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Bioautography and GC-MS analysis of anti-dermatophytic components in the methanol extract of *Musa paradisiaca* leaf

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ABSTRACT

Background: The search for new agents from medicinal plants is on the increase because of the development of resistance to available drugs.

Methods: The methanol leaf extract of *Musa paradisiaca* Linn was investigated to isolate its anti-dermatophytic components. The extract was fractionated to pool the active compounds into the polar solvent. The functional groups present in the extract were detected by FTIR, while chemical components of the extract were separated by TLC methods before determining the activity of the separated components by bioautography. The components were isolated and tested for their individual activity against the test dermatophytes.

Results: Infrared spectra indicated the presence of organic compounds of the phenolic group. All the test organisms had varying degrees of susceptibility on the bioautography, with *M. audouinii* being more susceptible. GC-MS analysis of the components indicated the presence of oxirane tridecyl, 12-methyl-E, E-2, 13 octadecadien-1-ol, cis-7, cis-11-Hexadecadien-1-yl acetate, n-Hexadecanoic acid, cis-11-Hexadecenal, 2-Hexadecanol and butylated hydroxytoluene which are known to possess antifungal properties.

Conclusion: The leaf of *M. paradisiaca* is confirmed to possess antidermatophtyt ic potentials which can be exploited to produce leads for the development of novel antifungal agents.

Keywords: *Musa paradisiaca*, Antidermatophyte, Bioautography, GC-MS

1.0 INTRODUCTION

From time immemorial, plants and plant products have been employed in the treatment of diseases. In fact, before the advent of orthodox medicine, man had completely relied on plants for its healthcare needs. The medicinal value of these plants are due to the present of bioactive compounds [1]. As such, activity-guided assays are important in the isolation of chemical compounds with medicinal value. Antifungal product research has become a necessity because of the drastic increase in resistance to available antifungal drugs due to the growing number of immunosuppressed patients stemming from the advent of aggressive cancer chemotherapy, organ transplantation, those affected by the human immunodeficiency virus and the appearance of multidrug-resistant fungal strains due to the huge utilization of fungicides for human and agricultural purposes [2]. Bioautography is a technique used to isolate leads by employing a suitable chromatographic process followed by a biological detection system [3]. By so doing, it is possible to identify the compounds responsible for the observed activity using Gas chromatography-mass spectroscopic analysis. The use of this method for antifungal research has been thoroughly reviewed by [4]. The antidermatophyte activity of *Musa paradisiaca* has previously been reported [5, 6], it is therefore necessary to isolate the components that are responsible for the observed activity.

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2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

Fresh leaves of *Musa paradisiaca* was used for this study. The test organisms; *Microsporum audouinii, Trichophyton rubrum, Trichophyton verrucosum, Trichophyton tonsurans* and *Epidermophyton floccosum* were obtained from the National Veterinary Research Institute, Vom, Plateau State.

2.1.2 Chemicals and Reagents

The chemicals and reagents used include; methanol, petroleum ether, chloroform, butanol, acetic acid, 2% sulphuric acid, silica gel G, methyl thiazolyl tetrazolium (MTT).

2.1.3 Materials and Equipment

The materials and equipment used include; beakers, test tubes, pipettes, petri dishes, glass slides, Whatmann No.42 filter papper, separating flask, retort stand, electronic water bath, aluminium-backed thin layer chromatographic plate, UV lamp, genius IF machine by Xenemetrix limited, Agilent 1990IS gas chromatography system, HP-5 MS column, Agilent 433UI ultra insert mass spectrometer detector.

2.2. Methods

2.2.1 Plant Collection and Authentication

Fresh plantain leaves were collected from the botanical garden of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. The botanical authentication was done at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Nigeria. The taxonomic keys for the identification of the plant were provided by the Herbarium, where a specimen sample with the specimen number UUPH51 (a) was deposited.

2.2.2 Preparation of Plant Material

The leaves were cut into smaller pieces without the midrib for easy drying. The plant material was air-dried on the laboratory bench. The dried leaves were powdered using a mortar and pestle. The powdery sample was packed into screw-capped bottles and labelled appropriately.

2.2.3 Extraction of plant material

The leaf of *Musa paradisiaca* L. was extracted by maceration using methanol as a solvent. This was prepared by soaking 500 g of the dried powdery sample in 5,000 mL of methanol for 72 hours, during which the mixture was intermittently shaken. It was filtered through Whatman No. 42 filter paper. The extract was concentrated to dryness using a water bath at a temperature of 40 ºC.

