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# EFFECTS OF LEAF EXTRACTS OF *Piper guineense* AND *Lasienthera africanum* ON FUNGAL PATHOGENS ASSOCIATED WITH SPOILT FRUIT FROM AKWA IBOM STATE

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## ABSTRACT

Effects of the extracts of *Piper guineense* and *Lasienthera africanum* on fungal pathogens associated with spoilt fruit from Akwa Ibom State were evaluated. Phytochemical screening of the plant extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides in varying amounts in both the ethanolic and aqueous extracts. FT-IR spectrum analyses of the ethanolic *Lasienthera africanum* extract revealed prominent absorption bands located at 3404, 2935, and 1600cm$^{-1}$ while the aqueous extracts had intense peak bands at 1631 and 1103 cm$^{-1}$. Absorption bands in the region of 3416, 2928, 1604, and 1122.cm$^{-1}$ for ethanolic extracts of *Piper guineense* and 3404, 2929, 1616, and 1078 cm$^{-1}$ for aqueous extracts were obtained. Inhibitory activities of the extracts against the mycelial growth and spore germination of an array of fungal that cause fruit rots showed that their antifungal potential was concentration dependent. Extracts of *Lasienthera africanum* at a concentration of 750mg/L proved highly effective with zones of inhibition that ranged from 11.7 - 19.3 mm for inhibiting mycelial growth and spore germination of all the test fungal isolates except *Trichoderma koningiopsis* and *Purpureocillium lilacinum*. Also, antifungal activity above 10 mm was observed in 500 mg/mL and 750 mg/mL of ethanolic extracts of *Piper guineense* on *Talaromyces verruculosus*, *Trichoderma koningiopsis*, *Penicillium citrinu*, *Fusarium solani*, *Mucor racemosus*, etc. showed the extract as a potential antifungal source. Evidently, both plant extracts have great potential as natural antifungals which can be safely used against post-harvest rot of fruit.

**KEYWORDS**: *Piper guineense*, *Lasienthera africanum*, Phytochemical Analysis, Antifungal Activities, FT-IR Spectrum, Preservative Purpose

## INTRODUCTION

Plants are said to be rich in secondary metabolites with varied chemical diversity thus a valuable resource for possible development of new pharmaceuticals. Plants provide excellent perceptions for the discovery of new therapeutic products, such as biofungicides development and nutraceuticals for the prevention of mycological illness. Several approaches to deter mycotoxin contamination on both pre-harvest and post-harvest have been employed not limited to the use of Genetically modified crops, synthetic fungicides, and a toxigenic fungus /bacteria to control toxigenic fungal strains, all tailored to control crop contamination occasion by phyto-pathogenic fungi; however, these are expensive to most resource poor farmers (Salako, 2002).

Synthetic fungicides application appears to be the most favoured method, but due to negative environmental hazards, long degradation periods, and toxigenic fungal strains developing resistance to most synthetic fungicides, there is a need for a search for an alternative less expensive and eco-friendly method to control fungal contamination both pre and post-harvest (Daferera *et al*., 2000). Many plant pathogens are now resistant to most of the synthetic fungicides currently in use (Udousoro and Ekanem, 2013; White *et al*., 2015). Also, the use of fungicides as pathogen control or preservatives is being discouraged due to their negative effects on human health. Hence, this necessitates the need for novel sources of antifungal agents, to replace ineffective fungicides. Local edible medicinal plant extracts from *P. guineense* and *L. africanum* may be possible sources since edible medicinal plants can be used to interfere efficiently with the growth of food-borne pathogens on fresh fruits and thus can be used as bio preservatives to inhibit the growth and colonization of fungal pathogenic and spoilage organisms.

*P. guineense* (Family: Piperaceae) is a plant tropical to West Africa (Uhegbu *et al*., 2015) popularly known as African black pepper. In Nigeria, it is commonly known as Uziza in Igbo and Iyere in Yoruba. Its seeds and leaves are used as spices in the preparation of certain types of foods such as the popular hot soup or pepper soup usually consumed by nursing mothers after childbirth to aid uterine contraction and consequently, placenta expulsion or the expulsion of remains in the woman's womb (Udoh *et al*., 1999). In traditional medicine, the leaves have been associated with the management of problems of infertility in women and for treating respiratory ailments.

