGGTCACCCTTA
CGGGAAAATTTTTTATTTATGTTAAAGCGCCTTACCTCTTAGGGTTTCCTCTGGGGGAAGTGATTGC
TTCTACACTGTGAAAATTTGGCTGAAAACTCAGACGGGCCGGGGTGGCCTATTTGGGGTTTGACCA
AGCCCCTCCGGGTTCGGGACCCCCTTCAAAAAAACCTGGGATTCGGTTTAAAAAGTTTAAAAAAAA
AAATTTTAAAAAGGGCTTTTGGTTCCGCTCGAGAAAAAGGGAAAAGGGGGACCGGGGGGGGGGA
ATTCGGGAATTCCGGCTTTTACCGCGTGGCTCCCGGGTTTTTTTTGAGGACCCCGTTTGTTTCCCC
CAACCCCACAAAAAATTTTTTTTTTGGAAGGGGCCCCGCGTTTTTTTTACTATAGAGCCCAAAAAGG
GGGTGTTTTTCTGCTCTGGGGTGGGGGAGTATATGCCGTCCTCGACCTTTTTTTTTTGTTCCCCCG
CGCGAGGAGCGAGAATAGATACGACACGTCTCTACATGATATATGAGCGAGAGGGTGTCTGCTAG
TTTAGCCTTGGAGTGGGGTTGAGAATAAGAGATC

Isolate 3 has 95.38% pairwise identity with Aspergillus flavus isolate Afl-S which has NCBI accession number KX270348.1. The e value is 5e-17 while your isolate sequences are as shown below:

*3. Isolate 3 sequences*

CAGGTTTGGGGCTCAAAGCTCGCCGACCTCTGCCCGTGTGTACTGTTTTGTTACGGGCTTCGCCG
GCCCGGTTACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGTGGTGCGCCAGATA
CATTTCACGCACGTTTTACCCGAAGAGTGTCATCGATCAACCACGCAACCCGTGACCACGGGGCAA
ATGAATGTGTGCATTGAGATGAACTGAAGCAGGGTACTCTCGGAAGACTATAGAGTGTAGCTGAG
TTAAGTTCGATGCTCAAGTTATGCATTGATGTAGAAGCGGCCTGGGCGACTTATCATGTAGACGAG
TACGTTGTGTAGGCTCTTAAAAGCTTAAAGATGATTTAGTTATCTGGGGGGCTGGAGTGGGCGATG
TGACCCTAGGCTGCCTGCAACTTTCCTTCCCGCTTTCGAGGAAACATATTACCAATAAACTAAGAG
GGTTTAAAATTCGACTTCCGCCCCTAGGAATCTGTGTTTTGGCCTTGCTTTGGTCCTCTTGAAGCAG
GGTTCTCCCGTGAATTTGCTGAAAACTAAACTGTTATGGTGGACGATGTGGGTTTATCTTGCCCTCT
GGTTCAGTTCACCTGGAAATGACTTGTAATTAGATTAAAAGATAGAAAAAAAGTTTAAGCATTGTTT
TGTGTTCCGTAAAGGCAGGGGAAAAGAGGGCCGGATGTGTTGGCGGGGGCCTGGGGCTTGGCCG
GCGGCCCGGGGTTTTTGAGGGGGGCCCCCCTGCCCGCCAAACCCCCCCTAATTTGTTTTCGG

Isolate 4 has 96.23% pairwise identity with Aspergillus niger strain NWU32 which has NCBI accession number MT079300.1. The e value is 8e-12 while your isolate sequences are as shown below:

