

## Pharmacognostic and Antifungal Evaluation of *Mitracarpus villosus* Extracts against Dermatophyte Isolates from Children in Makurdi, Nigeria

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### Abstract

Dermatophytosis is a major public health concern, especially in tropical regions. Increasing antifungal resistance necessitates the search for novel agents. *Mitracarpus villosus* is a medicinal plant used traditionally for skin infections. This study aimed to evaluate the *in vitro* antifungal potency of *M. villosus* extracts against dermatophytes isolated from school children in Makurdi, Nigeria. Clinical samples were collected from 384 primary school children with suspected dermatophytosis. Isolates were identified culturally, microscopically, and biochemically. *M. villosus* was extracted using aqueous, methanol, and ethyl acetate solvents via cold percolation. Phytochemical screening was performed qualitatively and quantitatively. The antifungal activity of extracts and standard drugs (Fluconazole, Ketoconazole, Nystatin, Griseofulvin) was assessed using agar well diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Fungicidal Concentration (MFC) assays. Data was subjected to ANOVA and DMRT. This was interpreted at  $P < 0.05$ . A total of 156 dermatophytes were isolated. *Microsporum canis* (30.13%) and *Trichophyton rubrum* (27.56%) were most prevalent. Phytochemical analysis revealed the presence of phenols, alkaloids, flavonoids, saponins, tannins, and terpenoids. Methanolic extract showed significant activity against *Epidermophyton floccosum* (19.67±0.67 mm inhibition zone). Fluconazole was the most effective standard drug. The mean MIC values for aqueous, methanolic, and ethyl acetate extracts were 50.00, 56.25, and 37.50 mg/ml, respectively. *Mitracarpus villosus* extracts possess notable antifungal activity against dermatophytes, validating its traditional use. The methanolic extract demonstrated promising efficacy, warranting further investigation for development as a topical antifungal agent.

**Keywords:** Dermatophytosis; *Mitracarpus villosus*; Antifungal activity; Phytochemistry; Minimum Inhibitory Concentration

### 1. Introduction

Dermatophytosis are superficial fungal infections caused by dermatophytes which are pathogens of humans and animals infecting keratinized tissues notably the skin, nails and hair <sup>1</sup>. The most common fungal infections of man are termed tinea infections or ringworm <sup>2</sup>. The major dermatophytes that cause tinea are *Trichophyton*, *Microsporum* and *Epidermophyton*. Also, *Candida* spp and dermatophytic molds have also been reported to cause dermatomycosis <sup>3</sup> Based on the mode of transmission, they are classified as anthropophilic (from humans), zoophilic (from animals) and geophilic (from soil). They are classified clinically by the sites of infections. *Tinea capitis* (head), *Tinea corporis* (body), *Tinea barbae* (beard), *Tinea faciei* (face), *Tinea curis* (groin), *Tinea unguium* (nails), *Tinea pedis* (foot), *Tinea manum* (hand). Other clinical variants such as *Tinea imbricate* and *psedoimbricata* have also been reported <sup>3</sup>. Although, several antifungal agents have been developed and used for internal and external treatment of dermatomycoses, increase in prevalence of dermatophytic infections have been seen in recent years globally, especially in developing countries due to poor socioeconomic status, high population densities and poor sanitary conditions among so many other factors <sup>4</sup>.

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Serious draw backs of the drugs currently available to treat fungal infection are reported and resistance to antifungal agents has increased in recent years and recurrence which could be attributed to global changing epidemiological trends and immune suppression. In addition, high toxicity, low efficacy and high cost of these drugs are limiting factors <sup>5, 6, 7</sup>. Remedy herbal medicines are handy for developing countries in treating common fungal diseases, as such, plants are rich in secondary metabolites that have been proven in vitro to contain antifungal properties. Globally, more than a billion fungal diseases are recorded yearly, especially among children. Dermatophytosis are among the most prevalent infections and constitute important public health problem, especially in Nigeria where 11.8 % of fungal infections are recorded annually. Also, resistance of some fungi species to fungicidal agents is a common health challenge that has been reported by <sup>8, 9, 10, 11</sup>. Relatively, they are many cases in Makurdi, Nigeria that are not adequately recorded or reviewed. Solution to this problem is the focus of this work. In the last few decades, there has been significant increase in the burden of fungal diseases which continues to pose a global threat to human health, plants and animals <sup>12</sup> Dermatophytosis is currently a disease of global importance and a public health problem all over the world especially in tropical and subtropical countries due to prevailing humidity and temperature as well as socioeconomic factors <sup>4</sup> The occurrence of fungal infections is increasing at an alarming rate especially among immunocompromised hosts such as AIDS patients, transplanted patients and neonates <sup>13</sup>. Antifungal drug resistance has dramatically increased, resulting to unsuccessful treatment of dermatophytic patients. The aim of the study is to evaluate antifungal potency of *Mitracarpus villosus* and other antifungal agents against human pathogenic dermatophyte isolates in Makurdi.

## 2. Methodology

### 2.1. Study Area

The study was carried out in Makurdi town (Fig.2), North central region of Nigeria. Makurdi town is located between latitude  $7^{\circ}37'0''$  and  $7^{\circ}47'0''$  North of the equator and Longitude  $8^{\circ}27'0''$  and  $8^{\circ}40'0''$  East. Makurdi town is a major link between the North and South of Nigeria. The River Benue which is the second largest River in Nigeria, bisects the town into two parts, North and South Banks. The climate is hot and humid. Makurdi, the capital of Benue State has grown into a town since its inception in the early 1930s and is experiencing rapid urbanization like many urban centres in the country <sup>14</sup>.