*Lasienthera africanum* called "Editan by Akwa Ibom and Cross River is a member of the order Celestrales which consists of thirteen families of trees and shrubs with simple leaves. It belongs to the family Icacinacea. These kinds of plants and shrubs are of both nutritional and medicinal importance (Sofowora, 1993). *L. africanum* is consumed as vegetables in the South-Eastern State of Nigeria, especially the Akwa-cross region. It is believed to have cooling effects on the body, purifying effects, and prevent internal bleeding. Fruits have high nutritional value as they are rich in sugars, minerals, vitamins, and other biotic molecules. They help in the repair and control of body processes. They also serve as a good source of income for the people. However, the contamination of fresh fruits with plant and human pathogens can cause considerable economic losses for the industry, apart from being the origin of food-borne diseases (Barros *et al*., 2012; Ebong *et al*., 2014). This study was therefore aimed at assessing the antifungal potential of ethanol and aqueous leaf extracts of two indigenous medicinal plants; *Piper guineense* and *Lasienthera africanum* in Akwa Ibom State, Nigeria against fungal pathogens associated with post-harvest rots of selected fruits in Akwa Ibom State, Nigeria.

## MATERIALS AND METHODS

### Isolation and identification of fungal pathogens from the fruits

Fungal isolates were obtained from diseased fruits of *Persia americana*, *Citrussinensis*, *Carica papaya*, *Annona muricata*, and *Lycopersicon esculentum*. The fruits were obtained from local markets in the three senatorial districts of Akwa Ibom State, Nigeria. Prior to use, the fruit were washed with clean water and surface sterilized in 10% hypochlorite solution. Sterile scalpel was used to cut from the diseased portions and aseptically plated on Potato Dextrose Agar (PDA) medium and incubated at 28$^{o}$C ± 2$^{o}$C. Pure cultures of each isolate were obtained after a series of inoculation onto PDA and incubation at 28$^{O}$C ± 2$^{O}$C for five days. Preliminary identification was carried out by wet-mounting the fungal mycelium on lacto phenol-cotton blue and observing under × 40 objective lens of the phase contrast microscope. Colony colour, growth pattern on plates, details of philiades and spores

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were used as identification parameters (Cheesebrough, 2005).

**Preparation of aqueous and ethanolic leaf extracts**

The leaves of *Piper guineense* and *Lasienthera africanum* were separately cut into pieces and dried in shades for 48hrs and then ground into a coarse powder. The powdered dried leaves were placed in maceration tanks and water or ethanol added for aqueous and ethanolic extractions respectively. The macerated leaves were allowed to stand for 24 h (aqueous) and 72 h (ethanol) before filtration. The liquid crude extract was first concentrated in a rotary evaporator and then in a water bath at $45^{O}$C until it was fully dried. The dried crude extract was then transferred into 100ml beakers, covered with aluminum foil, and stored in a refrigerator at $4^{O}$C till required.

**Phytochemical constituents and functional group characterization of the extracts**

The phytochemical constituents of the crude extracts of the plant leaves were analysed using the methods described by Trease and Evans (2009). The following phytochemical was screened for: alkaloids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones, resins, deoxy-sugar, protein, and phlobatanins. The functional groups in the extracts were determined using Fourier Transform Infra-red (FT-IR) spectrometer Varian 660 MidIR Dual MCT/DTGS Bundle with ATR.

**Determination of the inhibitory activity of extracts on fungal spore germination**

The fungal isolates were inoculated into sterile conical flasks containing 100 mL Potato.Dextrose Broth (PDB), covered with sterile cotton wool and aluminum foil and placed in a water bath with a shaker and gently shake for 48 hours at 30ºC. Fungal spores of each isolate were obtained by filtering with sterile filter paper (0.45µm – pore-size filter, Millipore). Inhibitory activity of the extracts on fungal spore germination was carried out using the agar-well, diffusion method.