*4. Isolate 4 sequences*

GGGGTTCATACCGATCCGAGCTAACCTGCGTGGATTGGTACCATGTTGGTTCGGCGGGCCGTCGG
GGGAATCTTTTGACCCACCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGGGGCCCGCGCTGG
TGAGCATCTGACAGAGCCCCACTGCTCGAGGATCGGACGCGGTGCGCAGCCCCTTTAGGCCCGTC
CAAACGAGAGAGGTGAGCGACCCATCACAAAAGGGTGAAATTTTTCAAAAATATATCTTTTGGTTC
CGGGATTCCCTCAGGAAATACCAGGAAAACGCAATAACCGAAGGAAAAATTCGAAAAATTCGCTGA
ATTCCGCAATTTTTAAAAGAAATTGCACCCCCCTGCATTCTTGGTCGATGCCGTGACCAAAAAGATC
TTTTGTTGAAAGATTTCAGTTTTTGTAAAGGATCGACTCACCCTCCTCTCTGGGGACGGGTCGAAA
GGAGGGGCTCCGCCCCGCACGAACCTGGGGGCTTAGGGGCTTTGCCAAGGGCCCGAAGGATGG
GCCGCCCAAGCAAAAGTTTCAAACATCCTTTCGAGGGTGGACTCCGAACAGCCGCATTCCCGCTG
AAACTAAACATGTATAGGGGGGAAACCTCCTGCGAGCATGTGAGGAGCCCTCAGGCTCGAGGATC
GGACGCGGTGCAAACCTGACTTTCGGACTGTCCAAACGGTGAGGCCTGGGTGATGAATGCAAATC
GGTAAATTTTTTAAAAAATGAATCTTTGGTTTCGGTTTCCAATGAAAACCCAGGGAAGTGAATAACC
TACGGGAAATTGAGAATTTACGTGAACCTCGATTTCTTTAAGCTCAATTCTTCCGCTGGTATTCCGG
GGGATGCTGCTCAGAAGTCAATTGTGGCCCTATGCCCGCTTGTGTGTGGCAGCGTCCCCCTCTCC
GGCGGACGTCTCAAAGTGAGTGTCGACCGGTCGATCTGCGAGCGAAGAGGCTTCGCAACTCCTCA
TAGATGGCGGCCATGCACGTTTCAACATCGTCAGATGACTCCAAAGACGCACCGCTGACTTAGAGT
CAAGCGGAAG

Isolate 5 has 100.00% pairwise identity with Aspergillus flavus strain BP4 which has NCBI accession number KF860890.1. The e value is 6e-103 while your isolate sequences are as shown below:

*5. Isolate 5 sequences*

ACGTGGGATCCCATAACGGATCAGAGGTAACCTGCGAAAGCATGTGTGCAATACGTGCGTTCGGC
GGGCCCGCCTCCTACGCCTTTGGGATTACTTGCCTAAGGAACTTAGCGAACGTGGGAATAATAGTT
GAGCCCGACCTCCCACCCGGGCTTACTGTAACCTTAAGTGGTTGAGAGGCCCCATACTTAAGGCC

GATCGGACGCCATTCTGAATAATTCCTAGAAATCTCATTTCTTTGCACTCTTCATGCATCCCCTGAC
CTACAGATCCATTGGTGGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACC
CGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTGTCAGCC
CCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGACGTCTGAGTTGATTGT
ATCGCAATCAGTTAAAACTTTCAACAATGGAAGAGTTATAACTGATTGCGATACAATCGACTCAGAC
GTCACTAGATCAGACAGAGTTTCTGGTGTCTCCAGGGGCCCCGGAGCCGGCGGCTGATCCCCCCC
GGAGCCCTTAAAGGCGGGCCCTTTGAAACCACTTATGTTACAGCAAACACGGGTGGGAGGATGGG
CTCCCTAGGAACTTTACACTCTGAAATGATCCTTCGGCGGGATCCCCTACGAAAACCTTGTTGCCA
CTTTTACTAAAAAAGCGTGTGACAGAGCTCTCTGCCTCGGCCCGGACGCGGTGAAACCCTAAATTT
AGACTGGTTGAAACGTGAGGCGGGTTGATTAATCAAATGGGAAAATTTTTAAAAATGGACCTTTGG
ATCCGGCTCCCATGAAAAACCAGGGAACTGAAAAACTAAGGGATTGAAGATTACGGAATCTCAATT
CTTGACGAACTGGGTCCCCGGATATCCGGGGGATGCTTGTCGAGTTCATTGCTGCCTTAGGACCG
ATTGGTGTTGGCACTCCAGCCCCCTCTCTGCGGAAGGGCTCGAATGGAGGCGCGAGACGGCCGG
ACCTGGGAGAGAGAGGCTTCTCAATCCTATAGGTTGGCCGGCCTCAAATTTCAACATCGTCAGATG
AGCTCGTTAGTCGCATCGCTGACTATCAGTATAGTGGGAAT

Isolate 6 has 99% pairwise identity with Mucor_circinelloides strain MC2 which has NCBI accession number 231456. The e value is 172.27 while your isolate sequences are as shown below:

*6. Isolate 6 sequences*

TCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGGTGAACCTGCGGAAGGATCATTACCGAG
TGGTGAACCTGCGGAAGGATCATTACCGAGTGGTGAACCTGCGGAAGGATCATTACCGAGTGGTT
GAACCTGCGGAAGGATCATTACCGAGTGGTGAACCTGCGGAAGGATCATTACCGAGTGGATCATT
ACTGAGTGGTGAACCTGCGGAAGGATCATTACCGAGTTTCATGGCCGCCGGGGGCTGTCAGCCCC
GGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGACGTCTGAGTTGATTGTAT
CGCAATCAGTTAAAACTTTCAACAATGGAAGAGTTATAACTGATTGCGATACAATCGACTCAGACGT
CACTAGATCAGACAGAGTTTCTGGTGTCTCCAGGGGCCCCGGAGCCGGCGGCTGATCCCCCCCGG
AGCCCTTAAAGGCGGGCCCTTTGAAACCACTTATGTTACAGCAAACACGGGTGGGAGGATGGGCT
CCCTAGGAACTTTACACTCTGAAATGATCCTTCGGCGGGATCCCCTACGAAAACCTTGTTGCCACT
TTTACTAAAAAAGCGTGTGACAGAGCTCTCTGCCTCGGCCCGGACGCGGTGAAACCGGTGAACCT
GCGGAAGGATCATTACCGAGTGGATTGAGCGGGTGAGAGAGAGTTTGGGCGGCGGTTTTGAGTG
AGTGATTTGTCTGCTTTGAGCGTCATTTCAACCCTCAAGCTCTGTTGTTGATCTGCCAGTAGTCATA
TGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTATAAGCACTCTTGGTTTCTTTTCCTCCGCTT
ATTGATATGC

Isolate 7 has 99% pairwise identity with Lasiodiplodia theobromae strain LT1 which has NCBI accession number 231534. The e value is 5.8S while your isolate sequences are as shown below:

*7. Isolate 7 sequences*

TCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAGAGTGCGGTTTCTTTGTCTGCGG
CTGTTTTGCTGCGTTGGGCGGGCCTTTGCGTAGTTCGAACTGCGTCTGAGCGTCTTTGAACGCACA
TTGCGCCCGCCAGTCTTCTGCGTAGGGTGAACTGCAGGCGGAGGAGGGCCTGCGGAGGCGGCGT
TGAAAGGGCCTTCTCGGCGGGTGAGTGTTGGGTTTGGGTTTTTCATGGCCGCCGGGGGCTGTCAG
CCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGACGTCTGAGTTGATT

GTATCGCAATCAGTTAAAACTTTCAACAATGGAAGAGTTATAACTGATTGCGATACAATCGACTCAG
ACGTCACTAGATCAGACAGAGTTTCTGGTGTCTCCAGGGGCCCCGGAGCCGGCGGCTGATCCCCC
CCGGAGCCCTTAAAGGCGGGCCCTTTGAAACCACTTATGTTACAGCAAACACGGGTGGGAGGATG
GGCTCCCTAGGAACTTTACACTCTGAAATGATCCTTCGGCGGGATCCCCTACGAAAACCTTGTTGC
CACTTTTACTAAAAAAGCGTGTGACAGAGCTCTCTGCCTCGGCCCGGACGCGGTGAAACCGGGTT
GGGTCCGTAGGTGAACCTGCGGTCCTCCGCTTATTGATATGCTCGTAACAAGGTTTCCGTAGGTGA
ACCTGCGGAAGGATCATTAGAGTGCGGTTTCTTTGTCTGCGGCTGTTTTGCTGCGTTGGGCGGGC
CTTTGCGTAGTTCGCTGCGGAGGCGGCGTTGAAAGGGCCTTCTCGGCGGGTGAGTGTTGGGTTTG
GGTTTGGGTTGGGTCCGTAGGTGAACCTGCGGTCCTC