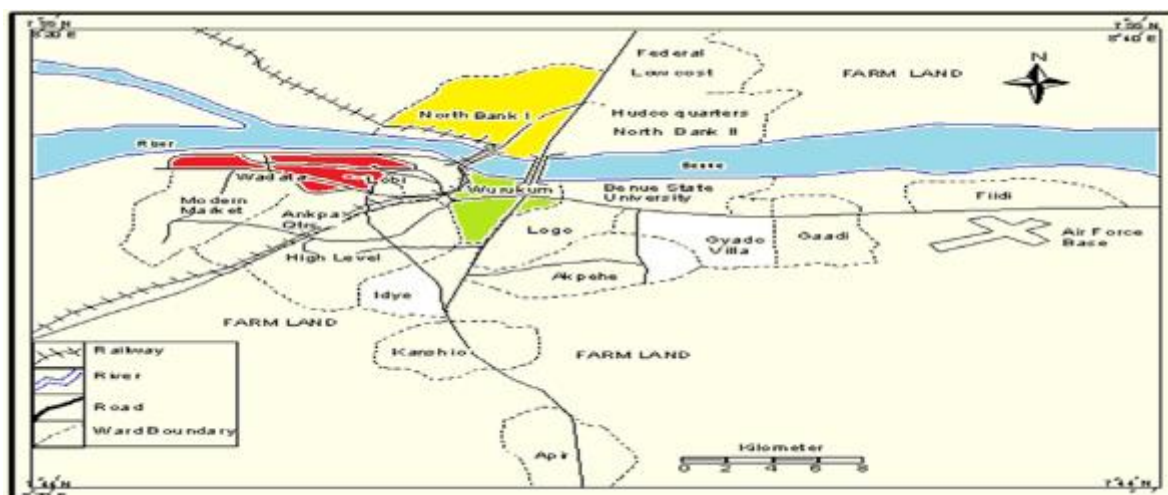


Figure 1 Map of Makurdi, the Benue State Capital

### 2.2. Ethical Clearance

Ethical clearance was obtained from Benue State Ministry of Health and sampling consent was sought from Makurdi local government education authority and school administrators.

### 2.3. Sample size determination

A total number of 384 samples were listed for this study. This number was arrived at using Rao soft online sample size calculator.

## 2.4. Collection of isolates

A total number of 384 samples were collected from pupils attending some selected primary schools in Makurdi. These pupils had visible clinical signs (erythema, alopecia, scaling, crusting, circinate lesions or follicular inflammation, pruritus, etc.) of dermatophyte infection on the skin, hair and nails. Samples were collected by scraping infected areas or lesions with surgical blade and swabbing with sterile swab sticks dipped in normal saline. They were properly labelled, packaged and transported to the Research Laboratory of the Department of Microbiology, JOSTUM for analysis

## 2.5. Collection and identification of *Mitracarpus villosus*

Fresh *M.villosus* plants were collected within Makurdi town (North bank). The plant was identified by experts in the Department of Botany, now plant science and Biotechnology, JOSTUM.

## 2.6. Collection of antifungal drugs

Four antifungal drugs namely Griseofulvin, Nystatine, Fluconazole and Ketoconazole were obtained from pharmacies in Makurdi.

## 2.7. Analytical preparations

### 2.7.1. Preparation of *Mitracarpus villosus*

The plant was washed and air dried at room temperature (25°C) for two weeks. It was then pulverised into powder and stored in an airtight bottle <sup>15</sup>

### 2.7.2. Media preparation

Sabouraud's Dextrose Agar (SDA), Potatoe Dextrose Agar (PDA), peptone water and Normal saline were used for this study. The media were all prepared according to the manufacturer's instructions.

## 2.8. Analytical Procedure

### 2.8.1. Determination of dermatophytes

Samples were divided into two portions; a portion of each sample was applied with KOH 10 – 20 % and lactophenol cotton blue solutions and examined microscopically for hyphae and conidia. The other portion was cultured on SDA and incubated at 25 ° C – 30 ° C for three weeks as described by <sup>17</sup>

### 2.8.2. Characterization of dermatophyte isolates

Samples were inoculated onto Sabouraud Dextrose Agar (SDA). Cycloheximide was added to inhibit saprophytic fungi and it was incubated at room temperature (25° C ± 2° C) for 4 weeks. The isolates were examined for morphological characteristics according to the method described by Khan *et al* <sup>16</sup>. Organisms were subcultured on Potatoe Dextrose Agar (PDA) at room temperature (25° C ± 2° C) for 4 – 7 days and stored in the refrigerator at 4° C for further use.

### 2.8.3. Biochemical test

Urease test was performed as described by Khan *et al.* <sup>16</sup>. Test tubes containing Christensen's urea agar were used to see the urease activity of the isolated dermatophyte species. The slant surface of the media was inoculated with pure culture of the test fungi and incubated at room temperature (25° C ± 2° C) for 7 days. Change in colour (pink colour) in the media indicated positive test result, no colour change was considered as negative.

### 2.8.4. Qualitative determination of phytochemical constituents of *Mitracarpus villosus* Extracts

The phytochemical components of *Mitracarpus villosus* plant extract was analysed according to the method described by Ishwa *et al.* <sup>17</sup>. The plant extracts were screened for alkaloids, flavonoids, saponins, terpenoid, phenols and tannins

### 2.8.5. Test for alkaloids

To determine the presence of alkaloids, 200 mg of the plant powder was added to 10 ml methanol followed by filtration. Then 2 ml filtrate was mixed with 2 ml HCl 1 %, and the mixture was heated at 37° C for 5 min. Dragendroff reagent was added, formation of orange precipitate indicated the presence of alkaloids <sup>17</sup>.

