

Profiling of phytochemicals and evaluation of antifungal activity of *Piliostigma* species on *Aspergillus* species causing tomato fruit rot

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Abstract. Mahmud AA, Danladi MD, Adi BA, Abdulrahman MD, Nafi'u BS, Mohammed UA. 2023. Profiling of phytochemicals and evaluation of antifungal activity of *Piliostigma* species on *Aspergillus* species causing tomato fruit rot. *Asian J Trop Biotechnol* 20: 62-68. Medicinal plants, such as *Piliostigma reticulatum* (DC.) Hochst. and *Piliostigma thonningii* (Schumach.) Milne-Redh. from the family Fabaceae are considered therapeutic due to the bioactive compounds present in their organs. These medium-sized, leguminous trees, native to the tropics and common in Nigeria, are rich in antifungal and antioxidant compounds. The study focused on the phytochemical components of these plants and evaluation of antifungal activity which was tested against *Aspergillus* fungi species isolated from rotten tomatoes. Identification and quantification of the plants' phytochemical constituents of ethanolic and aqueous extracts were conducted, assessing the antifungal activity of the extracts and determination of the extracts Minimum Fungicidal (MFC) and Inhibitory Concentrations (MIC). From the study, these extracts contained biologically active compounds, including alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and anthraquinones. The inhibitory effect of the ethanolic extracts ranged between (4-42mg/mL) and (5-31mg/mL) for *Aspergillus niger* and *Aspergillus fumigatus*, respectively. While aqueous extracts was recorded (6-20mg/mL) and (7-15mg/mL). The MIC values of ethanolic extracts have (6-8mg/mL) and (7-26mg/mL), and MFC values of (28-30mg/mL) and (22-28mg/mL) against *A. niger* and *A. fumigatus*. The aqueous extracts have MIC values (32-38mg/mL) and (36-59mg/mL), MFC values ranged (50-56mg/mL) and (38-65mg/mL) against *A. niger* and *A. fumigatus*, respectively. The study has shown that the plant extracts contained antifungal properties based on bioactive compounds found in their leaves and antifungal effects demonstrated against the tested organisms. The findings suggest the use of these plant extracts, which could serve as potential bio-fungicides when explored further. The study of the bioactive compounds of these plants provides a foundation for creating natural, safer medications and innovative bio-fungicide products. Subsequent investigations on the specified plants will be based on the framework established by this study. Future research, including in vivo testing and exploration of the mechanism of action, is recommended.

Keywords: Agriculture, extraction, isolation, medicinal plants, tomato

INTRODUCTION

Medicinal plants are globally targeted in agriculture and health to limit the usage of harmful chemicals in food. The World Health Organization defines a medicinal plant as any flora containing therapeutic substances in one or more organs (Behbahani et al. 2020; Memory et al. 2021). Medicinal plants are a rich source of bioactive compounds such as alkaloids, flavonoid, tannins, and phenolic compounds. These compounds can exert various therapeutic effects, from anti-inflammatory and antimicrobial to anticancer and antioxidant properties. Approximately 80% of individuals in developing countries employ non-modern medicine otherwise traditional medicine (Ali et al. 2018; Abdulrahman et al. 2022; 2023). As the world increasingly seeks sustainable solutions, using plant extracts as natural alternatives for managing fungal diseases in agriculture has gained significant attention (Abdulrahman et al. 2019; Abdulrahman et al. 2022).

Plants extracts contain bioactive compounds with a broad spectrum of antifungal activity, targeting multiple

fungal species. This versatility is advantageous as it reduces the need for specific fungicides tailored to individual pathogens, thereby simplifying disease management strategies (Da Silva et al. 2021). Furthermore, unlike synthetic chemical fungicides, plant extracts are generally considered safe for the environment. They are biodegradable and do not persist in the soil or water, minimizing the risk of long-term contamination and ecological disruption. Additionally, plant extracts often are non-toxic to beneficial organisms such as pollinators, predators, and decomposers, thus supporting the overall ecosystem (Ginovyann and Trchounian 2019).