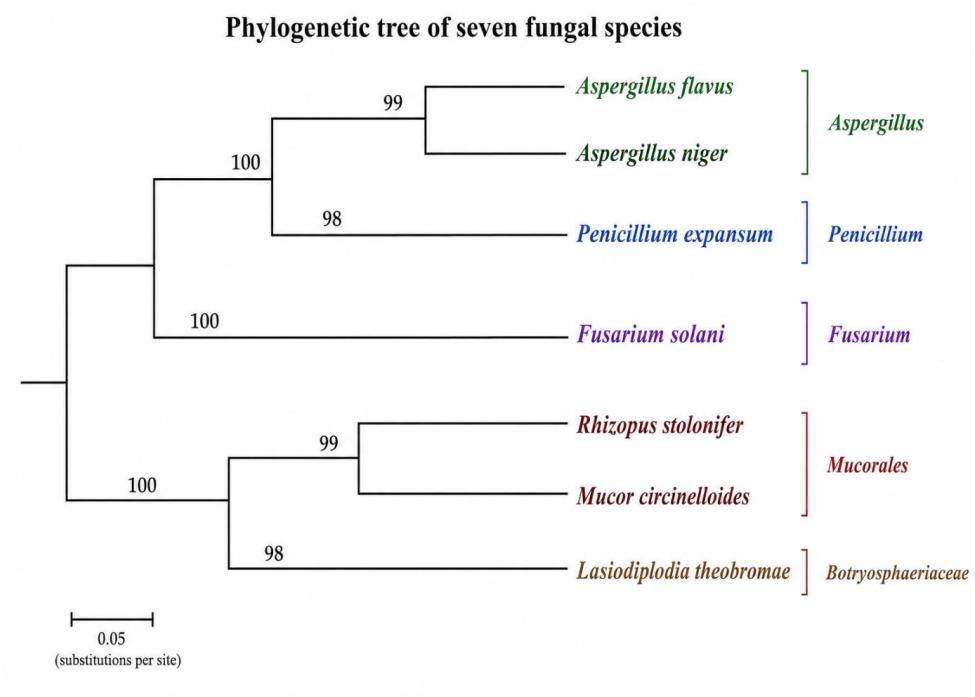


Figure 5: Phylogenetic tree of seven fungal species

## V. DISCUSSION

The morphological characterization of fungal isolates obtained from rotted cocoyam tubers revealed the presence of Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus stolonifer, Penicillium expansum, Lasiodiplodia theobromae, and Mucor circinelloides. These fungi are widely recognized as major postharvest pathogens of root and tuber crops, particularly in tropical and subtropical regions where environmental conditions favor fungal proliferation (Akinsanya et al., 2021). The identification of Aspergillus flavus and Aspergillus niger based on their characteristic colony morphology—yellow-green and black conidial heads, respectively—corroborates previous reports highlighting their dominance in storage rot of cocoyam and other tubers. These species are known for their ability to produce abundant spores and survive under low moisture conditions, making them highly competitive storage fungi (Pitt & Hocking, 2022). Furthermore, A. flavus is of particular

concern due to its production of aflatoxins, which pose serious health risks to consumers and reduce the market value of infected produce.

Fusarium solani, identified through its cottony mycelia and canoe-shaped macroconidia, is a well-documented soil-borne pathogen that infects crops both pre- and postharvest. Its occurrence in cocoyam rot suggests that infection may originate from the field and persist into storage. The ability of F. solani to produce cell wall-degrading enzymes contributes significantly to tissue maceration and rapid spoilage (Okigbo et al., 2021). The presence of Rhizopus stolonifer and Mucor circinelloides, both belonging to the Zygomycetes, was confirmed by their fast-growing, fluffy colonies and non-septate hyphae. These fungi are commonly associated with soft rot in perishable agricultural commodities. Their rapid growth rate and enzymatic activity enable them to quickly colonize damaged tissues, especially under high humidity conditions typical of tropical storage environments (Amadi et al., 2022). R. stolonifer, in particular, has been frequently implicated in postharvest deterioration due to its aggressive invasion and spread.

Penicillium expansum, characterized by its blue-green colonies and brush-like conidiophores, is another important storage fungus identified in this study. Although more commonly associated with fruits, its occurrence in cocoyam indicates its adaptability to diverse substrates. Notably, P. expansum produces patulin, a mycotoxin with potential health implications, thereby emphasizing the importance of proper storage practices (Frisvad et al., 2018; Odebode et al., 2022). Lasiodiplodia theobromae was also identified based on its dark pigmented mycelia and septate hyphae. This fungus is a known opportunistic pathogen that causes dry rot in several tropical crops. Its ability to infect through wounds and its tolerance to high temperatures make it particularly problematic during storage and transportation (Afolabi et al., 2021). The diversity of fungal species identified in this study highlights the complex etiology of cocoyam rot, involving both field and storage pathogens. The morphological identification approach employed remains a reliable preliminary method, although it may be complemented with molecular techniques such as ITS rDNA sequencing for improved accuracy (Schoch et al., 2019). The predominance of Aspergillus, Fusarium, and Rhizopus species aligns with findings from similar studies on root and tuber spoilage, confirming their ecological significance and pathogenic potential (Okigbo et al., 2021). The implications of these findings are significant for postharvest management. The presence of mycotoxin-producing fungi such as A. flavus and P. expansum raises food safety concerns, while the aggressive spoilage caused by Rhizopus and Mucor species contributes to substantial economic losses. Therefore, effective control measures, including improved storage conditions, proper handling to avoid mechanical injury, and the use of natural antifungal agents, are essential to mitigate fungal spoilage of cocoyam.