2.2.4 Fractionation of the Extract

The methanol extract of the leaf of *Musa paradisiaca* was partitioned with petroleum ether, chloroform and water according to the method of [7]. Twenty grams of the extract was dissolved in 200 mL of water before shaking vigorously in a separating flask. The mixture obtained was filtered using a filter paper to remove debris. Petroleum ether (200 mL) was then added to the mixture in a separating funnel, shaken vigorously and allowed to settle. The petroleum ether layer (on top) was removed and concentrated while a further 200 mL of chloroform was added to the aqueous layer and also vigorously shaken and allowed to settle. The aqueous and the chloroform layers were further separated, the chloroform portion was concentrated to dryness by allowing it to stand on the laboratory bench until all the solvent evaporated, while the aqueous layer was concentrated to dryness using the water bath at 40 °C.

2.2.5 Functional Group Determination

The functional groups present in the aqueous fraction of the methanol extract of the leaf of *Musa paradisiaca* was determined using Fourier-transform Infrared spectroscopy (FTIR). This was done at Multi User Central Science Research Laboratory, Ahmadu Bello University, Zaria. Characteristic peaks produced by different functional groups in the IR spectra helped to predict the molecular composition and structure of the compounds in the fraction. A little quantity of the sample was fed into the interferometer which produced optical signals. The signal was quickly measured and decoded by Fourier transformation and produced the FTIR spectrum.

2.2.6 Thin Layer Chromatography (TLC) of the Aqueous Fraction

The chemical constituents of the aqueous fraction of methanol leaf extract of *Musa paradisiaca* was separated on an aluminium-backed thin layer chromatographic plate (TLC silica gel 60 F254, 20 x 20 cm, 0.25 mm thick, Merck, Darmstadt, Germany). The TLC plates were developed under saturated conditions in a TLC chamber. The solvent system used was Butanol/Water/Acetic acid (7:2:1) which was developed in the Postgraduate Laboratory of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. The separated compounds were developed by spraying with 2 % sulphuric acid. The chromatogram was heated at 110 ^{o}C in an oven for 10 minutes after spraying to allow for optimal colour development. The developed plate was visualized with a UV lamp. The different bands representing the separated compounds were noted and their distance from the origin measured. The Rf value was calculated.

2.2.7 Preparation of Plates for Bioautography

Clean glass slides (1x3 inches) were precoated with Silica gel G (Loba Chemie, India) with thickness of 2.0 mm. After coating, the plates were allowed to dry at room temperature and then activated in the oven at 110 ^{o}C for 30 minutes.

2.2.8 Thin Layer Chromatography (TLC) - Bioautography

The method of [8] was employed for this process. About 0.1 mL of 100 mg/mL of the aqueous fraction was loaded onto the pre coated TLC plates in narrow bands using a sterile pipette. The plates were allowed to dry for 20 minutes before developing it in a chromatographic tank using Butanol/Water/Acetic acid (7:2:1) as the mobile phase. The developed plates were dried under a stream of fast moving air for 3 days to remove traces of solvents on the plates. For the bioautography, 5 mL of Saboraud Dextrose Agar (SDA) was poured into a Petri dish as a base and allowed to set. The TLC plate was then placed on the SDA base, 15 mL of SDA containing about 0.1 mL of the test organism was aseptically poured over it, allowed to solidify and pre diffuse for about 1-2 hours. The plate was then incubated at room temperature for 3-7 days. Inhibitions around the chromatographic spots, on the slide, in the form of clear zones, were noted. The plates were further sprayed on the surface with an aqueous solution of methyl thiazolyl tetrazolium chloride (MTT) and zones of inhibition were observed as clear zones against a purple background.

2.2.9 Separation of Antifungal Compounds

The aqueous fraction of the methanol extract of *M. paradisiaca* leaf was applied as a band on a silica gel pre coated 20 x 20 cm glass TLC plates and allowed to dry. The plates were eluded in a chromatographic tank using Butanol/Water/Acetic acid (7:2:1) as the mobile phase. After development, the plate was allowed to dry before scrapping of the different bands, separately. The compounds were extracted from the silica gel by washing with methanol. The supernatants were concentrated to dryness in a water bath at 40 ^{o}C. The dried samples were tested for purity by TLC. The four separated samples were labelled MPF1 – MPF4 respectively.