Piliostigma species, such as *P. reticulatum* (DC.) Hochst. and *Piliostigma thonningii* (Schumach.) Milne-Redh., are examples of medicinal plants (Memory et al. 2021; Ogbiko et al. 2021). These species belong to the Sub-family; Caesalpinioideae, in the Family; Fabaceae. These medium-sized, leguminous trees grow wild in the tropics and are common in Nigeria. Despite their similar morphology, *P. thonningii* and *P. reticulatum* have distinct characteristics, such as leaf size and texture (Ogbiko et al.

2021). The local names of *P. reticulatum* and *P. thonningii* are Kargo (or Kalgo) in Hausa (Northern Nigeria), Abafe in Yoruba language (Western Nigeria) and Okpo-atu in Igbo (Eastern Nigeria). Other names include Camel food (English), Pied de Chameau, Semalier (France), and Musacanca (Portugal) (Musa et al. 2015). These plants are also known as Barkee-hi in Fulfulde by Fulani in Northern Nigeria (Musa et al. 2015). These plants' leaves and pods are used as medicine in Nigeria to treat various diseases, including cough, diarrhea, and ulcers. Plant extracts, derived from various parts, offer a promising avenue for sustainable disease management, providing effective control while minimizing negative impacts on the environment and human health.

In agriculture, plant diseases caused primarily by various fungi result in considerable economic losses and pose health risks due to the mycotoxins produced. Fungal diseases significantly threaten global Agriculture, causing substantial early yield losses. Animal pests contribute to approximately 18% of agricultural crop losses, while microbial diseases and weeds account for 16% and 34%, respectively (Pawel et al. 2020). Fungal pathogens alone are responsible for about 70-80% of crop losses. The yearly economic impact of crop losses caused by fungal pathogens has reached a staggering £200 billion (Singh and Pulikkal 2022). Traditional disease control methods such as synthetic fungicides have proven effective but often with undesirable environmental and health consequences. Continuous and intensive use of synthetic fungicides has led to the emergence of resistant fungal strains, rendering many chemical control options ineffective. Among the fungal pathogens, *Aspergillus niger* and *Aspergillus fumigatus* are particularly destructive to tomatoes. *Aspergillus*, a genus with over 200 species, is economically significant despite not being a major cause of plant

diseases. Some species, such as *A. niger* and *A. flavus*, can cause diseases in susceptible plant varieties (Abdulrahman et al. 2019). These pathogens can contaminate crops at various stages, forming harmful mycotoxins (Nogueira et al. 2021). Tomatoes (*Solanum lycopersicum* L.), a significant crop worldwide and notably in Nigeria, are particularly vulnerable to fungal pathogens. These pathogens cause considerable spoilage and decrease in tomato Production, especially in comparison to temperate zones (Pawel et al. 2020).

Based on the search from the available databases such as Google Scholar, Hindawi, Pub Med, the majority of the available studies on pharmacological properties of these plants focused on antimicrobial effects of the leaves (Ogbiko et al. 2021). Hence, the study was necessary to fill the existing research gap on the antifungal effect of the plants. The present study was designed to profile some phytochemicals and evaluate antifungal activity (in vitro) of *Piliostigma* species leaves extracts on *Aspergillus* fungi, which could be a promising alternative to chemical fungicides. The successful application of the extracts can lead to a significant advancement in sustainable agriculture.

MATERIALS AND METHODS

Study area

Dutsin-Ma Local Government Area, Katsina State, Nigeria was the site where the research was conducted (Figure 1). The Local Government Area is located 60 Kilometers from Katsina Metropolis, it has an area of about 527km², and a population of 169,671 according to 2006 Nigerian Census. In terms of geographical coordinates, Dutsin-Ma lies between Latitude 12° 27' 18" North and 7° 29" East (Fanen and Olalekan, 2014).

