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Antifungal Activities of Unripe Plantain (*Musa paradisiaca*) Peels Extract in the Treatment of Skin Fungal Infection- Ringworm (*Tinea corporis*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ringworm, caused by dermatophytes, is a common fungal infection affecting millions of people worldwide each year. In recent years, there has been growing interest in natural remedies for the treatment of skin fungal infections due to concerns about drug resistance and side effects of conventional antifungal medications. Unripe plantain (*Musa paradisiaca*) peel, a byproduct of the plantain fruit, has been identified as a potential natural treatment option for ringworm due to its reported antimicrobial activities. Phytochemicals identified and isolated from the unripe plantain peel included: saponins, steroids, tannins, flavonoids, alkaloids, glycosides, terpenoids and phenols. Saponins and terpenoids have the highest concentration under ethanol and water

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contents. Most of the phytochemicals, appear to be in high concentrations under ethanol content and they include: saponins, flavonoids, alkaloid, terpenoids and phenol. Ethanol and water contents recorded absence of steroids. Macroscopic and microscopic examination of fungi were done from fungal skin infection, with the identification and isolation of four (4) fungi organisms: *Trichophyton* spp, *Aspergillus* spp, *Microsporum* spp and *Candida* spp. This work shows that all the four (4) isolated fungi are susceptible to ethanol extract and fluconazole. The results obtained during the course of 14 days revealed that all the four (4) isolated fungi are susceptible to ethanol extract and fluconazole and the water extract had MFCs (Minimum Fungicidal Concentrations) of ≥ 0.05 mg/ml for *Microsporum* spp, ≥0.01 mg/ml for *Trichophyton* spp and ≥ 0.25 mg/ml for *Aspergillus* spp *and Candida* spp. While these findings are promising, it is important to note that all studies were conducted in vitro, and further research is needed to evaluate the efficacy and safety of using unripe plantain peels for ringworm treatment in clinical settings. Additional studies are warranted to determine the optimal formulation and dosage of unripe plantain peel extract for effective treatment.

Keywords: Antifungal; peels; ringworm; skin fungal infection; unripe plantain extract.

1. INTRODUCTION

"Plantain (*Musa paradisiaca*) is a major food crops in the humid and sub-humid parts of Africa where its starchy fruits are generally cooked or fried before consumption and serves as major sources of energy for millions of people in these regions" (John, 2010). "It belongs to the natural order, plantaginaceae, which contains more than 200 species, twenty-five or thirty of which have been reported. The common plantain has broad, irregular oval leaves, abruptly contracted at the base into a long broad, channelled foot stalk. The fully grown blade is 1.3–2.4 meters long and about two- third as broad, usually smooth, with several parallel veins. Plantain grows more than any other plant in compacted soils, is abundant beside paths, roadside and other areas with frequent soil compaction" (Johann et al., 2007). It is also common in grassland and as a weed among crops.

Ringworm, caused by dermatophytes such as *Trichophyton*, *Microsporum*, and *Epidermophyton* species, is a common fungal infection affecting millions of people worldwide each year according to World Health Organization, (2019). Unripe plantain peel (*Musa paradisiaca*), a byproduct of the plantain fruit, has been identified as a potential natural treatment option for ringworm due to its reported antimicrobial activities (Qadim et al., 2013). Ogbe et al., (2020), opined that the peel of unripe plantain contains several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenols, which have been proven to exhibit antimicrobial properties. Research has shown that unripe plantain peel may inhibit the growth and proliferation of dermatophytes responsible for causing the infection (Ajijolakewu et al., 2021). Further studies are required to investigate the mechanisms of action and optimize the use of unripe plantain peel as an adjunct therapy for ringworm (Njoku et al., 2019).

Ringworm is a common fungal infection of the skin caused by dermatophytes such as *Trichophyton*, *Microsporum*, and *Epidermophyton* species (UpToDate, 2020). According to the World Health Organization, (World Health Organization, n.d.), ringworm affects around 20 million people worldwide each year, with an estimated 10% of the global population affected at some point in their life. Traditional antifungal treatments for ringworm include topical and oral medications such as terbinafine, clotrimazole, and griseofulvin. However, these treatments can be costly, have potential side effects, and resistance to some antifungal agents has been reported (Gupta & Cooper, 2008).

The use of natural products like extracts from plant as alternative therapies for disease treatment has gained attention in recent years (Afam-Ezeaku et al., 2022; Okigbo & Afam-Ezeaku, 2018). Unripe plantain (*Musa paradisiaca*) peel, a byproduct of the plantain fruit, has been identified as a potential natural treatment option for ringworm due to its reported antimicrobial activities (Sanchez, 2014). The peel of unripe plantain contains several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenols, which have been proven to exhibit antimicrobial properties (Adeyemi & Akanji, 2011). Several studies have explored the potential of unripe plantain peels in the treatment of ringworm, suggesting that it may inhibit the growth and proliferation of dermatophytes responsible for causing the infection.

One study by Owoseni et al., (2018), reported that silver nanoparticles synthesized with the extract of *Musa paradisiaca* (unripe plantain) leaves exhibited potent anti-dermatophyte activity. The authors proposed that bioactive compounds present in unripe plantain contributed to the antifungal activity by disrupting the structural integrity of fungal cell membranes. Another study by Ogbe et al., (2020), evaluated the phytochemical content and antifungal properties of unripe plantain peel extract. The study revealed the presence of various bioactive compounds known for their antimicrobial properties, and the extract exhibited the ability to inhibit fungal growth. Similarly, Ijeh et al., (2014), investigated the antimicrobial activities of different plantain varieties, including unripe plantain, against dermatophytes and found significant antifungal activity. Njoku et al., (2019) further demonstrated the antifungal activity of *Musa paradisiaca* (unripe plantain) peel extract, further highlighting the potential use of this natural product as an alternative therapy for ringworm.

"As a consequence of the resistance to antifungal agents, standards and protocols have been developed in order to ensure the delivery of herbal medicine services in a systematic manner" (World Health Organization, n.d). The antibacterial activities of the plantain (*M. paradisiaca*) have been demonstrated in a number of investigations, with a wide variety of organisms serving as test subjects (most commonly fungus and bacteria, but also parasites and viruses) (Abd Razik et al., 2012).