2.2.10 Antidermatophyte Assay of the Separated Components

The anti-dermatophytic activity of the separated samples was determined by the Kirby-Bauer disc diffusion method as described by [9]. The fungal inoculum was prepared by suspending five distinct fungal colonies from a 7 days culture on SDA in 5 mL of sterile distilled water. The turbidity was adjusted to the turbidity of 0.5 McFarland standard by serial dilution, equivalent to 5 x 10^6 cfu/mL. A 1 in 10 dilution of the standardized suspension was carried out in sterile distilled water to obtain an inoculum size of 5 x 10^5 cfu/mL. About 0.1 mL of the final dilution of the test organism was aseptically introduced into labelled Petri dishes. Twenty millilitres (20 mL) of sterilized and cooled Sabouraud dextrose agar medium was aseptically poured into each Petri dish and gently swirled to mix. The plates were allowed to set. Whatman No. 42 filter paper was cut into discs measuring 5 mm in diameter. The discs were sterilised in hot air oven before impregnating with different concentrations of the separated samples MPF1-MPF4 respectively. The impregnated discs were placed in each plate as labelled. The plates were incubated at room temperature for 3-7 days after which microbial growth inhibition zones around the discs was observed.

2.2.11 Determination of the Mineral Element Composition of the Separated Components

The mineral element composition of the separated component was determined using the genius IF machine by Xenemetrix Limited at the Multi User Central Science Research Laboratory, Ahmadu Bello University, Zaria. The protocols were carried out according to the manufacturers' instruction. The element were detected based on their atomic weight.

2.2.12 Gas Chromatoghraphy- Mass Spectroscopy (GC-MS) Analysis

The Mass spectroscopy analysis of the separated compound was performed at the Multi User Central Science Research Laboratory, Ahmadu Bello University, Zaria. The sample was analysed with an Agilent 19091S gas chromatography system coupled with a HP-5 MS column (30 m x 250 µm with 0.25 µm film thickness) and Agilent 433UI ultra inert Mass Spectrometer Detector with triple-axis detection. The sample volume was 2 µL. The injection port was maintained at a temperature of 250 ºC; the initial temperature was 40 ºC, and was increased at a rate of 5 ºC/min to 270 ºC and pressure of 11.089 psi. The flow rate was 1.4034 mL/min with an average velocity of 42.958 cm/sec. The holding time was 1.1639 minutes, while the total run time was 650 minutes. The Mass Hunter software was used for detection. The detection employed the National Institute of Standards and Technology (NIST 14) Libraries guidelines.

2.2.13 Identification of the Chemical Compounds

The results of the GC-MS was examined using the database of the National Institute of Standards and Technology (NIST 14) libraries. The obtained spectrum of the unknown components was compared with the spectrum of known components stored in the library.

2.3 Statistical method

The data obtained were means of double determinations.

3.0 RESULTS

3.1 Fourier-Transform Infrared Spectroscopy

The spectra obtained (Figure 1) showed a broad rounded peak at 3302.4 cm^{-1} which represents aromatic OH group. A carbon-hydrogen (C-H) bending is present at 2926.0 cm^{-1} while a sharp absorption bend at 1561.8 cm^{-1} indicates the presence of an aromatic double bond. The absorption peak at 1394.0 cm^{-1} suggests an oxygen containing compound which could be an ether linkage, C-O-C. Medium absorption band at 1039.9 cm^{-1} means the molecule could be a primary aliphatic amine. Weak absorption band at 898.3 cm^{-1} indicates finger print region for alkyl group.

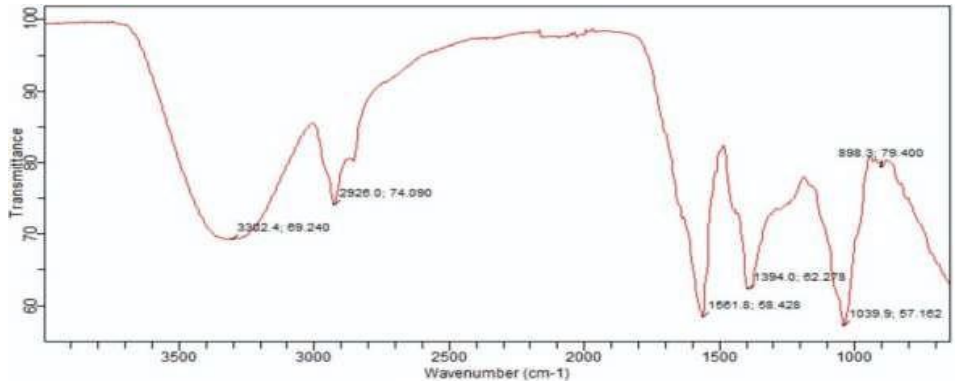

Figure 1: FTIR Spectra of the Aqueous Fraction of Methanol Leaf Extract of *M. paradisiaca* L.