#### 2.8.6. Test for phenol

Ferric chloride test was used. Few drops of 5 % FeCl<sub>3</sub> solution were added to 2-3 ml of the crude plant extracts. Formation of deep blue-black colour indicated the presence of phenols <sup>17</sup>.

#### 2.8.7. Test for saponins

About 2 g of the crude plant extracts was boiled in 20 ml of distilled water in a water bath and filtered. Then 10 ml of the filtered sample was mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable froth. The froth was mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins <sup>17</sup>.

#### 2.8.8. Test for flavonoids

A few drops of 1 % NH<sub>3</sub> solution was added to the crude plant extracts in a test tube. A yellow colouration confirms the presence of flavonoid compounds <sup>17</sup>.

#### 2.8.9. Test for terpenoids

About 5 ml of the crude plant extracts was mixed with 2 ml of CHCl<sub>3</sub> in a test tube. Then 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added and then boiled. Formation of a grey colour indicate the presence of terpenoids <sup>17</sup>.

#### 2.8.10. Test for tannins

0.5 g of the powdered plant was boiled in 20 ml distilled water followed by filtration. Then 0.1 % FeCl<sub>3</sub> was added to the filtrate and the mixture was monitored until a brownish-green or blue-black colour appeared, which showed the presence of tannins <sup>17</sup>.

#### 2.8.11. Quantitative analysis of phytochemical components of *Mitracarpus villosus*

The phytochemical constituents of crude *Mitracarpus villosus* extracts was quantified using spectrophotometric method and Gas Chromatography.

### 2.9. Assessment of antifungal activity

#### 2.9.1. Standardization of inoculum

The sub cultured isolates were collected with a sterile wire loop and suspended in 2 ml of sterile 0.85 % saline. Conidia suspensions was filtered with sterile guaze and the concentrations was compared with 0.5 MCFarland turbidity standard <sup>18</sup>.

#### 2.9.2. Determination of zones of inhibition

The ethanol, ethyl acetate and aqueous extracts were tested for antifungal activity using the agar well diffusion assay. Twenty millilitre of sterilized Sabourad Dextrose Agar medium was poured in 15 cm petri dish. Twenty microliter of inoculums suspension of each test organism were distributed evenly over the surface. A 6mm cork borer was used to make wells on the plate. Fifty microliter of each anti-fungal drug and the crude plant extract were placed into the wells. Sterile distilled water was used as control. The plates were incubated at room temperature (25° C ± 2° C) for 5-7 days and the zones of inhibition were observed. This was done in triplicates <sup>19</sup>.

#### 2.9.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined using the broth dilution technique as outlined by Doughari and Obidah <sup>20</sup>. A two-fold serial dilution of the aqueous, ethyl acetate and ethanolic extracts were done using potato dextrose broth. A 0.1 ml aliquot of the 48 hours broth culture of the test fungi was dispensed into each of the dilution test tube and incubated at room temperature for 48 hours. The tubes were examined for presence of growth. The minimum inhibitory concentration was taken as the lowest concentration that prevented fungi growth.

#### 2.9.4. Determination of minimum fungicidal concentration (MFC)

The minimum fungicidal concentration was determined as described by Doughari and Obidah <sup>20</sup>. This was carried out by pour-plating all test tubes that did not show fungi growth on a sterile potato dextrose agar and incubated at room temperature for 48 hours. After 48 hours of incubation, plates with no visible growth of fungi were considered fungicidal

and the plate with minimum concentration was recorded as the minimum fungicidal concentration of the particular extract against the fungi isolate.

### 2.10. Statistical analysis

The experimental results (data) were statistically analysed using one-way ANOVA with assistance of SPSS version 20.0 software (IB19, Armonk, N.Y, USA) and means were compared using Duncan multiple range test (DMRT). The results were expressed in terms of mean  $\pm$  standard deviation. All data presented were mean values of triplicate measurement (n=3) obtained from the separate runs

## 3. Results

A total of 384 primary school children were screened. Table 1. shows fungi counts (in CFU/swab) of fungi isolates across the study locations. LGEA Yakyior and LGEA Owner Occupier recorded the highest fungal counts with  $292.42 \pm 52.89$  and  $290.00 \pm 80.36$  respectively. Army Children School North Bank showed a relatively high count of  $208.17 \pm 54.86$ . St. Catherine's recorded a moderate count at  $202.50 \pm 28.07$ , while LGEA Wurukum and St. Mary's North Bank had similar moderate counts of  $184.17 \pm 18.40$  and  $180.83 \pm 28.96$ . NKST Wadata showed a lower count of  $157.50 \pm 17.67$  and Holy Ghost Primary School Wadata recorded the lowest count at  $148.33 \pm 18.62$ . There is no significant difference in the total viable count of isolates across the different primary schools.

The cultural, microscopic and biochemical characteristics of fungi isolates in this study are presented in Table 2. Four fungi species were identified namely *T. rubrum*, *T. mentagrophyte*, *E. floccosum* and *M. canis*. Table 3. shows the occurrence of various fungi isolates across the different school locations. LGEA Wurukum had the highest prevalence of 36 (23.08 %), followed by LGEA Owner Occupier 28 (17.95 %), St Mary's Primary School North bank 23 (14.74 %), NKST Wadata 20 (12.82 %), Holy ghost primary School 18 (11.54 %), St Catherine's Primary School 14 (8.97 %), LGEA Yakyior 10 (6.41 %) and Army Children School North Bank had the least prevalence of 7 (4.49 %). Out of the 156 positive samples (isolates) *M. canis* had the highest occurrence of 47 (30.13 %) followed by *T. rubrum* 43 (27.56 %), *T. mentagrophyte* 41(26.28 %) and the least was *E. floccosum* 25 (16.03 %). *E. floccosum* was absent in Army Children School North Bank and St. Catherine's Primary School Wurukum. *T. rubrum* was not found in St. Mary's Primary School, North-Bank.