To determine the inhibitory activity of the leaf extracts against fungal spore germination, $10^{6}$ conidia/ml of the fungal spores were dispensed into different sterile Petri dishes and freshly prepared PDA poured unto it. These were allowed to solidify. Four wells of 8mm per plate were made using a cork borer. One millilitre of aliquots of the leaf extracts at various increasing concentrations (250 mg/mL, 500 mg/mL, and 750 mg/mL) were added three wells while the fourth well received 1mL of the solvent dimethylsulfoxide (DMSO), and served as control. The experiment was carried out in triplicates and the plates were incubated at $28^{O}C \pm 2^{O}C$ for 72 h. The zones of inhibition were measured using a transparent meter rule.

**Use of the leaf extracts as preservative agents**

Fresh healthy fruits were purchased from the market and surface sterilized by washing in tap water and soaking in 10% hypochlorite for 30 min. The fruits were soaked inside antifungal leaf extracts at different concentrations (250 mg/mL, 500 mg/mL, and 750 mg/mL) for 20 min, allowed to air-dry under the laminar flow sheet while other fruits were left without the leaf extract. Fungal spores ($10^{6}$conidia/ml) were applied unto the surface of all the fruits using a sterile plastic spreader. The fruits were wrapped loosely with sterile aluminum foils and left at ambient temperature for 14 days. The appearance of fungal growth on the surface of the fruits was monitored.

**RESULTS**

**Percentage yields of plant extracts**

The extract yields ranged from 14.7 to 16.3 % and from 17.5 to 21.6 % for the aqueous and ethanolic extracts, respectively. Largest extract yield was detected in the *Lasienthera africanum* when ethanol was the extraction solvent used (Table 1).

Table 1: Percentage yields of the plant extracts

| | Extract yield (%) | |
|---|---|---|
| | **Aqueous extract** | **Ethanol extract** |
| *Lasienthera africanum* | 14.7 | 21.6 |
| *Piper guineense* | 16.3 | 17.5 |

**Phytochemical constituents of the extracts**

The phytochemical constituents identified in the ethanol leaf extracts of *P. guineense* were alkaloids, saponins, tannins, flavonoids, cardiac glycosides, deoxy-sugar, and proteins while both free and combined anthraquinones and phlobatanins were not detected. Similarly, alkaloids, saponins, tannins, flavonoids, cardiac glycosides, deoxy-sugar, and proteins were detected in the *L. africanum* ethanol extract. (Table 2). For the aqueous extracts, phytochemicals detected in the *P. guineense* were alkaloids, saponins, tannins, flavonoids, cardiac glycosides, deoxy-sugar, and proteins while anthraquinones, resin and phlobatanins were not detected. In the aqueous extracts of *L. africanum*, the phytochemicals detected were alkaloids, saponins, tannins, flavonoids, cardiac glycosides and deoxy-sugar, whereas the presence of anthraquinones, resin, proteins, and phlobatanins were not detected. (Table 3).

Table 2: Phytochemical constituents of ethanol leaf extracts of the plants

| Constituents | Tests | Observation/Inference | |
|---|---|---|---|
| | | *P. guineense* | *L. africanum* |
| Alkaloids | Picric test | +++ | + |
| | Drangendorff's | +++ | ++ |
| | Mayer's | ++ | + |
| Saponins | Frothing | + | + |
| | Sodium bi-carbonate | ++ | + |
| Tannins | Ferric chloride | + | ++ |
| | Bromine water | + | ++ |
| Flavonoids | Shinoda reduction | +++ | + |
| | Alkaline reagent | + | ++ |
| | Aluminum chloride | + | ++ |
| Cardiac glycoside | Salkowski's | + | + |
| | Lieberman's | ++ | + |
| | Keller-Killiani's | + | + |
| Anthroquinones | Borntrager's for free anthraquinones | - | - |
| | Borntrager's for combined anthraquinones | - | - |
| Resin | Acetone water | - | - |
| Deoxy sugar | Benedict | + | + |
| | Fehling | + | + |
| Proteins | Xanthoproteic | ++ | - |
| | Biuret | + | - |
| Phlobatanins | General | - | + |

'+++', '++', '+', and '- 'represent detected very high concentration, detected in moderately high concentration, detected