3.2 Thin Layer Chromatography

The thin layer chromatographic separation of aqueous fraction of the methanol leaf extract of *Musa paradisiaca* L. showed four (4) distinct spots on the plate. The Retention factors (Rf) of the different spots were calculated as presented on Table 1.

Table 1: Retention Factors (Rf) of the Components of Aqueous Fraction of the Methanol Leaf Extract of *Musa paradisiaca* L.

Bioactive component	Rf value
MPF1	0.19
MPF2	0.34
MPF3	0.56
MPF4	0.88

3.3 Bioautography

Antifungal activity of the separated components of the aqueous fraction of the methanol extract of the leaf of *Musa paradisiaca* L. on bioautography showed that spot 4 (Rf = 0.88) exhibited the greatest activity by producing

wider zones of microbial inhibition for the fungal isolates tested. Figure 2 shows the effect of the Separated Components of Aqueous Fraction of the Methanol Leaf Extract of *Musa paradisiaca* L. on Bioautography

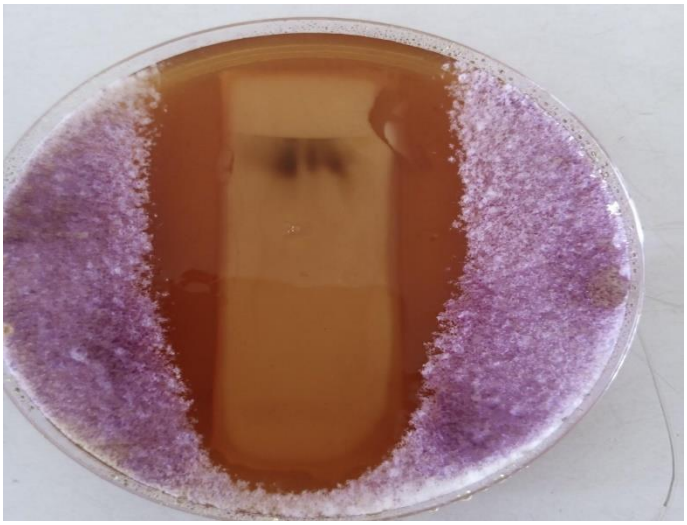

Figure 2: Effect of the Separated Components of Aqueous Fraction of the Methanol Leaf Extract of *Musa paradisiaca* L. on Bioautography

3.4 Anti-dermatophytic Activity of the Separated Components

The antidermatophyte activity of the separated components of the fraction showed that out of the four separated components, only MPF4 inhibited the growth of dermatophyte isolates used for the test. The result is presented on Table 2.

Table 2: Anti-dermatophytic Activity of the Separated Component MPF4

Conc. (mg/mL)	Zones of Inhibition (mm)				
	M. audouinii	*T. rubrum*	*T. verrucosum*	*T. tonsurans*	*E. floccosum*
10	15	14	10	12	13
5	12	12	8	10	11

3.5 Mineral Element Composition

Mineral elements were detected from the separated components of the aqueous fraction of the methanol extract of the leaf of *Musa paradisiaca* L. Carbon, Nitrogen and Potassium were the major components while others were in trace quantities. The mineral elements and their respective concentrations are presented on table 3.

Table 3: Mineral Elements and Concentrations

Element number	Element symbol	Element name	Atomic concentration	Weight concentration
6	C	Carbon	84.70	77.94
7	N	Nitrogen	11.48	12.32
19	K	Potassium	1.65	4.94
17	Cl	Chlorine	0.31	0.85
12	Mg	Magnesium	0.44	0.82
11	Na	Sodium	0.39	0.69
13	Al	Aluminium	0.27	0.56
15	P	Phosphorus	0.21	0.50
20	Ca	Calcium	0.15	0.47
14	Si	Silicon	0.22	0.47
16	S	Sulphur	0.14	0.35
22	Ti	Titanium	0.03	0.09