### 3.1. Phytochemical Analysis

Results of the qualitative phytochemical analysis of *M. villosus* samples using Aqueous, Methanolic and Ethylacetate solvents are presented in Table 4. *M. villosus* contains phenols, alkaloids, flavonoids, saponins, tannins and terpenoids. It is important to note that Saponins was absent in the qualitative screening of Ethylacetate plant extract.

Table 5 on the other hand, shows the results of the quantitative phytochemical analysis of the plant extract. The result shows that the aqueous extracts of *M. villosus* contained  $9.50 \pm 0.16$  mg/g of phenols,  $0.91 \pm 0.02$  mg/g alkaloids,  $2.24 \pm 0.02$  mg/g flavonoids,  $5.29 \pm 0.01$  mg/g saponins,  $7.87 \pm 0.23$  mg/g tannins and  $3.68 \pm 0.14$  mg/g of terpenoids. Methanolic extract of *M. villosus* contained phenols  $6.30 \pm 0.02$  mg/g, alkaloids  $0.10 \pm 0.02$  mg/g, flavonoids  $3.89 \pm 0.03$  mg/g, saponins  $4.14 \pm 0.09$  mg/g, tannins  $8.86 \pm 0.07$  mg/g and terpenoids  $5.34 \pm 0.02$  mg/g. while the Ethylacetate extract of *M. villosus* had concentrations of  $4.83 \pm 0.07$  mg/g of phenols,  $0.03 \pm 0.01$  mg/g of alkaloids,  $1.39 \pm 0.03$  mg/g of flavonoids,  $1.60 \pm 0.26$  mg/g of saponins,  $4.20 \pm 0.20$  mg/g of tannins and terpenoids  $2.8 \pm 0.01$  mg/g.

Table 6 Shows GC-MS results of methanolic extract of *M. villosus*. Retention time of the different eluents ranges between 1.182 Ethanimidic acid ethyl ester to 31.834 2 Isopropyl-5methyl cyclohexyl 3-(1-(4-chlorophenyl)-3 oxobutyl)-coumarin-4-yl carbonate. A total of 24 eluent were elucidated from this extract with varying molecular weight ranges from 87.07 to 524.19 g/mole.

The GC-MS of Aqueous extracts of *M. villosus* is as presented in Table 7. Here retention time ranges between 1.199 to 9.219 for ethanimidic acid ester and cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-. A total of seventeen eluents were elucidated with molecular weight between 87.07-280.31g/mole.

Data from the GC-MS analysis of Ethylacetate extracts of *M. villosus* presented in Table 8 showed that the retention time of the thirty eluents ranges from 1.205 to 20.202 for Ethanimidic acid, ethyl ester to Cyclohexanemethyl propanoate with molecular weight between 87.06 - 170.13 g/mole. The Table also gives the peak area, height and molecular formular of eluent.

### 3.2. Susceptibility of *M. villosus* Results

Table 9 shows the zones of inhibition of extracts *M. villosus*. The results shows that fluconazole had the highest zone of inhibition particularly against *T. rubrum* and *T. Mentagrophyte*, followed by Ketoconazole which is highly effective against *M. canis* and *E. floccosum*. Methanolic extract showed considerable inhibition against *E. floccosum*. Aqueous extract is moderately effective against *M. canis* and *T. Mentagrophyte* while ethylacetate extract is the least effective against *T. Mentagrophyte*. Nistatin is effective against *E. floccosum* but less against other fungi and griseofulvin is highly effective against *T. Mentagrophyte* but has no effect on *E. floccosum*.

Table 10 presents the minimum inhibitory concentrations (MIC) of *Mitracapus villosus* plant extracts on fungal isolates in milligrams per milliliter (mg/ml). The Table shows that *Mitracapus villosus* extracts (aqueous, methanolic and ethylacetate) exhibited inhibitory properties against the tested fungal isolates. The mean MIC values showed that aqueous extract had the highest mean inhibitory concentration of 50.00 mg/ml, followed by the methanolic extract 56.25 mg/ml, and the lowest ethylacetate extract 37.50 mg/ml. *E. floccosum* and *T. rubrum* showed variability in their sensitivity with aqueous and methanolic extracts having higher MIC values (100.00 mg/ml) compared to Ethylacetate (50.00 mg/ml). while the sensitivity for *M. canis* and *T. mentagrophytes* are constant across all three extracts (25.00 mg/ml).