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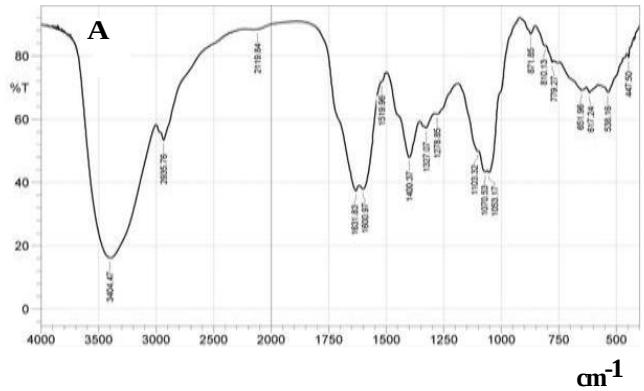
Table 3: Phytochemical constituents of aqueous leaf extracts of the plants

| Constituents | Tests | Observation/Inference | |
|---|---|---|---|
| | | *P. guineense* | *L. africanum* |
| Alkaloids | Picric test | ++ | + |
| | Drangendorff's | +++ | ++ |
| | Mayer's | ++ | + |
| Saponins | Frothing | + | + |
| | Sodium bi-carbonate | + | + |
| Tannins | Ferric chloride | ++ | + |
| | Bromine water | + | + |
| Flavonoids | Shinoda reduction | ++ | + |
| | Alkaline reagent | + | ++ |
| | Aluminum chloride | + | + |
| Cardiac glycoside | Salkowski's | + | + |
| | Lieberman's | ++ | ++ |
| | Keller-Killiani's | + | + |
| Anthroquinones | Borntrager's for free anthraquinones | - | - |
| | Borntrager's for combined anthraquinones | - | - |
| Resin | Acetone water | - | - |
| Deoxy sugar | Benedict | + | + |
| | Fehling | + | + |
| Proteins | Xanthoproteic | ++ | - |
| | Biuret | + | - |
| Phlobatanins | General | - | - |

'+++', '++, '+' and '- represent detected very high concentration, detected in moderately high concentration, detected in low concentration, and not detected, respectively

**Fourier Transform Infrared Spectroscopic Analysis of Extracts**

Absorption bands with corresponding functional groups of aqueous and ethanol leaf extracts of *L. africanum* are presented in Fig. 1. Prominent peaks around 3404, 2935 and 1600 $cm^{-1}$ were evident of –OH, -C-H stretching, and -C=C aromatic in both aqueous and ethanolic extracts of *L. africanum* respectively similar to report by Mohammed 2021 and Singh *et al.* 2021. However, the ethanolic extract has -C=O conjugated ketone which could be ascribed to the nature of the extractant (ethanol). Similarly, some very important bands in the aqueous extract at 1631 and 1103 $cm^{-1}$ were as a result of the presence of C=O stretching of ester. -CO group of lactones (Fig. 1).



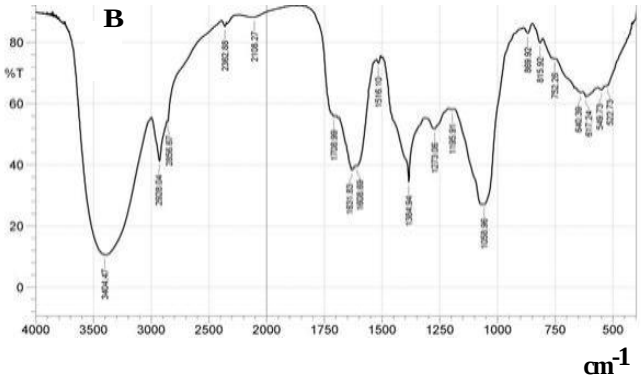




Fig. 1: Absorption bands of the *L. africanum*: A and B represent aqueous and ethanol extracts, respectively.