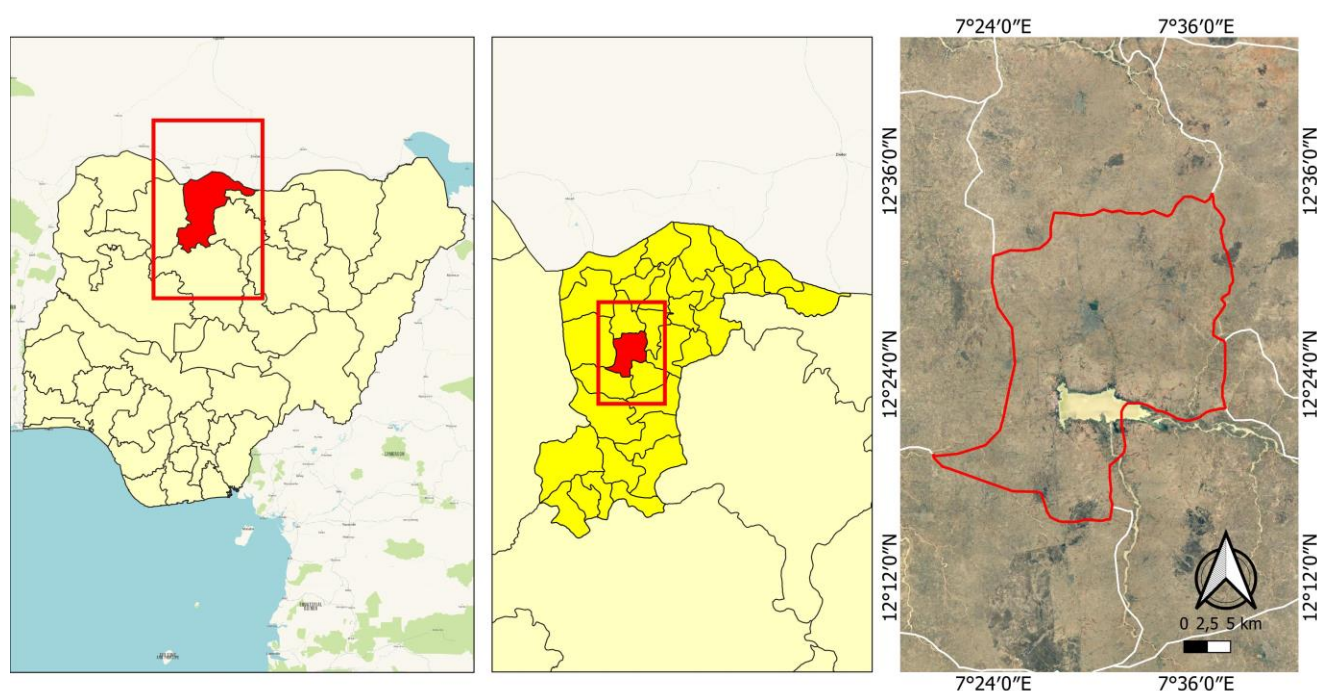


Figure 1. Dutsin-Ma local government area map, Katsina State, Nigeria

Collection of plants, identification and preparation

The *P. reticulatum* and *P. thonningii* leaves were collected from natural habitat at Dabawa Area, in Dutsin-Ma (12° 27' 18" N and 7° 29" E), Katsina State, Nigeria. The leaves were authenticated and identified by Plant Scientist at Department of Plant Science and Biotechnology, Federal University Dutsin-Ma, Katsina State, Nigeria. The voucher numbers for *P. reticulatum* FUDMA/PSB/00136 and *P. thonningii* FUDMA/PSB/00140 were obtained and deposited at the herbarium unit of Plant Science and Biotechnology Department, Federal University, Dutsin-Ma, Katsina State, Nigeria. The leaves were washed with distilled water, and then, air dried for 21 days. Finally, ground into a fine uniform powder using mortar and pestle following the procedure of (Ginovyan and Trchounian 2019).