Table 11 shows the minimum fungicidal concentrations (MFC) of *Mitracapus villosus* plant extracts against fungal isolates measured in mg/ml. The Table indicates that *Mitracapus villosus* extracts have fungicidal activity against the tested fungal isolates. The mean MFC values indicated that the aqueous and methanolic extracts have the same mean fungicidal concentration values of 75.00 mg/ml while ethylacetate extract had a slightly lower mean of 62.50 mg/ml. *E. floccosum* exhibited higher MFC values compared to *M. canis* and *T. mentagrophytes*

**Table 1** Fungi Counts of Isolates from Different Primary School Children in the Study Location

Location	Fungi Count (CFU)
St. Catherine	202.50 ± 28.07 <sup>ab</sup>
LGEA Yakyior	292.42 ± 52.89 <sup>a</sup>
NKST Wadata	157.50 ± 17.67 <sup>ab</sup>
Holy Ghost Primary School	148.33 ± 18.62 <sup>b</sup>
St. Mary's North Bank	180.83 ± 28.96 <sup>ab</sup>
LGEA Wurukum	184.17 ± 18.40 <sup>ab</sup>
LGEA Owner Occupier	290.00 ± 80.36 <sup>a</sup>
Army Children School (N/B)	208.17 ± 54.86 <sup>ab</sup>

df = 11, P < 0.05 Means with different superscript varies significantly between the rows

**Table 2** Cultural, Microscopic and Biochemical Characteristics of Fungal Isolates

Fungi Isolates	Cultural	Microscopic	Biochemical
<i>T. rubrum</i>	Velvety white cottony upper side and deep red to yellowish under side	Tear drop shaped microconidia, long smooth walled macroconidia, septate straight hyphae	Urease negative
<i>M. canis</i>	White cottony surface with deep yellow from reverse	Septate hyphae, spindle shaped macroconidia	-
<i>E. floccosum</i>	Flat, velvety powdery surface Upper side and yellowish underside	Septate hyphae, Club shaped macroconidia.	-
<i>T. mentagrophyte</i>	Cottony to granular colony, upper side whitish and underside brownish	Curved septate hyphae, grape-like clusters of microconidia	Urease positive

**Table 3** Occurrence of Fungal Isolates in Different Study Locations

School / Location	<i>M. canis</i> (%)	<i>T. mentagrophyte</i> (%)	<i>E. floccosum</i> (%)	<i>T. rubrum</i> (%)	Total (%)
St. Catherine's Primary School	6 (3.85)	4 (2.56)	0 (0.00)	4 (2.56)	14 (8.97)
L.G.E.A. Yakyior	3 (1.92)	0 (0.00)	4 (2.56)	3 (1.92)	10 (6.41)
NKST Wadata	5 (3.21)	4 (2.56)	3 (1.92)	8 (5.13)	20 (12.82)
Holy Ghost Primary School, Wadata	4 (2.56)	5 (3.21)	2 (1.28)	7 (4.49)	18 (11.54)
St. Mary's Primary School, North Bank	8 (5.13)	11 (7.05)	4 (2.56)	0 (0.00)	23 (14.74)
L.G.E.A. Wurukum	11 (7.05)	10 (6.41)	6 (3.85)	9 (5.77)	36 (23.08)
L.G.E.A. Owner Occupier	7 (4.49)	4 (2.56)	6 (3.85)	11 (7.05)	28 (17.95)
Army Children School, North Bank	3 (1.92)	3 (1.92)	0 (0.00)	1 (0.64)	7 (4.49)
Total	47 (30.13)	41 (26.28)	25 (16.03)	43 (27.56)	156 (100.00)

**Table 4** Qualitative Phytochemical Analysis of *M. villosus* Extracts

Phytochemicals	Aqueous Extract	Methanol Extract	Ethyl Acetate Extract
Phenols	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	-
Tannins	+	+	+
Terpenoids	+	+	+

Key += Present -= Negative

**Table 5** Quantitative Screening of phytochemicals of methanolic, Ethyl acetate and Aqueous extracts of *M.villosus*

Phytochemicals	Aqueous Extract	Methanol Extract	Ethyl Acetate Extract	P-value
Phenols	9.50 ± 0.16 <sup>a</sup>	6.30 ± 0.02 <sup>b</sup>	4.83 ± 0.07 <sup>c</sup>	0
Alkaloids	0.91 ± 0.02 <sup>a</sup>	0.10 ± 0.02 <sup>b</sup>	0.03 ± 0.01 <sup>c</sup>	0
Flavonoids	2.24 ± 0.02 <sup>b</sup>	3.89 ± 0.03 <sup>a</sup>	1.39 ± 0.03 <sup>c</sup>	0
Saponins	5.29 ± 0.01 <sup>a</sup>	4.14 ± 0.09 <sup>b</sup>	1.60 ± 0.26 <sup>c</sup>	0.001
Tannins	7.87 ± 0.23 <sup>b</sup>	8.86 ± 0.07 <sup>a</sup>	4.20 ± 0.20 <sup>c</sup>	0
Terpenoids	3.68 ± 0.14 <sup>b</sup>	5.34 ± 0.02 <sup>a</sup>	2.84 ± 0.01 <sup>c</sup>	0