Asoso et al., (2016), demonstrated "the antibacterial activity of plantain peel and fruit extracts against *Escherichia coli*, *S. aureus*, *Salmonella, Shigella, Klebsiella pneumonia,* and *Bacillus subtilis*. The researchers used the agar well diffusion method to conduct their research".

"Tinea corporis, also known as 'ringworm,' is a superficial dermatophyte infection of the skin, other than on the hands (*Tinea manuum*), feet (*Tinea pedis*), scalp (*Tinea capitis*), bearded areas (*Tinea barbae*), face (*Tinea faciei*), groin (*Tinea cruris*), and nails (Onychomycosis or *Tinea unguium*)" (Hsu et al., 2001). "*Tinea corporis* is most commonly caused by dermatophytes belonging to one of the three genera, namely, *Trichophyton* (which causes infections on skin, hair, and nails), *Microsporum* (which causes infections on skin and hair), and *Epidermophyton* (which causes infections on skin and nails)" (Nenoff et al., 2014). Sahoo & Mahajan, (2016) suggested that "Dermatophytes are grouped as either anthropophilic, zoophilic, or geophilic, depending on whether their primary source is human, animal, or soil, respectively" (Surendran et al., 2014). *Tinea corporis* is common and many other annular lesions can mimic this fungal infection, physicians must familiarize themselves with its etiology and its treatment (Czaika, 2013; Leung et al., 2019).

"Humans may become infected through close contact with an infected individual, an infected animal (in particular, domestic dog or cat), contaminated fomites, or contaminated soil" (Andrews & Burns, 2008). Infection may be acquired as a result of spread from another site of dermatophyte infection (e.g. *Tinea capitis, Tinea pedis,* Onychomycosis), (Nenoff et al., 2014; Shy, 2007). "Transmission among household family members is by far the most common route; children often become infected by spores shed by an infected household family member" (Leung et al., 2020). "Autoinfection by dermatophytes elsewhere in the body may also occur" (Gupta et al., 2003). "Transmission of the fungus is facilitated by a moist, warm environment, sharing of towels and clothing, and wearing of occlusive clothing (Alter et al., 2018). Predisposing factors include personal history of dermatophytosis (e.g. *Tinea capitis, Tinea pedis, Tinea cruris,* and *Tinea unguium*), concurrent affected family members, pets in the home, crowding in home, recreational exposure (e.g. wrestling and marital arts), hyperhidrosis, low β-defensin 4 levels, immunodeficiency, diabetes mellitus, genetic predisposition (in particular, *Tinea imbricata*), xerosis, and ichthyosis" (Singh et al., 2019).

1.1 Current Treatment Options for Ringworm

1.1.1 Non-pharmacologic measures

"As fungi thrive best in moist and warm environments, patients should be advised to wear light and loose-fitting clothing" (Weinstein & Berman, 2002). The skin should be kept clean and dry (Veraldi et al., 2018).

1.1.2 Pharmacotherapy

"The standard treatment of *Tinea corporis* is with topical antifungals and there is evidence of the superiority of topical antifungals over the use of placebo" (Rajagopalan et al., 2018). "Localized

or superficial *Tinea corporis* usually responds to topical antifungal therapy applied to the lesion and at least 2 cm beyond the lesion once or twice daily for 2–4 weeks. Commonly used topical antifungal agents include azoles (e.g. econazole, ketoconazole, miconazole, clotrimazole, miconazole, oxiconazole, sulconazole, sertaconazole, eberconazole, and luliconazole), allylamines (e.g. naftifine, terbinafine), benzylamine (butenafine), ciclopirox, and tolnaftate" (Abdul-Bari, 2012). "In this regard, nystatin, which is an effective treatment for Candida infections, is not effective for *Tinea corporis*. In a 2013 meta-analysis of 65 trials (trials with a common comparator and head-to-head trials) involving 14 topical antifungals, there was no significant difference among the antifungals regarding the outcome of mycologic cure at the end of the treatment" [34]. "Pairwise comparison of topical antifungals showed that butenafine, naftifine, and terbinafine were significantly more efficacious in sustaining the cured outcome. A 2014 Cochrane review suggests that individual treatments with terbinafine and naftifine are effective and have few mild adverse events" (Rotta et al., 2013). "Topical antifungal agents are generally well tolerated. Side effects are uncommon, except for rare instances of contact dermatitis. Common causes of treatment failure include poor compliance, drug resistance, reinfection from close contact and auto-inoculation, and misdiagnosis" (El-Gohary et al., 2014). "Some authors suggest the addition of a topical corticosteroid to the topical antifungal agent, especially in individuals with inflammatory dermatomycosis' (Hube et al., 2015).

"Systemic antifungal treatment is indicated if the lesion is extensive, deep (e.g. *Majocchi granuloma*), recurrent, chronic, or unresponsive to topical antifungal treatment; if the patient is immunodeficient; or if there are multiple site lesions" (Goldstein & Goldstein, 2020). "Randomized control trials support the efficacy of systemic treatment with oral antifungal agents" (Bhatia et al., 2019).

1.2 Potential Antimicrobial Activities of Unripe Plantain Peels

"Musa paradisiaca L., growing in tropical and subtropical countries, is used globally for its nutritional value. Phytochemical screening showed that *Musa paradisiaca* peel contains tannins, alkaloids, steroids, saponin, flavonoids, and carbohydrates, while cyanogenic glycoside was absent" (Okorondu et al., 2010). "The fruit, peels, and leaves of *M. paradisiaca* are used in traditional medicines" (Asoso et al., 2018).

"Banana peels have been reported as a good source of phenolic and flavonoid compounds" (Darsini et al., 2012).

"The extracts of peels from different cultivars of banana were observed to have good antioxidant activity, which is correlated with the presence of phenolic and flavonoid compounds" (Nagarajaiah & Prakash, 2011).

"Among the extracts of peels from different cultivars, plantain peel flour was shown to have the lowest level of extractable polyphenols, but the highest antioxidant capacity" (Agama-Acevedo et al., 2016). "Phenolic compounds in peel extracts of tanduk and nangka bananas, which were grown in West Java-Indonesia, were major contributors to their antioxidant activities" (Fidrianny et al., 2018). "The fatty acids that were observed in banana peel extract were responsible for its antimicrobial activity" (Brooks, 2019). In addition, in fully ripe bananas, peel and pulp had reported to have antibiotic and antifungal properties.