*P. guineense* extracts had fewer bands unlike *L. africanum* but with a little difference owing to the presence of -C=O stretching of ester. Significant bands at 3416, 2928, 1604 and 1122 $cm^{-1}$ of the aqueous extracts of *P. guineense* were ascribed to -OH, -C-H, -C=C, and -C=O (stretching of ester) in that order. The ethanolic extract on the other hand manifested peaks at 3404, 2929, 1616, and 1078 $cm^{-1}$ similar to that of aqueous extracts *P. guineense* except for the band at 1078 $cm^{-1}$ that was ascribed to the –C-O group in lactones (Fig. 2)

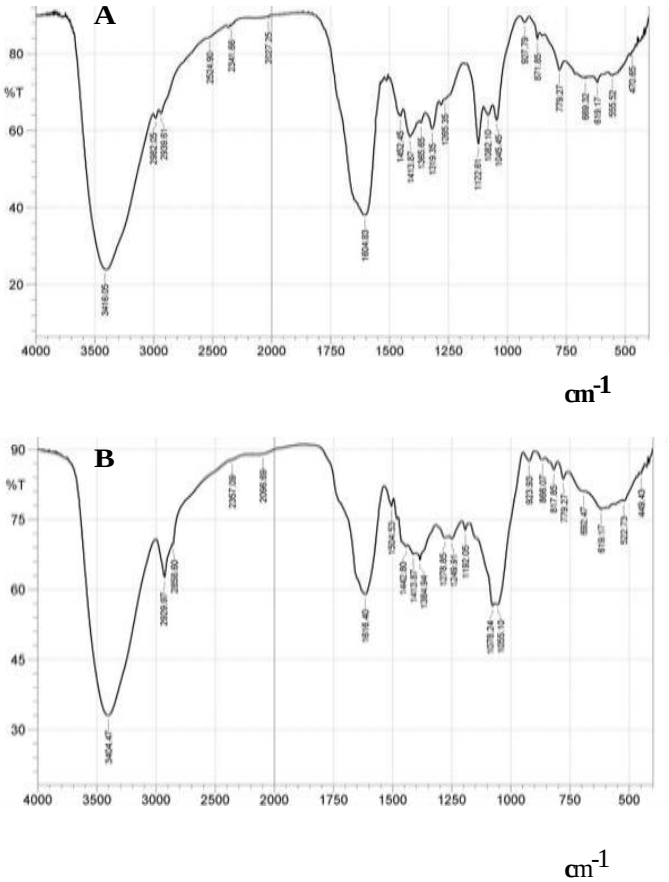



Fig. 2: Absorption bands of the *Piper guineense*: A and B represent aqueous and ethanol extracts, respectively

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**Antifungal activities of leaf extracts on spore germination of fungal isolates**

In presence of the *P. guineense* extracts, higher zones of inhibition against the test fungal isolates were observed with the ethanol extract than the aqueous extract. Degree of inhibition was directly proportional to the concentration of extract. No inhibition of *an theobromae* was observed, except when ethanol extract concentration of 750 mg/mL was used. None of the concentrations of the extract showed inhibition of the growth of *Moniliella suaveolens*. Growth inhibition of *Geotrichum candidum, Pichia kudriavzevii,* and *Fusarium culmorum was* only observed at extract concentrations higher than 250 mg/mL (Table 4). When the extracts of *L. africanum were* used, inhibition of *Talaromyces verruculosus, Trichoderma koningiopsis, Rhizopus oligosporus,* and *Mucor racemosus* were not observed at the different concentrations of the aqueous extract used for investigation. When the ethanol the extract was used, a concentration of 750 mg/mL was observed to inhibit all the fungal isolates investigated. Generally, the highest zone of inhibition of 20 mm (*Lasiodiplodia theobromae*) and 17 mm (*Moniliella suaveolens*) was reported at a concentration of 750 mg/mL for the ethanol and aqueous extract, respectively (Table 5).