Means with different superscript varies significantly between the rows

**Table 6** GC=MS of Methanolic Extracts of *M. villosus*

Peak	R.T (min)	Area	Height	Molecular Formula	M.M (g/mol)	Identified Compound
1	1.182	3,624,509.36	978,184.00	C <sub>4</sub> H <sub>9</sub> NO	87.07	Ethanimidic acid ethyl ester
2	1.256	2,571,640.73	832,473.01	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	90.06	1,2-Butanediol
3	1.394	134,338.45	63,639.92	C <sub>2</sub> H <sub>3</sub> ClO <sub>2</sub>	93.98	Methyl chloroformate
4	1.823	106,597.74	77,377.49	C <sub>4</sub> H <sub>6</sub> Cl <sub>2</sub> O <sub>2</sub>	55.97	Chloromethyl 2-chloropropanoate
5	1.891	982,949.52	484,675.73	C <sub>3</sub> H <sub>9</sub> FSi	—	Trimethylsilyl fluoride
6	2.595	47,691.90	20,713.85	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>	151.06	Oxime-, methoxy-phenyl-
7	3.362	44,379.63	4,772.41	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	174.10	2H-Pyran, 2-(tert-butylthio) tetrahydro-
8	4.747	87,084.32	9,212.49	C <sub>7</sub> H <sub>10</sub> O <sub>2</sub>	126.06	Cyclobutanone, 2-methyl-2-oxiranyl-
9	7.350	802,268.79	116,506.35	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	130.09	(R)-(-)-4-Methylhexanoic acid
10	8.060	564,909.22	372,691.35	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.25	Hexadecanoic acid, methyl ester
11	8.334	374,343.57	145,613.50	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	—	Undecanoic acid
12	8.689	494,611.76	233,570.90	C <sub>9</sub> H <sub>11</sub> Cl <sub>3</sub> NO <sub>3</sub> P <sub>2</sub>	348.92	Chlorpyrifos
13	9.296	755,727.72	171,258.58	C <sub>19</sub> H <sub>40</sub> O	284.30	2,6,10,14-Tetramethylpentadecan-6-ol
14	9.422	61,824.69	23,702.65	C <sub>16</sub> H <sub>28</sub> O	236.21	cis,cis-7,10-Hexadecadienal
15	9.484	144,818.91	70,561.74	C <sub>10</sub> H <sub>16</sub> O	152.12	2-Methyl-6-methylene-octa-1,7-dien-3-ol
16	9.736	403,995.57	55,441.70	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.24	Tetradecanoic acid, 12-methyl-, methyl ester
17	9.868	213,270.00	33,120.33	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.24	Tetradecanoic acid, 12-methyl-, methyl ester
18	10.995	37,395.56	11,902.18	C <sub>9</sub> H <sub>16</sub> O <sub>3</sub>	172.10	9-Oxononanoic acid
19	14.354	137,357.13	19,171.35	C <sub>9</sub> H <sub>16</sub> O <sub>3</sub>	172.10	9-Oxononanoic acid
20	16.603	282,058.34	35,923.72	C <sub>10</sub> H <sub>12</sub> N <sub>4</sub>	188.10	2,6-Bis(diazo)adamantine
21	17.427	64,718.55	10,350.07	C <sub>11</sub> H <sub>12</sub>	144.09	Benzene, 4-pentynyl-
22	18.239	121,893.80	7,823.88	C <sub>7</sub> H <sub>15</sub> NO	129.11	Isoxazolidine, 5-ethyl-2,4-dimethyl-, cis-
23	20.757	123,689.65	22,563.51	C <sub>14</sub> H <sub>22</sub> O	206.16	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
24	31.834	98,029.15	5,705.02	C <sub>30</sub> H <sub>33</sub> ClO <sub>6</sub>	524.19	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate

**Table 7** GC=MS of Aqueous Extracts of *M. villosus*

Peak	R.T (min)	Area	Height	Molecular Formula	M.M (g/mol)	Identified Compound
1	1.199	1,816,663.94	504,998.00	C <sub>4</sub> H <sub>9</sub> NO	87.07	Ethanimidic acid, ethyl ester
2	1.296	856,023.77	203,537.00	C <sub>2</sub> H <sub>3</sub> ClO <sub>2</sub>	93.98	Chloroformate
3	1.777	795,842.75	279,049.11	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>	132.04	1,2-Propanediol diformate
4	1.886	442,558.41	263,037.87	C <sub>3</sub> H <sub>9</sub> NO	75.06	(R)-(-)-2-Amino-1-propanol
5	2.223	614,193.17	126,276.48	C <sub>6</sub> H <sub>11</sub> N <sub>3</sub>	125.09	1H-Imidazole-4-ethanamine, 2-methyl-
6	2.458	849,873.30	192,071.52	C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>	176.05	Furan, 2,5-dimethyl-
7	2.630	614,623.82	127,488.69	C <sub>4</sub> H <sub>8</sub> S	88.03	Thiirane, 2,3-dimethyl-, trans-
8	2.939	960,677.60	154,359.46	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub>	110.08	(R)-(-)-1-Cyano-2-methylpyrrolidine
9	3.082	669,583.24	469,325.25	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.04	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
10	3.265	313,192.59	39,152.74	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116.16	Pentanoic acid, 4-methyl-
11	4.226	4,097,499.48	877,428.68	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.04	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
12	4.615	326,429.77	71,894.84	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub>	114.11	4-Piperidinethanamine
13	4.798	18,859,268.40	3,574,957.49	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	5-Hydroxymethylfurfural
14	5.290	322,535.57	60,764.94	C <sub>9</sub> H <sub>16</sub> O <sub>2</sub>	156.11	1-Allylcyclohexane-1,2-diol
15	5.903	6,779,549.76	758,973.54	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	1,2,3-Benzenetriol
16	7.264	3,955,972.28	227,674.74	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342.11	Sucrose
17	9.219	298,989.77	93,399.13	C <sub>20</sub> H <sub>40</sub>	280.31	Cyclohexane, dimethylhexyl)-4-(4-methylpentyl)- 1-(1,5-