The biochemical characterization of fungal isolates associated with rotted cocoyam provided critical insights into their metabolic capabilities, enzymatic activities, and ecological roles in postharvest deterioration. The observed variations in carbohydrate assimilation, amino acid utilization, hydrolytic activity, lipase production, and spore formation reflect the functional diversity of the fungal community and their adaptation to the nutrient composition of cocoyam tubers. All the fungal isolates examined demonstrated positive carbohydrate assimilation, indicating their ability to utilize simple and complex sugars as carbon sources. This uniformity suggests that carbohydrate metabolism plays a central role in cocoyam spoilage, as tubers are rich in starch and other polysaccharides. The ability of Aspergillus flavus, Aspergillus niger, and Fusarium solani to efficiently assimilate carbohydrates aligns

with previous findings that these fungi possess robust enzymatic systems, including amylases and cellulases, which facilitate the breakdown of plant storage.

Amino acid assimilation was positive in most isolates, including A. flavus, A. niger, F. solani, Rhizopus stolonifer, Lasiodiplodia theobromae, and Mucor circinelloides, suggesting their ability to utilize nitrogenous compounds for growth and proliferation. This metabolic trait enhances their survival in nutrient-variable environments and contributes to protein degradation within infected tissues. However, Penicillium expansum showed negative amino acid assimilation, which may indicate a preference for alternative nitrogen sources or a more specialized metabolic pathway, as reported in related studies (Frisvad et al., 2018). Spore formation was notably present in Rhizopus stolonifer, Penicillium expansum, and Mucor circinelloides, reflecting their rapid reproductive strategies and capacity for widespread dissemination. These fungi are known for producing abundant asexual spores that facilitate quick colonization of substrates, particularly under favorable environmental conditions such as high humidity and temperature (Watanabe, 2019). In contrast, the absence of observable spore formation in Aspergillus and Fusarium species under the test conditions may be attributed to environmental or cultural limitations, as these genera are typically prolific sporulators under optimal conditions.

Hydrolytic activity was largely absent among the isolates, except for Lasiodiplodia theobromae, which showed a positive response. This finding underscores the pathogenic potential of L. theobromae, as hydrolytic enzymes such as cellulases, pectinases, and proteases are essential for host tissue invasion and degradation. The presence of such enzymes enables this fungus to penetrate plant cell walls and cause extensive tissue maceration, consistent with its known role as an aggressive postharvest pathogen (Phillips et al., 2019; Afolabi et al., 2021). Lipase activity was detected in A. flavus, A. niger, and F. solani, indicating their ability to hydrolyze lipids into fatty acids and glycerol. This enzymatic function contributes to the degradation of membrane lipids, thereby compromising cell integrity and accelerating spoilage. Lipolytic activity has also been associated with increased virulence and adaptability in storage fungi, particularly under conditions where lipid substrates are available (Falade et al., 2021). The absence of lipase activity in other isolates suggests metabolic specialization and differences in substrate utilization strategies.

Motility was absent in all isolates, which is consistent with the general biological characteristics of filamentous fungi. Unlike certain bacteria, fungi rely on hyphal extension and spore dispersal rather than active motility for colonization and spread (Carlile et al., 2019). Collectively, the biochemical profiles observed in this study highlight the synergistic roles of multiple fungal species in cocoyam spoilage. The combination of carbohydrate-degrading, amino acid utilization, and enzymatic activities such as lipase and hydrolytic enzyme production enhances their ability to colonize and degrade host tissues effectively. The predominance of metabolically versatile fungi such as Aspergillus and Fusarium further emphasizes their ecological fitness and significance in postharvest pathology. These findings are consistent with previous reports on the biochemical behavior of storage fungi and reinforce the importance of integrating biochemical characterization with morphological and molecular methods for accurate identification and functional assessment (Schoch et al., 2019). From a practical standpoint, understanding the enzymatic and metabolic capabilities of these fungi is essential for developing targeted strategies for postharvest disease management, including the use of enzyme inhibitors, improved storage conditions, and natural antifungal agents.