Extraction of plants' Leaves

Cold maceration technique was employed during the extraction of the plants' leaves. Two solvents, ethanol and water were opted for the extraction of *P. thonningii* and *P. reticulatum* leaves. About 100g of the leaves powder of each plant was immersed with 500 mL of each solvent separately. Each suspension was prepared in a bottle with intermittent shaking and soaked for 24 hours. All extracts were filtered separately using a Whatman No.50 filter paper, the water filtrates were heated on a hot plate at 100°C to allow the water vapor to evaporate, while the ethanol extracts were kept for a week to allow the ethanol to escape leaving behind the solid extracts. All the extracts were put to an oven at 25°C for a week, after which they were subject to phytochemical tests to identify and quantify some selected phytochemical constituents of the plants following the procedure (Oladunmoye et al. 2018). Thus, the percentage yield of the extracts was calculated:

Percentage (%) = weight of dried extract (g) / weight of dried plant sample (g) × 100. For *P. reticulatum* ethanol extract = $50/100 \times 100 = 50\%$, for *P. thonningii* ethanol extract = $45/100 \times 100 = 45\%$, for *P. reticulatum* water extract = $30/100 \times 100 = 30\%$, for *P. thonningii* water extract = $25/100 \times 100 = 25\%$.

Qualitative and quantitative phytochemical tests

Plant aqueous and ethanolic extracts were tested to identify the presence of five (5) phytochemical constituents, such as flavonoids, alkaloids, saponins, tannins, and cardiac glycosides followed by the quantity identification (Afolayan et al. 2018).

Collection of tomato samples and isolation of fungi

Rotten tomatoes were purchased from Dutsin-Ma Wednesday Market in Dutsin-Ma, Katsina State, Nigeria. It was then transported to the Federal University Dutsin-Ma Biology laboratory under a cold chain. The edges of the rotting tomato samples were cut into thin sections (2mm in diameter), which were then sterilized for 2 minutes with 1% Sodium hypochlorite, immersed in 70% ethanol for an additional minute, and then rinsed in distilled water. The thin slices were placed on Petri dish with Potato Dextrose Agar (PDA) media that have been mixed with the antibiotic

Gentamycin, and they were cultured for 7 days at a temperature of 28°C. Then, aseptically performing multiple colony development transfers from PDA plates resulted in the creation of a pure culture (Asma et al. 2018).

Morphological and molecular identification of the fungi

For macroscopic identification, the appearance, variation in medium color and development of the colony were noted on the Petri dish. For microscopic identification; a sterile inoculating loop was used to apply a thin smear of fungal isolates from 5-7 day old culture aseptically to a clean glass slide. The mixture was then combined with a drop of Lacto phenol cotton blue, coated with a cover slip, and examined with a ×100 magnification light microscope. The conidia, spores, and hyphae shapes were noted according to Yau et al. (2020). Molecular identification of the fungi at the species level, DNA extraction, gene amplification, and sequencing were performed (Iyanyi et al. 2021).

Determination of antifungal activity

About 10,000 µg/mL of the stock solutions of the leaves extract (both ethanolic and aqueous) of each plant was dissolved in Dimethyl Sulfoxide (DMSO) in a 1% (weight/volume) concentration, the following concentrations were prepared from the stock solutions 10, 50, and 100mg/mL, respectively (Afolayan et al. 2020). The antifungal activity of the fungi was assessed using the poisoned food method (Tasneem et al. 2021). Potato Dextrose Agar (PDA) was employed as the culture medium. Dimethyl sulfoxide (DMSO) at a concentration of 1% (weight/volume) was used to dissolve the plant extracts. Following this, 0.1mL (containing 1mg of the specific plant extract being tested) was transferred to a sterile Petri dish and allowed to harden. Each Petri dish's centre was inoculated with a 5mm block of each fungus's mycelium. The mycelium block was established by applying a corkscrew to the developing region of a 5-day-old culture of the tested fungi on a PDA medium. To maximize contact between the culture medium and the fungal mycelium, the fungi blocks were positioned at the centre of each Petri dish in an inverted posture. The fungi's mycelium growth was then gauged. This experiment was carried out thrice (Erhonyota et al. 2020; Tasneem et al. 2021).