**Table 8** GCMS of Ethyl Acetate Extracts of *M. villosus*

Peak	R.T (min)	Area	Height	Molecular Formula	M.M (g/mol)	Identified Compound
1	1.205	3,435,310.33	729,293.45	C <sub>4</sub> H <sub>9</sub> NO	87.06	Ethanimidic acid, ethyl ester
2	1.331	1,800,475.57	331,969.98	C <sub>6</sub> H <sub>14</sub> O <sub>2</sub>	118.09	meso-3,4-Hexanediol
3	1.428	1,731,367.99	290,187.26	C <sub>6</sub> H <sub>12</sub> O	100.08	Oxirane, 2,3-diethyl-
4	1.829	1,821,102.40	328,749.80	C <sub>5</sub> H <sub>10</sub> O	86.07	1-Methylcyclopropanemethanol
5	1.880	2,659,151.88	527,139.55	C <sub>5</sub> H <sub>10</sub> O	86.07	1-Methylcyclopropanemethanol
6	2.029	1,209,300.69	143,410.96	C <sub>7</sub> H <sub>16</sub> O	116.12	1-Pentanol, 3,4-dimethyl-
7	2.178	1,108,340.44	186,110.98	C <sub>7</sub> H <sub>16</sub> O	116.12	1-Pentanol, 3,4-dimethyl-
8	2.595	52,252.95	24,491.57	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	130.09	Butanol, 3-methyl-, acetate
9	3.786	95,643.37	23,658.20	C <sub>8</sub> H <sub>14</sub>	110.10	2-Methyl-1,5-heptadiene (c,t)

10	3.917	60,423.58	10,817.90	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.04	1,2-Cyclobutanedicarboxylic acid, trans-
11	4.198	209,423.18	40,401.46	C <sub>9</sub> H <sub>17</sub> NO <sub>4</sub>	203.11	N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane
12	4.718	1,771,006.05	231,060.56	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	5-Hydroxymethylfurfural
13	5.313	4,282,216.00	12,937.46	C <sub>7</sub> H <sub>10</sub> O <sub>2</sub>	126.06	4-Ethyl-2-hydroxycyclopent-2-en-1-one
14	5.611	56,587.08	14,279.94	C <sub>7</sub> H <sub>9</sub> FN <sub>2</sub> O <sub>2</sub>	172.06	4-Fluoro-1-methyl-5-carboxylic acid, ethyl ester
15	5.886	3,407,022.64	452,049.25	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	1,2,3-Benzenetriol
16	6.006	934,971.80	18,282.82	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03	1,2,3-Benzenetriol
17	6.990	1,539,728.61	75,282.82	C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>	208.09	Ethyl α-D-glucopyranoside
18	7.327	740,237.99	108,720.89	C <sub>11</sub> H <sub>16</sub> O <sub>4</sub>	212.10	—
19	8.054	279,570.34	108,720.89	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.25	Hexadecanoic acid, methyl ester
20	8.460	398,253.16	233,464.39	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	186.16	Undecanoic acid
21	9.284	408,932.01	136,942.61	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.27	Hexadecanoic acid, ethyl ester
22	9.673	455,557.13	82,127.29	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.27	9-Octadecenoic acid (Z)-, methyl ester
23	9.874	1,505,025.75	82,127.29	C <sub>15</sub> H <sub>28</sub>	208.21	1-Cyclohexylnonene
24	10.103	301,774.56	112,790.72	C <sub>15</sub> H <sub>28</sub>	208.21	1-Cyclohexylnonene
25	10.955	75,002.45	112,790.72	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.30	Octadecanoic acid, 17-methyl-, methyl ester
26	11.693	205,163.02	17,646.44	C <sub>15</sub> H <sub>28</sub>	208.21	1-Cyclohexylnonene
27	13.398	86,377.37	10,429.80	C <sub>12</sub> H <sub>24</sub> O	184.18	Z-2-Dodecenol
28	19.693	455,629.19	455,629.19	C <sub>10</sub> H <sub>16</sub> O	152.12	2-Methyl-6-methylene-octa-1,7-dien-3-ol
29	20.202	88,221.28	11,455.12	C <sub>8</sub> H <sub>18</sub> O	130.13	2-Heptanol, 5-methyl-
30	20.202	88,221.28	11,455.12	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	170.13	Cyclohexanemethyl

**Table 9** Zones of Inhibition of *M. villosus* Plant Extracts and Mycotic Agents on Fungi Isolates in mm

Treatment / Antimicrobial Agent	<i>M. canis</i> (mm)	<i>T. mentagrophyte</i> (mm)	<i>E. floccosum</i> (mm)	<i>T. rubrum</i> (mm)
Aqueous extract	20.33 ± 0.88 <sup>bc</sup>	19.33 ± 0.88 <sup>b</sup>	14.33 ± 2.73 <sup>b</sup>	14.67 ± 1.45 <sup>cd</sup>
Methanol extract	24.67 ± 0.88 <sup>b</sup>	21.33 ± 0.88 <sup>b</sup>	19.67 ± 0.67 <sup>a</sup>	12.33 ± 0.88 <sup>d</sup>
Ethyl acetate extract	18.67 ± 0.88 <sup>c</sup>	14.33 ± 0.88 <sup>c</sup>	12.33 ± 0.88 <sup>b</sup>	16.33 ± 0.88 <sup>c</sup>
Fluconazole	32.67 ± 3.28 <sup>a</sup>	24.33 ± 1.20 <sup>a</sup>	22.67 ± 0.33 <sup>a</sup>	29.67 ± 0.88 <sup>a</sup>
Ketoconazole	31.33 ± 0.67 <sup>a</sup>	20.33 ± 0.33 <sup>b</sup>	21.33 ± 0.33 <sup>a</sup>	19.33 ± 0.88 <sup>b</sup>
Nystatin	17.33 ± 0.88 <sup>c</sup>	15.67 ± 0.33 <sup>c</sup>	19.67 ± 0.88 <sup>a</sup>	15.67 ± 0.33 <sup>c</sup>
Griseofulvin	20.33 ± 0.33 <sup>bc</sup>	25.00 ± 0.58 <sup>a</sup>	0.00 ± 0.00 <sup>c</sup>	21.00 ± 0.58 <sup>b</sup>
P-value	0.000	0.000	0.000	0.000