"Both the plantain peel and fruit extracts exhibited antibacterial potentials on Gram positive and Gram negative bacteria most especially with methanol extract. However, the bacterial species were more susceptible to plantain peel extracts than fruit extracts" (Brooks, 2019; Wayne, 2008). Similar result was reported by (Ighodaro, 2012). "Effects proving this might be the higher percentage of hydrocarbon, monoterpene and oxygenated monoterpene appreciated for their antibacterial potentials in the peel than fruit. It could also be noted that hence methanol extract exhibited higher antibacterial activity; it then signified that methanol has the potential of extracting the antibacterial substances from the plantain samples than other solvents" (Brooks, 2019). The antibacterial results obtained are similar to that reported by Ahmad, (Ahmad & Beg, 2001). Some literatures have reported information on the presence of bioactive molecules in many plants, which have served as food and medicine in health care for man. Since the event of this scientific research on such discovery has been till date. The ideal about such research is to find lasting solutions to replacing synthetic antibiotics with naturally available phytochemicals present in plants for their low toxicity, low cost and readily available

for human employment in disease treatment. Fagbemi et al., (2009) has reported “ethanolic and aqueous extract of unripe *M. sapientum* fruit. In this study similar result was obtained with *M*. *parasidiaca* peel and fruit extracts”. Repon et al., (2013) has reported “on the antibacterial activity of *M. sapientum* on some pathogenic bacteria”.

1.3 Current Knowledge Gap and Rationale for the Study

A study by Asoso et al., (2016) shows that “higher antibacterial effects than known synthetic antibiotics were exhibited on test bacterial species. The methanol extract of both peel and fruit had higher inhibition value on test bacteria than ethanol and acetone extracts. The microbes against which the extracts were effective are pathogens already implicated in the etiologic and severity of human diseases. Thus, the plant extract may be useful in antibacterial application. As a natural health product, *M. parasidiaca* preparations as food may be accepted more readily than prescription drugs for some patient groups, particularly in some communities afflicted with varying incidence of bacterial diseases and a paucity of culturally acceptable treatment options. This result showed that *M*. *parasidiaca* though taken as food for carbohydrate source could serve as agent of bacterial inhibition”.

While there are anecdotal claims about the benefits of using unripe plantain peels as a natural treatment for ringworm, it’s important to note that scientific research and clinical studies in this area are limited. However, the components found in unripe plantain peels may provide certain potential benefits in treating ringworm. A study by Umar et al., (2014), investigated the antimicrobial activity of unripe plantain extracts against various dermatophytes, including those causing ringworm. The researchers found that the extracts showed inhibitory effects against the tested dermatophytes, indicating its potential use in the treatment of ringworm.

Another study by Okhuoya et al., (2012) examined the antifungal properties of unripe plantain peel extract against dermatophytes causing ringworm. The researchers observed significant inhibition of dermatophyte growth when treated with the extract, suggesting its potential as an antidermatophytic agent.

This study aims to investigate the antimicrobial activities of unripe plantain peels against ringworm-causing fungi.

The objectives of this study include:

1. To isolate and identify the fungal strains causing ringworm infections.
2. To extract active compounds from unripe plantain peels.
3. To evaluate the antimicrobial activities of the extracted compounds.
4. To compare the effectiveness of unripe plantain-based treatments with conventional antifungal medications.

2. MATERIALS AND METHODS

2.1 Study Area

This work was conducted at Alpha laboratory Awka, Anambra State. Anambra State is located in the south-eastern part of Nigeria and situated between latitudes 5° 32' and 6° 45' N and longitude 6° 43' and 7° 22' E respectively. The research is based on *in vitro* antifungal activity of plantain peel extracts on ringworm causing fungi.

2.2 Materials Used

The materials used for the study included plant specimens (unripe plantain peels), Whitman's filter paper No 42, beakers, volumetric flasks, measuring cylinder, spatula, inoculating loop, Bunsen burner, aluminum foil, cotton wool, scalpel, microscope, sterile polythene bags, masking tape, Petri dishes, blotting paper, sodium hypochlorite, test tubes and rack, micro pipette, funnels, slide, cover slip.

2.3 Sample Collection

The medicinal plant used in this study was the plantain peel. They were obtained from Awka, Anambra State, Nigeria and identified at the Botany Department of Nnamdi Azikiwe University, Awka. The samples were immediately transported to the laboratory for use.

2.4 Sample Preparation

The samples were sliced into very small sizes and sun dried for 7 days to constant weight. This was futher ground into fine powder and stored in an air tight plastic container for extraction.

2.5 Extraction

Extraction was done with ethanol using the Soxhlet extractor, and by cold maceration with fresh distilled water.

2.5.1 Soxhlet extraction

250g of the dried plant powder was put in a Soxhlet apparatus to which enough ethanol (70 % v/v) was added to submerge the powder and continuously extract it, until the extracting solvent became clear in the thimble indicating that the extraction of the phytochemicals soluble in the solvent ethanol was complete. The volume was noted; the extract was dried in an oven at a temperature of 70 °C and the weight of the dry ethanol extract taken.

2.5.2 Cold maceration

250g of powder sample was weighed into a glass and extracting solvent (water) was added until the medicinal plant residues were fully immersed. The vessel was closed with a tight-fitting glass cover and the contents in the vessel were shaken after every 4 h except at night, and left to stand for three days (72 h) but with subsequent agitation until this period was over. The contents of the flask were then strained through two clean pieces of cotton cloth placed on top of filter paper, both supported by a funnel, and the extracted solution (miscella) collected in a flask with a tight-fitting cover. The maximum yield of water extract was obtained by squeezing the marc (solid residue) in the top clean dry piece of cloth, while drippings of liquid extract were allowed to pass through the second clean dry piece of cloth, to the contents of the flask through Whatman's filter paper. The volume of the yield was noted. Both the ethanol and water extracts were transferred to a hot-air oven for drying at temperatures between 50 °C and 70 °C, and later transferred into a desiccators for further drying.