Determination of MIC and MFC of plants' extracts

Broth dilution method determined the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). All antifungal tests were performed in Potato Dextrose Broth (PDB) mediums. Serial dilutions of plant extracts, starting from 1-60 mg/mL, were used. Tubes were inoculated with 1 mL of fungal spore suspension and incubated at a temperature 28±2°C for 72 hours. The MIC and MFC were determined according to changes in optical density following conidial germination 72 hours after inoculation using a UV-Spectrophotometer (Model AE-450, 2003, Japan). Fungal growth was indicated by turbidity and a "pellet" on the bottom of the tube. The MIC value was determined as the lowest plant extract concentration that inhibits the fungi's visible

growth. At the same time, MFC was the concentration by which the extracts inhibited the growth of the fungi at 99.9% level. The experiment was conducted in triplicate (Babatunde et al. 2023).

Statistical analysis

Variance Analysis (ANOVA) was employed to analyze the data, a General Linear Model with the MINITAB Statistical Package version 17. Means separation was done using the Tukey Kramer comparison test at ($P \leq 0.05$).

RESULTS AND DISCUSSION

High yield of ethanolic extracts

Several technical procedures have been used to extract medicinal plants. Some new methods are still being developed and existing methods are being modified (Sasidharan et al. 2011; Azwanida 2015). Choosing an appropriate extraction method is very important and in some cases depends on the intended use of the extract. The extraction method used; cold maceration, was opted because it mirrors traditional herbalist techniques. The choice of water and ethanol in the current study was based on the fact that, ethanol and water are being used in preparation of food for consumption (Abdulrahman et al. 2023). The high polarity of ethanol, one of the solvents used for the extraction, could explain its polarity when compared with aqueous extracts. The highest yield was recorded from ethanol extraction (50% and 45%) from *P. reticulatum* and *P. thonningii* ethanol extracts (Table 1). This might be attributed to high polar solvents' ability to extract more phytochemical constituents from plant materials. Ethanol's strong polarity allows it to extract more phytochemicals than water. The difference in phytochemicals identified from the plant extracts is insignificant, as only alkaloids were recorded to be absent in the aqueous extracts (Table 1). The research agrees with Abdulrahman et al. (2022) who reported a high yield of ethanol extract from the study of in vitro biological investigations on *Syzygium polyanthum* (Wight) Walp. cultivars. Similarly, the research disagrees with Namadina et al. (2020), who reported a high yield of aqueous extract against ethanol extract in the study of the phytochemical and antibacterial activities of *Vitellaria paradoxa* C.F.Gaertn. stem bark and root extracts against some clinical isolates of respiratory tract infections. According to Abdulrahman et al. (2022) and Ferreira et al. (2022), the variation in the extract yield might be due to the extraction

type, the extraction solvent's nature, and the solvent's ability to extract more compounds from the extract.

Phytochemical constituents of the plant extracts

A qualitative preliminary test was carried out on some selected phytochemicals of *Piliostigma* species leaves extracts, which revealed the following phytochemicals, namely flavonoid, alkaloids, saponins, tannins, cardiac glycosides and anthraquinones except for alkaloids, that have been absent in both aqueous extracts of the plants (Table 1). On the other hand, the quantity of these phytochemicals has been reported, with flavonoid recording the highest quantity in both plants (150mg/mL and 136mg/mL), followed by Tannins, which also recorded (130mg/mL and 128mg/mL) (Table 2). The high Flavonoid content in the plants might be the reason why the plants leaves are being used to treat cough, and various transmissible diseases in Dutsin-Ma. Because it is a well-known fact that flavonoid have been known to treat various antimicrobial activities according to researchers such as, Mithilesh et al. (2013), Ruvimbo and Stanley (2017), Pratiwi et al. (2021), Aboudou et al. (2022), and Ferreira et al. (2022). In addition, tannins have also been reported to possess in vitro antimicrobial activities on various organisms (Wada et al. 2019).