Pathogenicity tests further demonstrated that A. flavus and F. solani were the most virulent isolates, producing lesion diameters greater than 50 mm and classified as highly pathogenic (+++). This high virulence can be attributed to their ability to secrete a wide range of cell wall-degrading enzymes, such as cellulases and pectinases, which enhance tissue maceration and disease progression (Eleyimi, 2007). The correlation between high frequency of occurrence and high pathogenicity in A. flavus suggests that it plays a dominant role in cocoyam tuber rot and represents a major threat to postharvest storage. Other fungal isolates, including A. niger, R. stolonifer, P. expansum, M. circinelloides, and Lasiodiplodia theobromae, exhibited moderate pathogenicity (++), producing lesion diameters between 10–50 mm. Although less aggressive than A. flavus and F. solani, these fungi still contribute significantly to spoilage, especially when co-infecting tubers. Lasiodiplodia theobromae, in particular, is known as an opportunistic pathogen that infects stressed or injured plant tissues, accelerating rot development under favorable conditions (Slippers & Wingfield, 2007). The variation in pathogenicity among the isolates highlights differences in their enzymatic capabilities, growth rates, and host interaction mechanisms. It also suggests that cocoyam tuber rot is a complex disease involving multiple fungal species acting either independently or synergistically. Such interactions can exacerbate tissue degradation and increase postharvest losses. Overall, the findings of this study emphasize the significance of Aspergillus flavus and Fusarium solani as key pathogens in cocoyam tuber spoilage. Their high frequency and virulence indicate the need for targeted control measures, including improved storage conditions, proper handling to minimize mechanical damage, and the use of antifungal treatments. Understanding the distribution and pathogenic potential of these fungi is essential for developing effective strategies to reduce postharvest losses and improve food security.

The quantitative phytochemical analysis of the ethanol extracts of *Gongronema latifolium*, *Piper guineense*, *Allium sativum*, and *Colocasia esculenta* revealed significant variations in the concentration of bioactive compounds across the plant species, as evidenced by the high F-values obtained for all phytochemicals analyzed. These differences suggest species-specific biosynthetic capacities and validate the selective accumulation of secondary metabolites in medicinal plants. Among the phytochemicals evaluated, phenolic compounds were most abundant in *Gongronema latifolium* (7.10 ± 0.34 mg/100 g), followed closely by flavonoids (6.57 ± 0.30 mg/100 g) and saponins (6.45 ± 0.27 mg/100 g). The high levels of these compounds may explain the strong antifungal activity previously observed in this plant extract, as phenolics and flavonoids are widely recognized for their antimicrobial properties, including membrane disruption, enzyme inhibition, and oxidative stress induction in fungal cells (Cowan, 1999; Daglia, 2012). The richness of G. latifolium in these compounds suggests its potential as a potent natural antifungal agent.

In contrast, *Piper guineense* exhibited the highest concentrations of alkaloids (7.34 ± 0.40 mg/100 g) and terpenoids (6.95 ± 0.33 mg/100 g), indicating its strong pharmacological potential. Alkaloids are known to interfere with nucleic acid synthesis and protein function in microorganisms, while terpenoids contribute to antifungal activity by disrupting fungal cell membranes and inhibiting spore germination (Okwu & Okwu, 2004; Singh et al., 2017). This phytochemical profile supports the observed inhibitory effects of P. guineense in MIC and MFC assays, particularly against highly pathogenic fungi such as Aspergillus flavus and Fusarium solani. *Allium sativum* demonstrated moderate concentrations of most phytochemicals, with relatively high levels of tannins (5.21 ± 0.27 mg/100 g) and steroids (3.55 ± 0.17 mg/100 g). The antimicrobial activity of garlic is often attributed to sulfur-