2.6 Phytochemical Screening

a. Preliminary Phytochemical Screening

The extracts will be subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents by the following methods according to (AOAC, 2010).

2.6.1 Test for Alkaloids

The extracts were treated with dilute (10%) hydrochloric acid and filtered. The filtrates were treated with various alkaloidal reagents.

a. **Mayer's test:** The extracts were treated with Mayer's reagent (Potassium mercuric iodide). Appearance of cream colour indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.

b. **Wagner's test:** The extracts were treated with the Wagner's reagent (Iodine solution) the appearance of brown colour precipitate indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.

2.6.2 Phenolics

0.5 g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 mins and filtered while hot. Then 1ml of Ferric chloride solution was added. Formation of blue-black or brown colouration indicated the presence of phenol.

2.6.3 Test for terpenoids

5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids

2.6.4 Test for cardiac glycosides

Keller-killani test: When a pinch of the extracts were dissolved in the Glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides in methanolic and aqueous extracts.

2.6.5 Test for flavonoids

a. **Shinoda's test:** The extracts were dissolved in alcohol, to that one piece of magnesium followed by conc. hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids in methanolic and aqueous extracts.

b. **Ferric chloride test:** To the extracts, few drops of neutral ferric chloride were added. Blackish red colour was observed in methanolic and aqueous extracts.

2.6.6 Test for Saponins

a. **Foam Test:** The extracts were diluted to 20 ml with distilled water and shaken well in a graduated cylinder for 15 minutes. The formation of foam in the upper part of the test tube indicates the presence of saponins in each extract.

b. Demonstration of emulsifying properties: 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

2.6.7 Test for steroids

a. Salkowski reaction: To 2 ml of extract, added 2ml chloroform and 2 ml of conc. H_2SO_4 shake well. Chloroform layer showed red color and acid layer showed greenish yellow fluorescence.

b. Liebermann-Burchard test: When the extracts were treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, absence of green colour indicates the absence of steroids in all extracts.

2.6.8 Test for tannins

a. Lead acetate solution: When the extracts were treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins in methanolic and aqueous extracts.

b. Ferric chloride solution: When the extracts were treated with Ferric chloride solution, NaOH and AgBr Solution appearance of green colour precipitate indicates the presence of tannins in methanolic and aqueous extracts.

2.7 Quantitative Phytochemical Screening

2.7.1 Steroids

One gram (1 g) of the extract will be macerated with 20 ml of ethanol. Two milliliters (2 ml) of chromagen solution will be added to 2 ml of the filtrate and allowed to stand for 30 minutes. Absorbance will be read at 550 nm. A standard will be made following the same procedure at different concentrations using steroid hormone, a standard curve of absorbance vs concentration will be plotted and the concentration of steroid in the extract extrapolated from the standard curve.

2.7.2 Saponins

The extracts (1g) each will be macerated with 10ml of petroleum ether and decanted into a beaker. Another 10ml of petroleum ether will be added into the beaker and the filtrate heated to evaporate into dryness. The residues will be dissolved in 6ml of ethanol. The solutions (2ml) will be then put into test tubes and 2ml of chromagen solution added. The mixtures will be allowed to stand for 30 minutes and absorbance will be read at 550nm. A standard will be made following the same procedure at different concentrations using ursolic acid. A standard curve of absorbance vs concentration will be plotted and the concentration of saponin in the extracts extrapolated from the standard curve.

2.7.3 Alkaloids

An aliquot of (0.5g) of the extract will be dissolved in 96% ethanol and 20% H_2SO_4 and filtered, the filtrate (1ml) will be added to 5ml of 60% tetraoxosulphate (VI) acid and allowed to stand for 3 hours after which reading will be taken spectrophometrically at 565nm wavelength. A standard will be made following the same procedure at different concentrations using caffeine, a standard curve of absorbance vs concentration plotted and the concentration of alkaloids in the extracts extrapolated from the standard curve.

2.7.4 Quantitative test for terpenoids

Dried plant extract 100mg (wi) was taken and soaked in 9ml of ethanol for 24 hour Indumathi et al., 2014. The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100).

2.7.5 Determination of tannins by titration (person method, 1974)

20g of sample was weighed in a conical flask and 100mls of n hexane or petroleum ether was added and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes for the solvent to evaporate. It was then re-extracted by soaking 100ml of 1% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected.

25 ml of ammonium hydroxide were added to the filtrate to precipitate the alkaloids. The alkaloid was heated with electric hot plate to remove some of ammonium hydroxide still in solution. The remaining volume was measured and 5ml of

this was taken and 20ml of ethanol was added to it. It was titrated with 0.IM NaOH using 1ml of phenolphthalein as indicator until a pink end point is reached. Tannin content was calculated in percentage ($C^1V^1 = C^2V^2$) molarity.

Data

C_1 = Concentration of Tannic acid
C_2 = Concentration of Base
V_1 = Volume of Tannic acid
V_2 = Volume of Base

Therefore;

$$C_1 = \frac{C_2 V_2}{V_1}$$

$$\%\ of\ tannic\ acid\ content\ = \frac{C_1 x\ 100}{Weight\ of\ sample\ analyzed}$$

2.7.6 Flavonoids

The extracts (1g) each will be macerated with 20 ml of ethylacetate for 5min and filtered. To (5ml) filtrate will be added 5 ml of dilute ammonium, shaken for 5min, the upper layer will be collected and the absorbance read at 490 nm. A standard will be made following the same procedure at different concentrations using quercetin as standard. A standard curve of absorbance vs concentration will be plotted and the concentration of flavonoid in the extract extrapolated from the standard curve.

2.7.7 Phenol

Defatting of 2 g wood powder sample was carried out for 2 hours in 100cm^3 of ether using a soxhlet apparatus. The defatted sample (0.50g) was boiled for 15 minutes with 50cm^3 of ether for the extraction of the phenolic components. Exactly 10cm^3 of distilled water, 2cm^3 of 0.1N ammonium hydroxide solution, and 5cm^3 of concentrated amyl alcohol were also added to 5cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505nm. 0.20g of tannic acid was dissolving in distilled water and diluted to 200 ml mark (1 mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which 2cm^3 of NH_3OH, 5cm^3 of amyl alcohol, and 10cm^3 of water were added. The solution was made up to 100cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 50 nm.