Table 4: Antifungal activities of ethanol and aqueous extracts of the *P. guineense* on fungal spore germination

| | Ethanol extract (mg/mL) | | | Aqueous extract (mg/mL) | | |
|---|---|---|---|---|---|---|
| Fungal isolates | 250 | 500 | 750 | 250 | 500 | 750 |
| *Talaromyces verruculosus* | 11.0 ± 2.00 | 16.3 ± 2.31 | 19.3 ± 1.53 | 8.7 ± 1.15 | 13.3 ± 1.15 | 16.3 ± 0.58 |
| *Lasiodiplodia theobromae* | NZ | NZ | 10.7 ± 1.53 | NZ | NZ | NZ |
| *Trichoderma koningiopsis* | 10.0 ± 1.00 | 13.3 ±1.53 | 16.7 ±1.53 | 9.3 ± 1.53 | 11.0 ± 0.00 | 13.3 ± 1.53 |
| *Penicillium citrinum* | 11.7 ± 1.53 | 13.7 ± 0.58 | 15.7 ± 0.58 | 10.3 ± 0.58 | 12.7 ± 0.58 | 15.3 ± 0.58 |
| *Pichia kudriavzevii* | NZ | 9.0 ± 1.73 | 13.0 ± 1.73 | NZ | 8.0 ± 0.00 | 10.7 ± 1.15 |
| *Rhizopus oligosporus* | 10.0 ± 1.73 | 12.3 ± 0.58 | 17.0 ± 2.65 | 10.0 ± 0.00 | 11.7 ± 0.58 | 12.7 ± 1.15 |
| *Aspergillus niger* | 10.0 ± 2.00 | 14.0 ± 1.00 | 18.3 ± 0.00 | 9.3 ± 0.58 | 12.0 ± 1.00 | 15.0 ± 0.00 |
| *Fusarium culmorum* | NZ | 9.00 ± 1.73 | 13.0 ± 1.73 | NZ | NZ | 12.0 ± 1.00 |
| *Fusarium solani* | 10.7 ± 2.31 | 14.3 ± 1.53 | 18.0 ± 0,00 | 9.7 ± 0.58 | 13.8 ± 1.53 | 15.3 ± 0.58 |
| *Aspergillus carbonarius* | 8.7 ± 0.58 | 12.7 ± 0.58 | 16.0 ± 2.00 | 8.00 ± 0.00 | 11.3 ± 0.58 | 14.0 ± 1.15 |
| *Aspergillus paraciticus* | 10.3 ± 1.15 | 13.7 ± 0.58 | 15.0 ± 2.65 | 11.0 ± 1.73 | 13.0 ± 1.00 | 14.0 ± 0.00 |
| *Moniliella suaveolens* | NZ | NZ | NZ | NZ | NZ | NZ |
| *Mucor racemosus* | 11.7 ± 1.53 | 14.0 ± 1.73 | 17.6 ± 0.58 | 10.7 ± 1.53 | 15.0 ± 1.73 | 18.3 ± 1.53 |
| *Aspergillus sclerotiorum* | 12.3 ± 0.58 | 16.0 ± 1.00 | 19.3 ± 2.31 | 11.0 ± 1.00 | 14.3 ± 0.58 | 19.0 ± 1.73 |
| *Geotrichum candidum* | NZ | NZ | 11.7 ± 1.53 | NZ | NZ | 9.0 ± 0.00 |
| *Candida tropicalis* | 1.0 ± 1.00 | 13.7 ± 1.53 | 16.0 ± 1.73 | 8.7 ± 0.58 | 13.0 ± 1.73 | 15.0 ± 1.73 |
| *Absidia corymbifera* | 10.3 ± 0.58 | 13.0 ± 0.00 | 16.3 ± 0.58 | 9.7 ± 1.53 | 10.0 ± 1.00 | 14.0 ± 1.00 |
| *Candida utilis* | 8.7 ± 0.68 | 11.0 ± 0.00 | 14.0 ± 1.73 | 9.0 ± 1.00 | 10.0 ± 1.73 | 11.7 ± 1.15 |
| *Aspergillus aculeatus* | 9.3 ± 1.15 | 13.0 ± 1.75 | 17.7 ± 1.15 | 10.0 ± 0.00 | 12.3 ± 2.08 | 16.0 ± 2.00 |
| *Aspergillus nomius* | NZ | NZ | NZ | NZ | NZ | NZ |
| *Purpureocillium lilacinum* | 10.3 ± 0.58 | 11.0 ± 1.73 | 17.0 ± 0.00 | NZ | 8.3 ± 0.58 | 12.0 ± 1.73 |
| *Candida pseudotropicalis* | NZ | NZ | NZ | NZ | NZ | NZ |