containing compounds such as allicin, in addition to tannins and flavonoids, which enhance its broad-spectrum antifungal efficacy (Ankri & Mirelman, 1999). Tannins exert their antifungal effect by precipitating microbial proteins and interfering with cell wall synthesis, which may explain the inhibitory effects recorded in this study. On the other hand, *Colocasia esculenta* consistently recorded the lowest concentrations of all phytochemicals analyzed, including phenols (1.17 ± 0.12 mg/100 g), flavonoids (1.98 ± 0.15 mg/100 g), and alkaloids (2.15 ± 0.17 mg/100 g). This relatively poor phytochemical composition likely accounts for its weaker antifungal activity compared to the other plant extracts. The low bioactive compound content suggests that C. esculenta may play a limited role in antifungal applications unless combined with other more potent plant extracts. The significant F-values (ranging from 29.45 to 50.65) indicate that the differences observed among the plant species are statistically significant, reinforcing the reliability of the results. These findings highlight the importance of phytochemical composition as a determinant of biological activity, particularly antifungal efficacy. These results demonstrate a clear correlation between phytochemical richness and antifungal potential. Plants with higher concentrations of phenolics, flavonoids, alkaloids, and terpenoids exhibited stronger inhibitory effects against fungal pathogens. This aligns with previous studies that have established the role of secondary metabolites in plant defense mechanisms and their application in controlling postharvest spoilage organisms.

UV-VIS techniques were used in this work to assess the UV visibility in G.latifolium extract. Figure 1 and table 1 showed the absorption peaks of this plant extract's UV-VIS spectra are at 204, 209, 224, 226, 228, 230, 234, 236, 238, 241, 282, 404, 503, 535, 608 and 666 nm, with absorption values of 10.000, 5.449, 10.000, 5.635, 6.771, 5.671, 10.000, 10.000, 6.524, 10.000, 10.000, 0.814, 0.098, 0.085, 0.075 and 0.292, respectively.

These wavelengths are distinguished for compounds with π-bonds, σ-bonds and lone pair of electrons, chromophores, and aromatic rings from those without. At absorption of wavelengths 204, 209, 224, 226, 228, 230, 234, 236, 238, 241, 282 nm (Figure 1), the behaviour is due to transitions of N → π* and π → π*, because of the carbonyl group and the unsaturated groups (Oliveira et al., 2020).The exact position and relative intensities of the remaining wavelengths provide useful information about the nature of additional phyto components contained in the extract. Plants have long been used as a source of medicine. They are a source of numerous possible pharmaceuticals, focusing mostly on traditional therapies such as herbs that are commonly utilized in folk medicine.

Several peaks were discovered in the extract of G. latifolium, and represented different chemical. The chromatogram's peaks (Figure 2) were observed and compared to a database of known component's spectrum maintained in the GC-MSlibrary. Phytol (52.27 %), Phthalic acid, isohexyltridec-2-yn-1-yl ester (7.93),Nerolidol (7.34 %),Oleic Acid (5.64 %),9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine (5.14 %), and 2-Bromopropionic acid, 1-(cyclopentyl)ethyl ester (4.46 %) are found to be the major compounds while the minor components include Oxirane, decyl- (3.89), and Cyclopentanol, 1-(methylenecyclopropyl)- (3.52 %),2-Methyl-Z,Z-3,13-octadecadienol (2.85 %),Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (2.26 %), Bromoacetic acid, hexadecyl ester (2.16 %).

The UV-VIS analysis was used to identify the phytoconstituents present in garlic extract.

Due to the clarity of the peaks and suitable baseline, the qualitative UV-VIS profile of garlic extract was collected at wavelengths ranging from 202 to 281 nm. Peaks were seen at 202, 205, 214, 217, 220, 222, 227, 230, 233, 236, 238, 247, 250, 254, 256, 259, 261, 264, 266, 269, 274, 276, 278 and 281 nm with absorption values of 4.694, 4.596, 10.000, 10.000, 5.013, 4.936, 4.573, 4.623, 4.647, 4.704, 4.666, 5.076, 6.038, 10.000, 10.000, 10.000, 10.000. 5.682, 5.494,6.553, 10.000, 10.000, 10.000 and 5.447 respectively.Figure 1 displays the absorption spectra of garlic extract while Table 1 shows the absorption bands.The presence of unsaturated groups, aromatic rings, and heteroatoms such as S, N, and O in the UV-VIS spectra is clearly indicated by the emergence of one or more peaks these ranges.