2.8 Antifungal Bioassay

2.8.1 Specimen collection

Cotton swabs, soaked in 70% ethanol, were used to clean the infected area. Lesions were scrapped by a sterile scalpel and collected in sterile disposable sterile Petri dishes and transferred to laboratory for mycological examination. Specimens were collected and sealed in sterile dry Petri dishes; they were labeled with the patient's name, age, sex, date of collection, and site of infection. The samples were divided into two portions: one for microscopic examination and one for culture.

2.8.2 Direct microscopical examination

"On a clean glass slide, a part of each skin scrapings was placed. Ten percent (10%) of Potassium Hydroxide (KOH) was used to digest the keratin material. The solution was then covered with a clean glass and gently heated for one minute. The slide was microscopically examined for fungal spores and hyphae under 10X and 40X magnifications" (Larone, 2011).

2.8.3 Isolation of dermatophytes

"To isolate dermatophytes, skin scrapings were cultured on Sabouraud dextrose agar (SDA) containing chloramphenicol (0.05mg/ml) and cyclohexamide (0.5mg/ml). The inoculated plates were incubated at 27°C for up to six weeks. They were regularly observed for differentiated colonies. Growth rate, obverse, reverse pigmentation of the recovered colonies were reported" (Kane et al., 1997).

2.8.4 Identification of dermatophytes

"Identification of dermatophytes was based on the colonies characteristic i.e macroscopically (color of the surface and reverse, topography, and texture) and microscopic features of the isolates. A small sample of each fungal mycelium free of the medium was examined using 10X and 40X objectives. The laboratory Handbook of Dermatophytes was used for fungal identification" (Hungerford et al., 1998).

2.8.5 Dissolution of extracts

The dried ethanol and aqueous extracts were weighed; 2.0g of each extract was dissolved in 2ml of RPMI and made to 1 g/ml. From these solutions, different strengths of starting concentrations (0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml, 0.0313mg/ml, 0.0156 mg/ml, 0.00781 mg/ml, and 0.00391mg/ml) were

prepared by serial dilutions. These concentrations were used to test the antifungal activity to obtain both the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC).

2.8.6 Testing of extracts for antifungal activity (Agar - well diffusion method)

In the freshly prepared and sterilized SDA Agar medium, a pinch amount of streptomycin was added and mixed well. Then these 20ml of SDA Agar Medium was poured into each petri dish and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a disc made was place in the petri dish. Then these plates were incubated at 27°C for 48-72 hours. After incubation period the results were observed and measured the diameter of inhibition zone around the each well.

2.8.7 Determining minimum inhibitory and minimum fungicidal concentrations

"The minimum inhibitory concentrations (MICs) for dermatophytes were defined as the lowest drug concentration that inhibited visible growth of the fungi. The minimal fungicidal concentrations (MFCs) for dermatophytes were determined by sub-culturing fungal isolates from each test tube with no visible growth onto an SDA slant. The slants were incubated at 30 °C for 14 days while monitoring for fungal growth. The MFC was defined as the lowest concentration of drug that yielded a negative subculture" (Tatsumi et al., 2001).

2.8.8 Reading of minimum inhibitory and minimum fungicidal concentrations

The MICs of both plant ethanol and water extracts were determined visually from the first day the growth control (negative control) showed fungal growth/turbidity, and 72 h later. Growth/turbidity in each test tube was given a score or code by comparing its contents with those of the positive control (clear). The MFCs of both ethanol and water extracts were determined by picking 100 µl of dermatophytes from each test tube and sub-culturing this onto an SDA slant, incubating at 25–30 °C, and visually observing for filamentous growth on the SDA slants in all test tubes while comparing with the positive and negative controls. Growth on each SDA slant was coded as FGo where filamentous growth was observed, or as NFGo for no filamentous growth observed. The MICs were determined by dermatophytes growth (turbidity) in each test tube while comparing with the positive control, and were scored numerically as follows:

0 = optically clear/no visible growth,
1 = approximately 25 % growth control,
2 = approximately 50 % growth control,
3 = approximately 75 % growth control and
4 = very turbid (no reduction in growth control) according to CLSI, 2008 guidelines.

3. RESULTS

In this study, upon assessment considerable number of fungi were identified and isolated from the samples of phytochemical in unripe plantain peel.

Table 1 shows that eight (8) phytochemicals were identified and isolated from the unripe plantain peel collected. Saponins and terpenoids have the highest concentration of ethanol and water content. Ethanol is in high concentration in most of the phytochemicals, including saponins, Flavonoids, alkaloid, terpenoids and phenol. Ethanol and water are absent in steroids.

Table 2 shows that flavonoids have the highest composition of 24.37mg/100g while steroid has the least composition with 1.01mg/100g.

Table 3 shows the macroscopic and microscopic examination of fungi isolated from fungal skin infection. A total of four (4) fungi organisms were identified and isolated.

Table 1. Qualitative phytochemical

Phytochemicals	Ethanol	Water
Saponins	+++	+++
Flavonoid	+++	++
Alkaloid	+++	-
Tannin	++	+
Steroids	-	-
Terpenoids	+++	+++
Glycosides	++	+
Phenol	+++	+

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

Table 2. Quantitative phytochemical composition

Phytochemicals	Quantities (mg100g)
Alkaloids	21.33
Saponins	11.60
Tanins	14.34
Flavonoids	24.37
Steroids	1.01
Terpenoid	6.74

**Values are mean scores ± Standard deviation of three (3) replicates*

Table 3. Macroscopic and Microscopic Examination of fungi isolated from fungal skin infection

Code	Macroscopic appearance	Microscopic appearance	Organism identified
1	White colony and circular growth on SDA plate	Small and cylindrical shaped macroconidia occurring in cluster	*Trichophyton* spp
2	Blue-black mould on SDA plates	Conidiophores terminate in a ball like structure	*Aspergillus* spp
3	White to yellow downy and powdery colony on with SDA plate	Spindle shape micro conidia terminal knob	*Microsporum* spp
4	Cream to yellow colony with smooth and dry texture	Conidial masses rises hyphae forming grape-like cluster along the hyphae	*Candida* spp