Values represent zones of inhibition and ± standard deviation of means in mm. NZ = No Zone of inhibition

Table 5: Antifungal activities of ethanol and aqueous extracts of the *L. africanum* on fungal spore germination

| Fungal isolates | Ethanol extract (mg/mL) | | | Aqueous extract (mg/mL) | | |
|---|---|---|---|---|---|---|
| | 250 | 500 | 750 | 250 | 500 | 750 |
| *Talaromyces verruculosus* | NZ | 10.7 ± 2.08 | 15.3 ± 1.15 | NZ | NZ | NZ |
| *Lasiodiplodia theobromae* | 13.3 ± 1.15 | 16.7 ± 1.15 | 20.0 ± 2.65 | 11.7 ± 0.58 | 13.3 ± 1.53 | 14.3 ± 0.58 |
| *Trichoderma koningiopsis* | NZ | NZ | NZ | NZ | NZ | NZ |
| *Penicillium citrinum* | 11.7 ± 1.15 | 14.7 ± 1.15 | 17.7 ± 2.08 | 9.7 ± 0.58 | 12.0 ± 0.00 | 15.0 ± 1.73 |
| *Pichia kudriavzevii* | 10.3 ± 1.15 | 16.0 ± 2.08 | 18.0 ± 1.73 | 8.7 ± 1.15 | 12.0 ± 1.73 | 13.7 ± 2.08 |
| *Rhizopus oligosporus* | NZ | NZ | 10.0 ± 0.00 | NZ | NZ | NZ |
| *Aspergillus niger* | 11.0 ± 1.73 | 14.7 ± 1.53 | 17.3 ± 1.53 | 9.3 ± 1.53 | 12.3 ± 1.53 | 15.3 ± 0.58 |
| *Fusarium culmorum* | 11.7 ± 1.15 | 13.7 ± 1.15 | 17.3 ± 0.58 | 8.7 ± 0.58 | 11.3 ± 1.15 | 15.3 ± 1.53 |
| *Fusarium solani* | 12.0 ± 1.73 | 16.0 ± 2.00 | 19.0 ± 1.73 | 9.0 ± 0.00 | 10.3 ± 0.58 | 14.3 ±1.53 |
| *Aspergillus carbonarius* | 10.7 ± 1.15 | 12.0 ± 1.0 | 15.0 ± 1.73 | 8.30 ± 0.58 | 10.0 ±1.00 | 15.3 ± 1.15 |
| *Aspergillus paraciticus* | 11.3 ± 1.53 | 14.3 ± 1.53 | 16.0 ± 1.73 | 9.30 ± 0.58 | 11.7 ± 0.58 | 15.3 ± 1.53 |
| *Moniliella suaveolens* | 11.7 ± 0.58 | 13.3 ± 0.58 | 18.7 ± 1.53 | 12.0 ± 1.00 | 15.0 ±1.73 | 17.0 ± 2.65 |
| *Mucor racemosus* | NZ | 10.7 ± 0.58 | 14.3 ± 1.53 | NZ | NZ | NZ |
| *Aspergillus sclerotiorum* | NZ | NZ | 11.7 ± 1.15 | NZ | NZ | NZ |
| *Geotrichum candidum* | 12.0 ± 1.00 | 14.7 ± 2.08 | 18.3 ± 3.21 | 9.00 ± 0.00 | 12.7 ± 1.53 | 15.0 ± 1.73 |
| *Candida tropicalis* | 10.7± 1.00 | 14.7 ± 1.00 | 17.3 ± 0.58 | 10.3 ± 0.58 | 12.3 ± 0.58 | 16.3 ± 1.53 |
| *Absidia corymbifera* | 12.2 ± 0.58 | 15.7 ± 1.53 | 20.0 ± 1.73 | 11.0 ± 1.15 | 13.7 ± 1.15 | 16.3 ± 0.58 |
| *Candida utilis* | 11.7 ± 0.58 | 15.0 ± 1.00 | 18.3 ± 1.53 | 10.3 ± 0.58 | 14.3 ± 0.58 | 17.0 ± 1.73 |
| *Aspergillus aculeatus* | 11.0 ± 1.15 | 13.7 ± 1.15 | 17.0 ± 1.00 | 9.30 ± 1.15 | 12.3 ± 1.53 | 14.3 ± 0.58 |
| *Aspergillus nomius* | 10.3 ± 0.58 | 12.7 ± 0.58 | 15.3 ± 1.53 | 9.0 ± 0.00 | 11.3 ±1.15 | 13.7 |
| *Purpureocillium lilacinum* | NZ | NZ | NZ | NZ | NZ | NZ |
| *Candida pseudotropicalis* | 9.3 ± 1.53 | 13.3 ± 1.53 | 16.0 ± 1.73 | 8.7 ± 0.58 | 10.0 ± 2.65 | 13.7 ± 1.52 |