Minimal inhibitory and fungal concentrations

All the test organisms responded to the extracts. The values ranged from (6-38 and 7-59 mg/mL) for *A. niger* and *A. fumigatus*. At the same time, the MFC recorded is (28-56 to 22-65mg/mL), respectively (Table 3). The lowest recorded MIC value of (6.00 mg/mL) was achieved on *A. niger*, followed by the value of (7.00 mg/mL) for *A. fumigatus*. The highest values of (56 mg/mL and 65 mg/mL) were observed on *A. niger* and *A. fumigatus*. The MIC/MFC tests demonstrated in the study has shown that, the ethanolic and aqueous extracts of *P. reticulatum* and *P. thonningii* have antifungal properties based on the *invitro* testing of various concentrations of the plant leaves 10, 50, and 100mg/mL (Table 3). The findings of the study support the conclusion of Babatunde et al. (2023) that, the antimicrobial effectiveness of plant extracts depends on concentration. Furthermore, when comparing the MIC and MFC values, a lower value suggests a strong capacity of the plant extracts to combat fungi (Babatunde et al. 2023). It is apparently clear from the study that, the MIC values were consistently lower than the MFC values, suggesting that, the extracts have antifungal properties. Distilled water was used as the control in the experiment which exhibited no inhibition against the fungi (Table 3).

Table 1. Some selected phytochemical constituents of the plants' extracts

| Plants extracts | Plant phytochemicals | | | | | |
|-----------------|----------------------|-----------|----------|---------|--------------------|----------------|
| | Flavonoids | Alkaloids | Saponins | Tannins | Cardiac glycosides | Anthraquinones |
| PREE | + | + | + | + | + | + |
| PTEE | + | + | + | + | + | + |
| PRAE | + | - | + | + | + | + |
| PTAE | + | - | + | + | + | + |

Note: PREE: *Piliostigma reticulatum* ethanol extract, PRAE: *Piliostigma reticulatum* aqueous extract, PTEE: *Piliostigma thonningii* ethanol extract, PTAE: *Piliostigma thonningii* aqueous extract. The +/- represent presence or otherwise absence of the phytochemical

Antifungal activity

Antifungal activity of three (3) concentrations of the plants leaves extracts (10,50, and 100 mg/mL) were evaluated against the tested fungi which was determined by measuring the diameter of mycelia growth inhibition zone of the fungi by poisoned food technique on PDA medium (Table 4). The results showed the extracts' inhibitory effect, ranged from (4-30 mm) against *A. niger* and (4-31 mm) against *A. fumigatus*. Ethanol extracts of *P. reticulatum* and *P. reticulatum* aqueous extracts were the most effective antifungal, followed by aqueous extract of *P. reticulatum* and aqueous extract of *P. thonningii*. A significant difference was recorded from the inhibition of the various extracts against the tested fungi. The study agrees with Perveen et al. (2020) on the study of green and sol-gel synthesis of ZnO nanoparticles and evaluation of antibacterial activity against various pathogens and phytochemical, evaluation of toxicity, antioxidant and antibacterial activities against active secondary metabolites of *P. thonningii* stem bark.

PCR amplification and sequencing

Genomic DNA from the fungal isolates was extracted with an Accu Prep Genomic DNA extraction kit from Bioneer. The genomic DNA of the fungal isolates shows clear bands on the gel when viewed under UV light (Figure 2). ITS1-forward (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4-reverse (5'-TCCTCCGCTTATTGATATGC-3') primer pairs were used to amplify the DNA fragments through Polymerase Chain Reaction (PCR). The expected amplicon size was between 500-700bps. The band size for ITS is 580. The fungal isolates' ITS 1 and 4 gene sequences were determined to be 543 and 586 base pairs. The alignment scores of the sequences were greater than 200 base pairs, which are ideal for identifying of microorganisms. Alignment scores were represented by the red lines in each chromatogram. The isolates were blasted on the NCBI database and identified as: *Aspergillus* species.

DNA sequence produces a nucleotide base sequence of a particular organism and a chromatogram that tells how good the Sequencing is conducted. Chromatogram produces from the sequencing is a four-color chromatogram that is usually generated from the sequence sequence that displays the sequencing run results, where a specific color indicates each nitrogenous base; a chromatogram shows how good a sequence is.