Table 4. Antimicrobial Activities and Comparison of the Extracts

Isolate	Extract	Zone of Inhibition (Mm)	Result
***Aspergillus* spp**	Ethanol extract	12.33 ± 0.120^{a}	S
	Water extract	10.16 ± 0.030^{b}	I
	Fluconazole	12.00 ± 1.00^{a}	S
***Microsporum* spp**	Ethanol extract	25.00 ± 0.460^{a}	S
	Water extract	12.00 ± 0.350^{b}	S
	Fluconazole	32.27 ± 0.360^{c}	S
***Trichophyton* spp**	Ethanol extract	17.33 ± 0.200^{a}	S
	Water extract	7.16 ± 0.080^{b}	R
	Fluconazole	18.00 ± 1.300^{a}	S
***Candida* spp**	Ethanol extract	27.00 ± 1.430^{a}	S
	Water extract	12.00 ± 0.430^{b}	S
	Fluconazole	32.27 ± 0.160^{c}	S

**Values are mean scores ± Standard deviation of three (3) replicates*
For each fungus row, values in the same column with different superscript are significantly different at p<0.05
N/B: R = Resistant, I = Intermediate, S = Susceptible;

Table 4 shows that all the four (4) isolated fungi are susceptible to ethanol extract and fluconazole.

Trychophyton spp. is resistant to water extract while *Aspergillus* spp. is intermediate to water extract.

Table 5. Percentage turbidity (mean) of aqueous and ethanol extracts against ringworm fungal isolates

Dilution (mg/ml)	Aqueous extract				Ethanol extract			
	***Aspergillus* spp**	***Microsporum* spp**	***Trichophyton* spp**	***Candida* spp**	***Aspergillus* spp**	***Microsporum* spp**	***Trichophyton* spp**	***Candida* spp**
1.00	50	50	0	50	25	0	50	0
0.50	50	50	25	50	50	0	50	25
0.25	75	50	50	100	50	0	50	50
0.10	75	50	50	100	75	50	50	50
0.05	100	100	100	100	100	100	100	100

The lowest average percentage turbidity was exhibited by the aqueous and ethanol extracts against Trichophyton spp, followed by Candida spp, then Aspergillus spp and finally Microsporum spp. The cell entries (0-100) indicate the percentage turbidities (a measure of fungal growth of the four strains), resulting from the effect of the two extracts at the corresponding dilutions. All tests were done in triplicate

Table 6. Minimum fungicidal concentrations of aqueous extract (Mg/mL)

Fungi	Minimum fungicidal concentrations of aqueous extract						
	1.00	**0.50**	**0.25**	**0.10**	**0.005**	**-ve ctrl**	**+ve ctrl**
Aspergillus spp	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo
Microsporum Spp	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo
Trichophyton Spp	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo
Candida spp	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo

Fgo denotes that filamentous growth was observed, while Nfgo denotes that no filamentous growth was observed. The results obtained during the course of 14 days revealed that the water extract had MFCs of ≥ 0.05 mg/ml for Microsporum spp, ≥0.01 mg/ml for Trichophyton spp and ≥ 0.25 mg/ml for Aspergillus spp and Candida spp. There was filamentous growth in the negative control (- ve ctrl) and no filamentous growth in positive control (+ve ctrl)

Table 7. Minimum fungicidal concentrations of ethanol extract (Mg/mL)

Fungi	Minimum fungicidal concentrations of aqueous extract						
	1.00	**0.50**	**0.25**	**0.10**	**0.005**	**-ve ctrl**	**+ve ctrl**
***Aspergillus* spp**	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo
***Microsporum* spp**	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo
***Trichophyton* spp**	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo
***Candida* spp**	Nfgo	Nfgo	Nfgo	Nfgo	Fgo	Fgo	Nfgo

Fgo denotes that filamentous growth was observed, while Nfgo denotes that no filamentous growth was observed. The ethanol extract indicated MFCs of ≥ 0.5 mg/ml for Microsporum spp and Trichophyton spp and ≥ 0.25 mg/ml for Aspergillus spp and Candida spp as result of subculturing 200 µl of dermatophytes from each serially diluted test tubes onto SDA slants

3.1 Means Plots

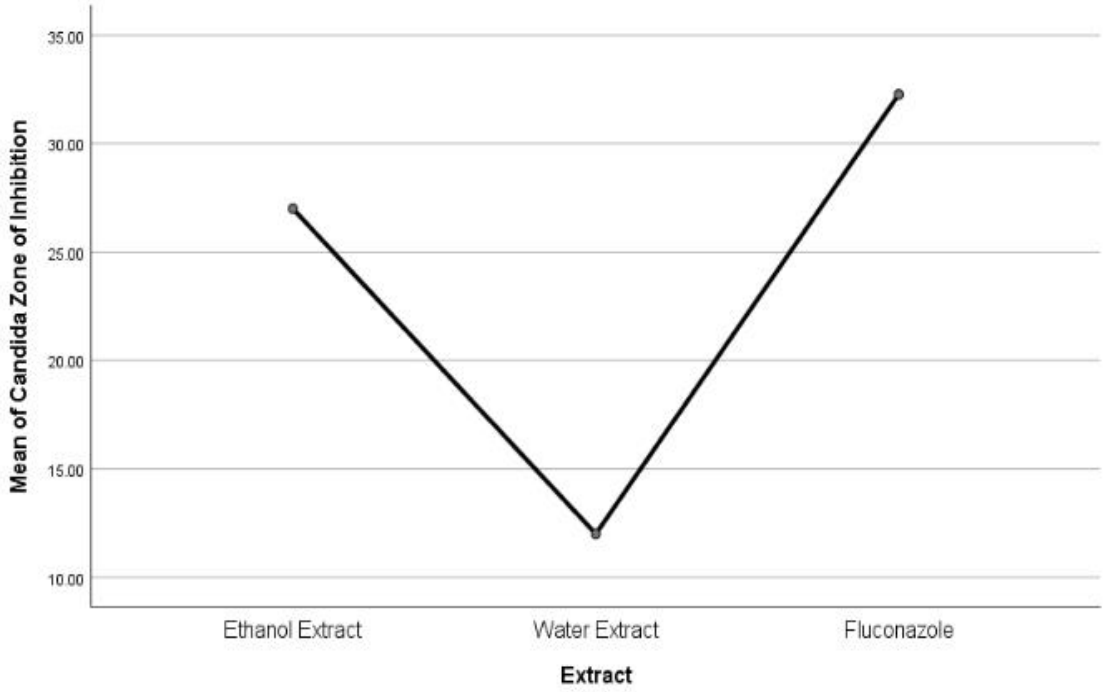

Fig. 1. Chart showing the mean plot of *Candida* spp zone of inhibition against the extracts