3.6 GC-MS of the Separated Component

GC-MS analysis of the separated component detected seven major compounds which were identified. The identified compounds and their molecular formula are presented on table 4 and the spectra (Figure 3)

Table 4: GC-MS Analysis of the Separated Components

Retention Time	Library Identity	Peak Area	Molecular Formula
19.3896	Butylated hydroxyl toluene	0.221	$C_{15}H_{24}O$
29.8454	n-Hexadecanoic	6.4986	$C_{16}H_{32}O_2$
30.0734	2-Hexadecanol	2.6477	$C_{16}H_{34}O$
31.9499	Cis-7,Cis-11-Hexadecadein-1-yl acetate	16.966	$C_{18}H_{32}O_2$
32.783	Cis-11-Hexadecanal	4.7446	$C_{16}H_{30}O$
33.1536	Oxirane,tridecyl	24.0448	$C_{15}H_{30}O$
33.5612	12-Methyl-E,E-2,13-Octadecadien-1-ol	12.4385	$C_{19}H_{32}O_2$

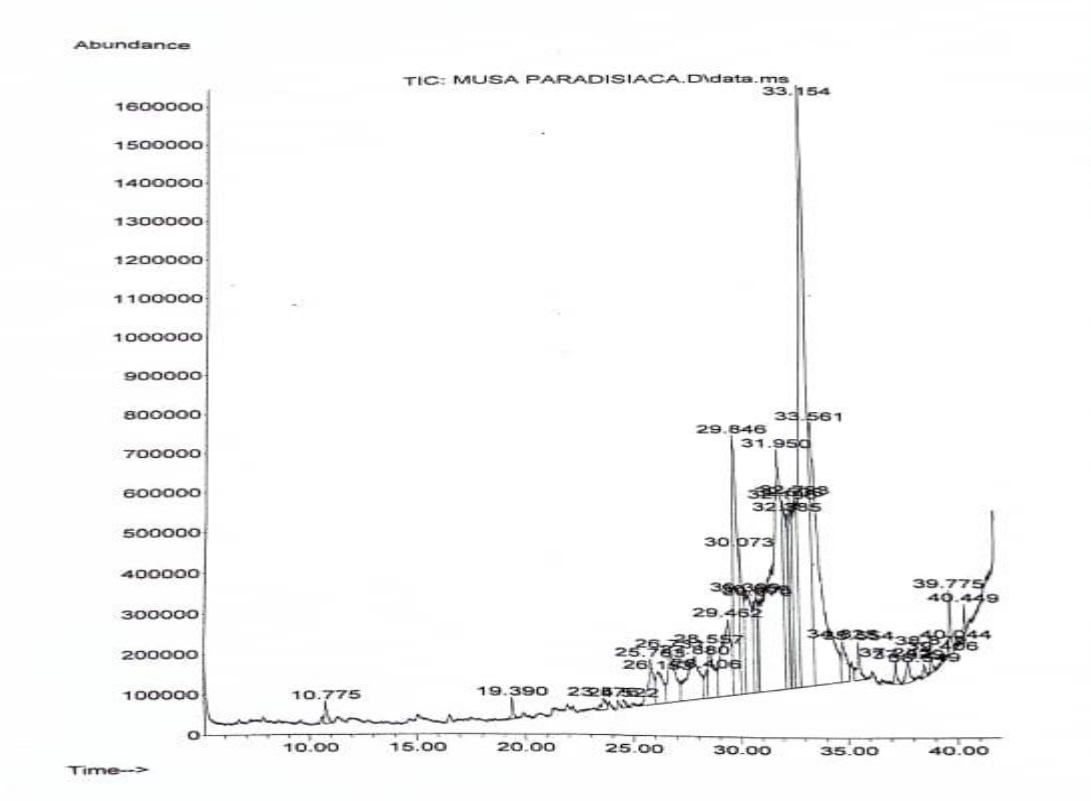

Figure 3: GC-MS spectra of the separated component of the Aqueous Fraction of the Methanol Leaf Extract of *M. paradisiaca*