Values represent zones of inhibition and ± standard deviation of means. NZ = No Zone of inhibition

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**Use of leaf extracts as bio-preservatives**

Fruits that had undergone treatments with the respective extracts were observed to remain relatively healthy up to fourteen days of post-treatment storage while the unprotected fruits developed fungal growth showing signs of spoilage within five days of storage.

## DISCUSSION

*Piper guineense* and *Lasienthera africanum* leaf extracts exhibited antifungal activities on the various rot pathogens used in this study. The choice of these two plants was informed by an earlier report on their antimicrobial activities (Abo *et al*., 2011; Ire and Eruteya, 2017). Antifungal potentials observed in these selected medicinal plants were concentration-dependent in line with earlier reports (Ire and Eruteya, 2017). At a higher extract concentration of 750mg/mL, more active ingredients were expected to be present which translated to higher activity. The activity observed at 750 mg/mL and all the other concentrations can be attributed to the effect of the extract and not the solvent, as there was no zone of activity when only the solvent was used. Antifungal activity above 10 mm was observed in 250 mg/mL, 500 mg/mL, and 750 mg/mL of ethanolic extract of *Piper guineense* on *Talaromyces verruculosus*, *Trichoderm koningiopsis*, *Penicillium citrinu*, *Fusarium solani*, *Mucor racemosus*, etc. showed the extracts a potential antifungal source. However, antifungal activity was less in aqueous extract than in ethanolic extract of *Piper guineense*, especially at low concentrations of 250 mg/mL and 500 mg/mL. This difference in activity based on solvent was in line with the report of Abo *et al*., 2011; Their profile also agreed with reports by previous researchers (Abo *et al*., 2011; Ire and Eruteya, 2017). Generally, low activity of $\leq$ 15 mm was observed across the plant extracts used against fungal spores in this study was due to higher resistance a fungal spore usually shows compared to that of vegetative cells. This level of inhibition observed makes the extract be possible sources of potent preservatives (Mostafa *et al*., 2017).

Antifungal activities observed in this research can be due to the presence of high concentration of alkaloids in *P. guineense* (Maatalah *et al*., 2012). Presence of moderate to the high concentration of cardiac glycosides in this study might have contributed to the observed antifungal activity also (Ajaib *et al*., 2018). Arekemase *et al*. (2015) assessed the presence of these phytochemicals and reported their impacts to act as preservatives due to their antimicrobial activity. This further aligns with the use of these plants in medicine is due to the presence of bioactive constituents, some of which were detected in this study e.g. flavonoids, tannins, and alkaloids, present in the leaves. Naturally, some phytochemicals in some fruits have been proven to reduce the growth of fungi associated with fruit rots (Whitehead *et al*., 2015) and can be applied in a more effective way to prevent the rot of fruits.

## CONCLUSION

High concentrations of *Lasienthera africanum* and *Piper guineense* showed activity against the mycelial growth and spore germination of an array of fungi that caused rots in fruits from Akwa Ibom State. The inhibition that was concentration specific had the highest concentrations showing maximum inhibition in the mycelial growth, and spore germination, followed by lower concentrations. These plants thus have potentials to be used as new natural fungicide for the management of fungal diseases of fruits, following further research.

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