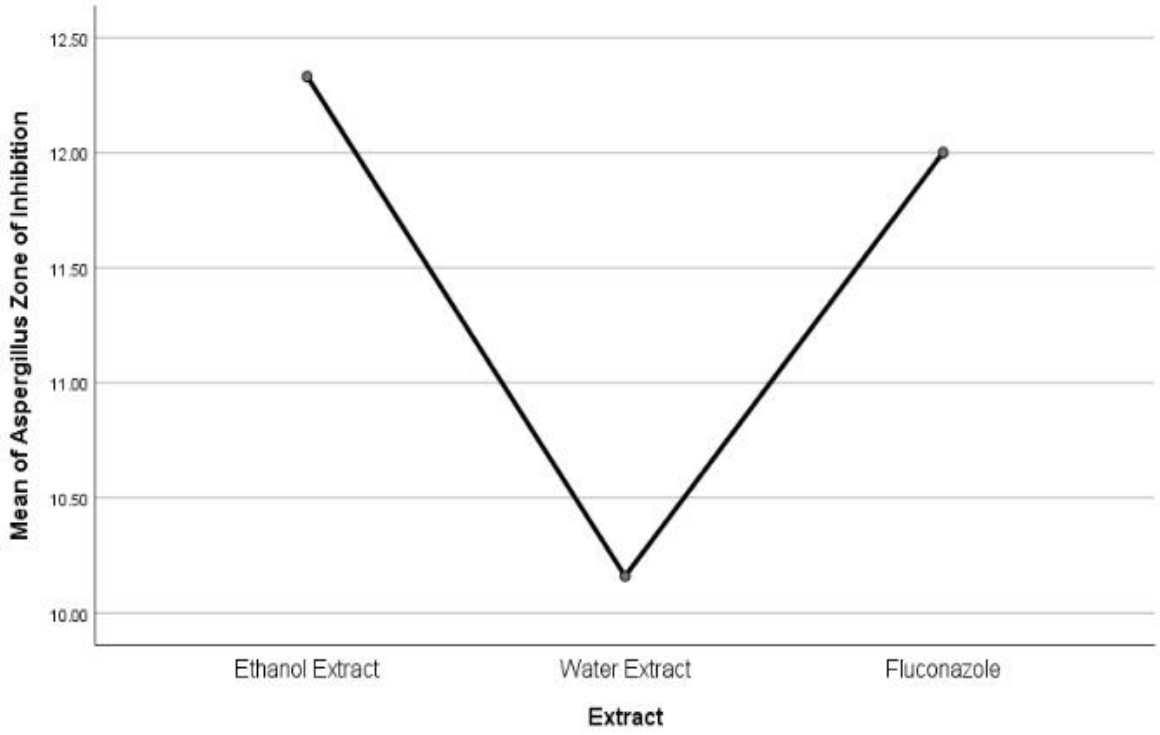

Fig. 2. Chart showing the mean plot of *Aspergillus* spp zone of inhibition against the extracts

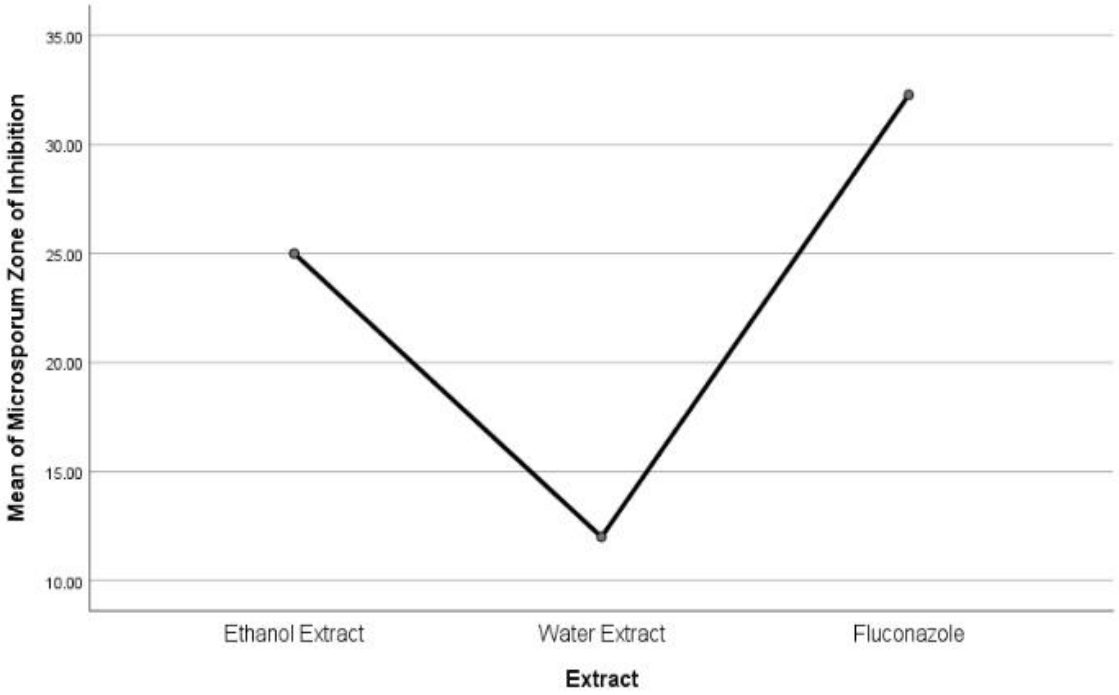

Fig. 3. Chart showing the mean plot of *Microsporum* spp zone of inhibition against the extracts