The green color represents the base Adenine, the blue color represents Cytosine, and blackcolor represents Guanine and the red color represents Thymine.

The results of the blasts showed the identity of the fungal organisms as *A. niger* and *A. fumigatus* from isolate 1 and 2. Table 5 shows the taxonomic affinities of the fungal isolates retrieved from Basic Local Alignment Search Tool (BLAST) database.

Macroscopic and microscopic identification of the organism used in the study were not entirely relied upon during the course of the study. The morphological characteristics noted cannot be successfully identified and characterized the fungal organisms up to specie level. This

is why the Polymerase Chain Reaction (PCR) and Sequencing were necessary to concisely reveal the identity of the *Aspergillus* species. Molecular techniques of identification are the most suitable method to provide a complete data on any organism.

Table 2. Quantity of some selected phytochemical constituents of the plant extracts

| Phytochemicals | <i>P. reticulatum</i> (mg/mL) | <i>P. thonningii</i> (mg/mL) |
|--------------------|----------------------------------|---------------------------------|
| Flavonoid | 150.00 | 136.00 |
| Alkaloids | 120.00 | 97.60 |
| Saponins | 115.80 | 125.00 |
| Tannins | 130.00 | 128.00 |
| Cardiac glycosides | 93.40 | 86.50 |
| Anthraquinones | 90.20 | 62.40 |

Table 3. Determination of MIC and MFC of plants' extracts in mg/mL

| Plant Extracts | <i>A. niger</i> | | <i>A. fumigatus</i> | |
|---------------------------|-----------------|-------|---------------------|-------|
| | MIC | MFC | MIC | MFC |
| PREE | 6.00 | 28.00 | 7.00 | 22.00 |
| PTEE | 8.00 | 30.00 | 26.00 | 28.00 |
| PRAE | 38.00 | 50.00 | 59.00 | 65.00 |
| PTAE | 32.00 | 56.00 | 36.00 | 38.00 |
| Distilled Water (Control) | 0.00 | 0.00 | 0.00 | 0.00 |

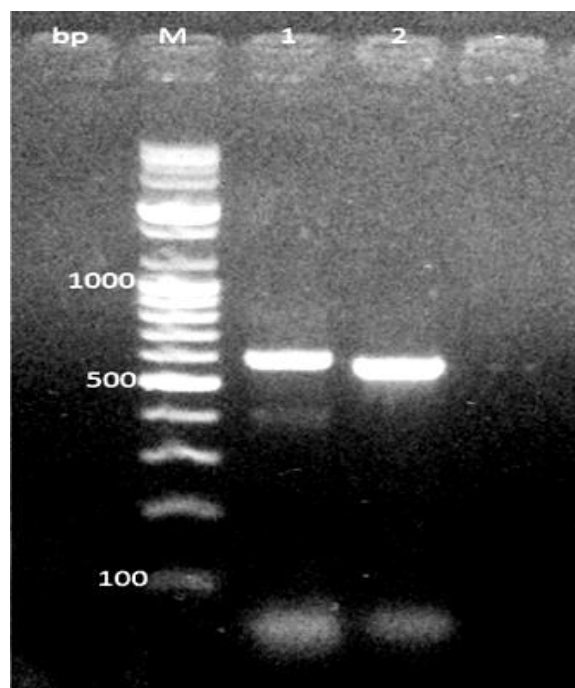


Figure 2. Agarose gel electropherogram showing PCR amplicon. Note: M; represents the DNA ladder, the numbers 1 and 2 represent the fungal isolates, 1and 2 represents *A. niger* and *A. fumigates*, respectively