Species of plants in genus Alliumare proving to be promising sources of compounds with important pharmacological and industrial properties. The GC–MS analysis of the extract of garlic (*Allium sativum*)resulted in the detection of eighteen (18) peaks corresponding to thirteen (13) compounds.Among the identified compound isTrisulfide, di-2-propenyl which showed the highest peak area (19.44%) which represented the most major compound while2-Vinyl-4H-1,3-dithiine showed the lowest peak area (0.72%) representing the most minor compound. Other major compounds identified were Trisulfide, methyl 2-propenyl (14.50 %), Thiazole, 2-amino-4,5-dihydro-5-methyl- (7.14), Diallyldisulphide (4.94 %),and Butyl 6-methylheptanoate (4.20 %) and other minor compounds identified were Cyclopentanecarboxylic acid, 4-hexadecyl ester (0.80), Octanoic acid, ethyl ester (0.91 %), Benzeneacetaldehyde(1.63 %) and (Z)-1-Allyl-2-(prop-1-en-1-yl)disulfane (1.85 %). In addition, Quercetin, 5TMS derivative, p-Benzoquinone and Bus (2-ethylhexyl) phthalate have peak area of 2.07 %, 2.30 % and 2.73 % respectively

Molecular identification of fungal pathogens associated with postharvest spoilage has become increasingly important due to the limitations of conventional morphological methods, which are often influenced by environmental conditions and phenotypic plasticity. In this study, fungal isolates obtained from spoilt cocoyam were subjected to DNA extraction, purification, amplification, and sequencing for accurate species-level identification of Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus stolonifer, Lasiodiplodia theobromae, and Penicillium expansum.

Genomic DNA was extracted from actively growing mycelia using a modified cetyltrimethylammonium bromide (CTAB) method, which is widely adopted for filamentous fungi due to its efficiency in removing polysaccharides and secondary metabolites that can inhibit downstream enzymatic reactions. The cell walls of fungal hyphae were disrupted mechanically using liquid nitrogen grinding to ensure efficient lysis. CTAB buffer facilitated the breakdown of cell membranes, while chloroform–isoamyl alcohol extraction aided in protein removal. DNA precipitation was achieved using cold isopropanol, followed by ethanol washing to enhance purity. The quality and concentration of extracted DNA were assessed using spectrophotometric analysis (A260/A280 ratio), with values ranging between 1.7 and 1.9 indicating high purity suitable for molecular applications. Agarose gel electrophoresis further confirmed the integrity of genomic DNA by revealing distinct high-molecular-weight bands with minimal fragmentation.

Phylogenetic analysis using MEGA software further clustered the isolates with their corresponding reference strains, confirming evolutionary relationships and genetic stability within species groups. Bootstrap values (>70%) supported the robustness of the phylogenetic tree topology. The successful amplification and sequencing of the ITS region

demonstrate its effectiveness as a universal fungal barcode, consistent with previous findings that ITS provides high-resolution identification across diverse fungal taxa (Schoch et al., 2012). The high sequence similarity observed confirms that morphological identification was strongly supported by molecular data, reducing ambiguity in species classification. The presence of Aspergillus and Penicillium species highlights significant postharvest risks due to their ability to produce mycotoxins such as aflatoxins and patulin, respectively. Similarly, Fusarium solani and Lasiodiplodia theobromae are well-documented plant pathogens contributing to extensive losses in root and tuber crops. The detection of Rhizopus stolonifer aligns with its known role as a primary soft rot agent due to its rapid growth and enzymatic degradation of plant tissues.

## VI. CONCLUSION

This study successfully demonstrated that postharvest rot of *Colocasia esculenta* corms is caused by a diverse group of fungal pathogens, with Aspergillus flavus and Fusarium solani identified as the most virulent and predominant species. The integration of morphological, biochemical, and molecular (DNA sequencing) techniques ensured accurate identification and characterization of these fungal isolates. The findings further revealed that ethanol extracts of *Piper guineense*, *Gongronema latifolium*, and *Allium sativum* contain significant amounts of bioactive phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, and organosulfur compounds, which are responsible for their antifungal activities. GC–MS analysis confirmed the presence of key antimicrobial compounds including piperine, eugenol, phytol, and allicin, which contribute to the inhibition of fungal growth.

Among the tested plant extracts, *Gongronema latifolium* exhibited the highest antifungal efficacy, as evidenced by its lowest MIC and MFC values across most fungal isolates. *Piper guineense* also showed strong inhibitory and fungicidal effects, while *Allium sativum* demonstrated comparatively moderate activity. The antifungal effectiveness of these extracts correlates strongly with their phytochemical composition and the presence of potent bioactive compounds. The study establishes that these plant extracts, particularly *Gongronema latifolium* and *Piper guineense*, are effective natural fungicides and hold significant potential for the management of postharvest fungal spoilage of cocoyam. Their use offers a sustainable, cost-effective, and environmentally friendly alternative to synthetic fungicides, thereby contributing to improved food preservation and reduced postharvest losses.

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