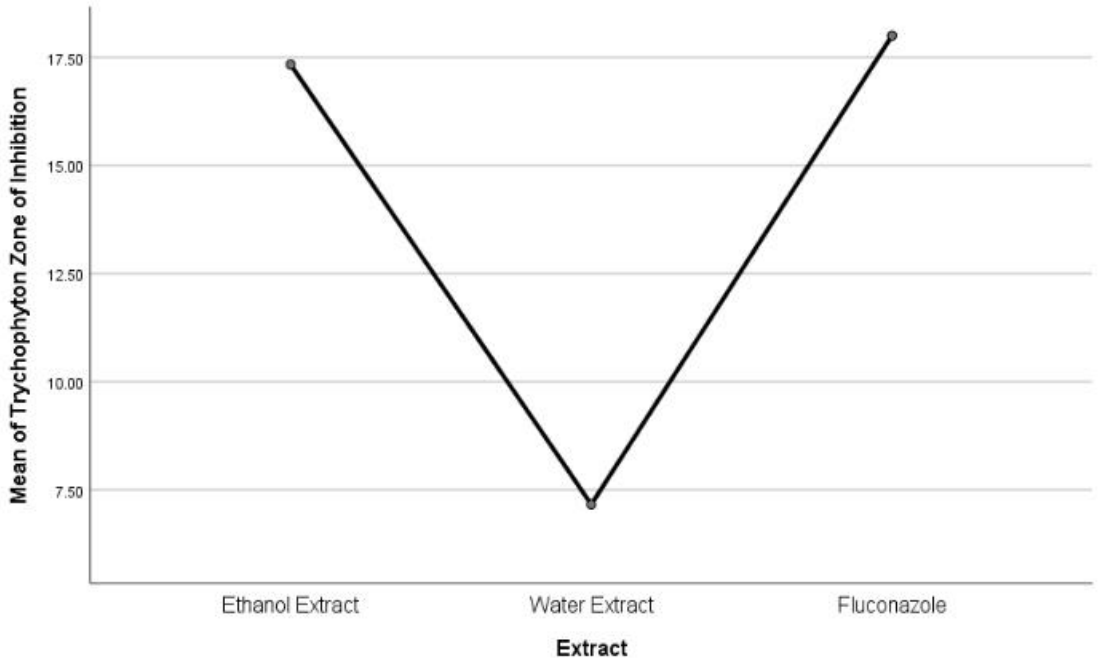

Fig. 4. Chart showing the mean plot of *Trychophyton* spp zone of inhibition against the extracts

4. DISCUSSION

Unripe plantain peel was sliced into very small sizes and sun dried for seven days to constant weight. This was further ground into fine powder and stored in an air tight plastic container for extraction. The extracts will be subjected to preliminary chemical screening for their presence

or absence of active phytochemical constituents by the following methods according to (AOAC. 2010).

The result showed that eight (8) phytochemicals were identified and isolated: saponins, steroids, tannins, flavonoids, alkaloids, glycosides, terpenoids and phenols from the unripe plantain peel collected. Saponins and terpenoids have the highest concentration when treated with ethanol and water content. Ethanol content recorded high concentration in most of the phytochemicals, including saponins, Flavonoids, alkaloid, terpenoids and phenol. Ethanol and water contents recorded absence in steroids. The macroscopic and microscopic examination of fungi isolated from fungal skin infection from Table 3 shows an identification and isolation of four (4) fungi organisms: *Trichophyton* spp, *Aspergillus* spp, *Microsporum* spp and *Candida* spp. Table 4 shows that all the four (4) isolated fungi are susceptible to ethanol extract and fluconazole. The results obtained during the course of 14 days revealed that all the four (4) isolated fungi are susceptible to ethanol extract and fluconazole and the water extract had MFCs of ≥ 0.05 mg/ml for *Microsporum* spp, ≥0.01 mg/ml for *Trichophyton* spp and ≥ 0.25 mg/ml for *Aspergillus* spp *and Candida* spp. "Localized or superficial *Tinea corporis* usually responds to topical antifungal therapy applied to the lesion and at least 2 cm beyond the lesion once or twice daily for 2–4 weeks. Commonly used topical antifungal agents include azoles (e.g. econazole, ketoconazole, miconazole, clotrimazole, miconazole, oxiconazole, sulconazole, sertaconazole, eberconazole, and luliconazole), allylamines (e.g. naftifine, terbinafine), benzylamine (butenafine), ciclopirox, and tolnaftate" (Abdul, 2012).

This also agrees with the studies of Ogbe et al. (2020) and Njoku et al. (2019) that the phytochemical content, antifungal properties and antimicrobial properties of unripe plantain peel extract exhibited the ability to inhibit fungal growth especially for ringworm.

5. CONCLUSION

The antimicrobial activities of unripe plantain peels against dermatophytes and *Candida* species, the causative agents of ringworm, have been demonstrated in this study. Unripe plantain peels have shown significant inhibitory effects against these fungal species. Although further research is needed to establish the optimal formulation and dosage of unripe plantain peel extract for effective treatment, the findings suggest that unripe plantain peels may be a promising natural remedy for ringworm. Clinical trials are necessary to validate these findings and explore the practical applications of unripe plantain peels in the treatment of ringworm. While this natural remedy may provide an alternative to conventional antifungal medications, it should not be used in place of medical treatment unless under the guidance of a healthcare provider. Further research efforts may help to develop new therapeutic options for individuals with ringworm or other fungal infections.

6. RECOMMENDATION

If you are considering using unripe plantain peels as a complementary treatment for ringworm, it's important to:

- Consult a healthcare professional: Speak with a healthcare provider, preferably a dermatologist or a traditional medicine practitioner, to obtain their expert advice and guidance.
- Follow medical advice: Continue with any prescribed antifungal medications or therapies recommended by the healthcare professional. The use of unripe plantain peels should be in addition to medical treatment, not as an alternative.
- Conduct a patch test: Before applying unripe plantain peel extract topically, perform a patch test on a small area of unaffected skin to check for any adverse reactions or allergies.
- Monitor progress and seek medical help if necessary: Regularly evaluate the progress of the treatment. If there is no improvement or if the condition worsens, consult the healthcare professional immediately.

Ultimately, self-treatment should be approached with caution, especially when it involves natural remedies. Collaborating with healthcare professionals ensures the most effective and safe management of ringworm.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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