Table 4. Determination of antifungal activity in mg/mL

| Concentrations(mg/mL) | <i>A. niger</i> | | | <i>A. fumigatus</i> | | |
|-------------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | 10 | 50 | 100 | 10 | 50 | 100 |
| Plant extracts | | | | | | |
| <i>P. reticulatum</i> ethanol | 4.0.0 ^a ± 0.9129 | 20.0 ^a ± 1.041 | 42.0 ^a ± 0.6455 | 13.0 ^a ± 0.7638 | 23.0 ^a ± 1.041 | 31.0 ^a ± 0.7638 |
| <i>P. thonningii</i> ethanol | 7.0 ^b ± 0.9129 | 16.0 ^{ab} ± 1.041 | 30.0 ^b ± 0.6455 | 5.0 ^b ± 0.7638 | 18.0 ^b ± 1.041 | 21.0 ^b ± 0.7638 |
| <i>P. reticulatum</i> aqueous | 6.0 ^b ± 0.9129 | 12.0 ^b ± 1.041 | 20.0 ^c ± 0.6455 | 9.0 ^c ± 0.7638 | 15.0 ^{bc} ± 1.041 | 14.0 ^c ± 0.7638 |
| <i>P. thonningii</i> aqueous | 12.0 ^b ± 0.9129 | 7.0 ^c ± 1.041 | 10.0 ^d ± 0.645 | 7.0 ^c ± 0.7638 | 12.0 ^c ± 1.041 | 10.0 ^d ± 0.763 |
| Distilled Water (control) | 0 | 0 | 0 | 0 | 0 | 0 |

Note: Values are means ± standard error. Means followed by different letter within a column (superscripts) are significantly different at $P \leq 0.05$

Table 5. Taxonomic affinities of the sequences retrieved from NCBI database

| Specie name | % identity | Accession number |
|---------------------|------------|------------------|
| <i>A. niger</i> | 99.63% | MH0375421 |
| <i>A. fumigates</i> | 97.97% | MT4974471 |

To conclude, the study revealed some phytochemicals in both aqueous and ethanolic leaves extracts of *P. reticulatum* and *P. thonningii* with only Alkaloids that recorded absent in all the aqueous extracts. Similarly, the antifungal activity tested *in vitro* on the fungi has shown the efficacy of the extracts against the tested fungal species. The ethanolic extracts were the most effective antifungal followed by the aqueous extracts. The ethanol was the best solvent for the extraction of the plants leaves due to the high extract recorded from the extraction. From the available data obtained from the antifungal activity of the extracts, the study suggests the use of these plants extracts as potential biofungicides when exploration of mechanism of action is investigated.

Recently, there is an absurge of deteriorating activity from the fungal species on plant produce worldwide according to Memory et al. (2021). However, the primary motivation for seeking a safer, natural alternative to synthetic chemical fungicides currently used to control fungal pathogens stems from the considerable risks the chemical fungicides pose to human health, plant life, animals, and the environment. Additionally, the development of resistance strain by fungi has rendered the available synthetic chemicals increasingly ineffective. Consequently, the quest for a safer alternative has become necessary. Plants have long been used in disease management due to the vast array of secondary metabolites they produce, making them a promising option. While the use of plant extracts for fungal disease management holds immense promise, several challenges must be addressed. Standardization of extraction methods, formulation development, and optimization of application techniques are crucial to ensure consistent efficacy and practical implementation. Furthermore, more research is needed to identify and understand the specific bioactive compounds responsible for antifungal activity in these plants. The study recommends a thorough research on the use of medicinal plants extracts for the treatment of various

agricultural diseases. This needs to be explored further in our quest for a more effective and safer biochemical fungicides. The current study is a stepping stone and examined the chemical element and antifungal effect of *P. reticulatum* and *P. thonningii* against *Aspergillus* fungi. To our knowledge, there is no previous documentation in the literature regarding the specific antifungal activity of these two *Piliostigma* species in Dutsin-Ma, Katsina State Nigeria, which underscores the need for this research. Given their richness in secondary metabolites and the demonstrated antifungal activity against the tested fungi as shown from this research, these plants may serve as safer antifungal alternatives to synthetic chemical fungicides. The study recommends further research on antifungal effects on the other plant parts aside from the leaves. In addition, more *in vivo* studies need to be conducted in order to ascertain the plant potential as well as to develop the most effective extracts. Efforts should also be made to isolate and characterize more biologically active compounds from other parts of the plant.

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