4.0 DISCUSSION

Infrared spectroscopy is an easy way of identifying the presence of functional groups in a sample. The functional groups are identified based on the peaks that are obtained. The characteristic peaks observed in the IR spectra of aqueous fraction of the methanol leaf extract of *M. paradisiaca* confirm the presence of organic compounds in

the separated component. For instance, a broad rounded peak at 3302.4 cm^{-1} suggests an OH stretching frequency for phenols and the peak at 1561.8 cm^{-1} suggests the presence of a chelated group normally found in flavonoids. Several studies have reported that the biological activities of plant extracts are due to the presence of saponins, flavonoids and tanins [10, 11], which all belong to the phenol group of compounds. The bioactive components showed different degrees of activity against the different test isolates according to the different bands of compounds. *M. audouinii* was the most susceptible test organism, followed by *T. rubrum*, *E. floccosum* and *T. tonsurans*, while the least susceptible was *T. verrucosum*. The activity was observed to increase with increase in the retention factor with the last band (MPF4) showing a higher activity. Several authors have reported the TLC-bioautography of different parts of *Musa paradisiaca* against bacteria [10, 12, 13]. However, none is available on the bioautography of the leaf against fungi. The four components were tested for their individual antidermatophytic activity after separation by TLC method. It was observed that only MPF4 had sufficient antidermatophytic activity even at low concentrations. As such, MPF4 can be used as a lead component for the development of new antidermatophytic agents. Mineral elements are important components of plants, and are essential for the reproduction, growth and development of plants. However, there is concern among researchers that plants might accumulate heavy metals at significantly high levels, which can be toxic and pose health risks [14]. Thus, there is need to determine the mineral element composition of plant materials intended for human or animal use. In the chemical analysis of the separated component of aqueous fraction of the methanol leaf extract of *M. paradisiaca*, the three major mineral elements detected were carbon (77.94 %), nitrogen (12.32 %) and potassium (4.94 %), while other elements were in trace quantities. High level of potassium has been reported by [15] (in the pseudostem wastes), [16] (in the pulp of plantain) and [17] (in the Leachate). Apart from magnesium which is necessary for plant photosynthesis, no heavy metal was detected, indicating the safety of the separated component. For the development of new pharmaceuticals from plant, Gas chromatography-mass spectroscopy (GC-MS) analysis is very important for the detection of chemical constituents. In this study, GC-MS was employed for the detection of the bioactive components that are responsible for the observed antidermatophytic activity. The major compounds detected were, oxirane tridecyl, 12-methyl-E, E-2, 13 octadecadien-1-ol, cis-7, cis-11-Hexadecadien-1-yl acetate, n-Hexadecanoic acid, cis-11-Hexadecenal, 2-Hexadecanol and butylated hydroxytoluene. This is in agreement with the findings of [18], who reported the detection of n-Hexadecanoic acid and octadecadienoic acid, among others, from the hyroethanolic leaf extract of *M. paradisiaca*. [19], also identified n-hexadecanoic acid as one of the bioactive compounds in the ethyl acetate leaf extract of *Musa paradisiaca*. Oxirane tridecyl is a member of the phytol group of compounds. This compound has been found to possess antimicrobial, anti-inflammatory and anticancer properties [20]. N-hexadecanoic acid, also known as palmitic acid, has been reported to possess anticancer, antiinflammatory, hepatoprotective and hypoglycemic activity [18]. The antifungal activity of this compound has been reported by [19] against systemic candidiasis. 12-methyl-E, E-2, 13 octadecadien-1-ol, cis-7, cis-11-Hexadecadien-1-yl acetate, cis-11-Hexadecenal, 2-Hexadecanol are fatty acids which have been reported to play a great role as antifungal agents by absorbing the fungus since they have lipophilic nature [22]. Fatty alcohols exhibit the ability to interact with proteins, lipids and other biomolecules. They have been demonstrated to engage with phospholipids within cell membranes, consequently influencing their structure and function. It modulates the activity of proteins [23]. In the other hand, butylated hydroxytoluene is a naturally occurring phenolic antioxidant. The antifungal activity of this compound has been studied by [24], who reported the inhibition of the growth of *Aspergillus* species. Thus, the antidermatophyte activity of the separated bioactive component of the leaf of *Musa paradisiaca* is due to the antifungal properties of the identified compounds.

5.0 CONCLUSION

The results obtained from this research indicates that the leaf of *Musa paradisiaca* possesses components with activity against dermatophytes. This can be exploited as leads in the formulation of antifungal agents for the treatment of diseases caused by dermatophytes.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Contributions of Authors

Ememobong G. Asuquo-design, laboratory analysis and manuscript writing.Edet E. Akpanenang and Ugochukwu D. Onele- laboratory analysis. Chinweizu E. Udobi- supervision and editing of manuscript. Chibuzor N. Nwosu-proof reading and final editing of manuscript.

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