df = 11, P=0.05

**Table 10** Minimum Inhibitory Concentrations of *M. villosus* Plant Extracts on Fungi Isolates in mg/ml

Isolates	Aqueous Extract	Methanol Extract	Ethyl Acetate Extract	Mean ± SEM
<i>M. canis</i>	25.00	25.00	25.00	25.00 ± 0.00 <sup>b</sup>
<i>T. mentagrophyte</i>	25.00	50.00	25.00	33.33 ± 8.33 <sup>b</sup>
<i>E. floccosum</i>	50.00	50.00	50.00	50.00 ± 0.00 <sup>b</sup>
<i>T. rubrum</i>	100.00	100.00	50.00	83.33 ± 16.67 <sup>a</sup>
Mean ± SEM	50.00 ± 17.68 <sup>a</sup>	56.25 ± 15.73 <sup>a</sup>	37.50 ± 7.22 <sup>b</sup>	—

P= 0.05 Means with different superscript varies significantly between the rows

**Table 11** Minimum Fungicidal Concentrations of *M. villosus* Plant Extracts on Fungal Isolates in mg/ml

Fungal Isolates	Aqueous Extract	Methanol Extract	Ethyl Acetate Extract	Mean ± SEM (mg/mL)
<i>M. canis</i>	50.00	50.00	50.00	50.00 ± 0.00 <sup>b</sup>
<i>T. mentagrophyte</i>	50.00	50.00	50.00	50.00 ± 0.00 <sup>b</sup>
<i>E. floccosum</i>	100.00	100.00	100.00	100.00 ± 0.00 <sup>a</sup>
<i>T. rubrum</i>	50.00	50.00	50.00	83.00 ± 16.67 <sup>a</sup>
Mean ± SEM	75.00 ± 14.43	75.00 ± 14.43	62.50 ± 12.50	—

P= 0.05. Means with different superscript varies significantly between the rows

#### 4. Discussion

This study observed that *M. canis* and *T. rubrum* were the most prevalent dermatophytes followed by *T. mentagrophytes* and *E. floccosum* was the least. This result is similar with work done by Dogo *et al.*<sup>21</sup> who carried out a study on the prevalence of *Tinea Capitis* among school children in Nok Community, Kaduna State, Nigeria. They observed that *T. rubrum* (28,8 %) and *M. canis* (22.7 %) were the most prevalent isolated dermatophytes in the study. The low prevalence of *E. floccosum* in this study agrees with findings by Adesiji *et al.*<sup>22</sup> who carried out a study in Osogbo, Nigeria. The study observed that *T. rubrum* (34 %), *T. mentagrophytes* (31 %) and *M. canis* (18 %) had higher prevalence compared to *E. floccosum* (6 %). Another study in southwest states of Nigeria by Ukhureigbe *et al.*<sup>23</sup> also observed that *M. canis* (n=21; 28 %) was the most identified dermatophyte, *E. floccosum* was low (n= 4; 5.3 %) and *T. rubrum* (n=2; 2.7 %) was the least identified dermatophyte. This disagrees with the current study which observed higher prevalence of *T. rubrum* 41 (26.28 %) although low prevalence of *E. floccosum* was similar. These differences in the different prevalences might be as a result of geographic variations amid other socioeconomic factors.

The distribution of identified species suggests a diverse presence of dermatophytes in the sampled population. The identified species *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, and *M. canis* are all known to cause dermatophytosis. This agrees with studies by Ezihe *et al.*<sup>24</sup> who noted that a variety of dermatophytes are responsible for dermatophytosis in Bauchi metropolis. Coulibaly *et al.*<sup>25</sup> also stated that dermatophytes prevalence and pattern of infection varies worldwide, this reflects differences in geographic distributions and other epidemiological factors such as age, sex, season and socioeconomic factors of this disease. The identification of species like *T. mentagrophytes* and *M. canis* which can infect both humans and animals is similar to the work of Ezihe *et al.*<sup>24</sup> who identified two zoophilic species *M. canis* and *T. mentagrophytes* out of the five identified species in their study. This highlights the need for integrated approaches to control zoonotic diseases and the importance of maintaining proper hygiene and implementing preventive measures especially in communal environments such as schools.

*Mitracarpus villosus* is a plant with rich diverse pharmacological potentials such as antioxidant, antimicrobial, antidiabetic and anti-inflammatory properties. These properties have been reported to be as a result of effects of the rich bioactive compounds eliciting the earlier mentioned pharmacological activities. Findings of this study observed the presence of phytochemical constituents such as tannins, phenols, alkaloids, terpenoids, flavonoids and saponins. This agrees with Ezihe *et al.*<sup>24</sup> in his review on the medicinal uses, phytochemistry and pharmacological activities of *Mitracarpus* species (Rubiaceae). The different extraction methods yielded varying concentrations of phytochemicals. This complexity suggests the potential for a variety of pharmacological activities, including antioxidant, anti-

inflammatory, and antimicrobial effects. This agrees with studies by Oguntona *et al.*<sup>26</sup>, who stated that *M. villosus* is an important medicinal plant widely known for its broad spectrum of pharmacological activities and biological uses. The study also showed the phytochemical analysis of the methanolic extract of *M. villosus* which was similar to findings of this study.

Gas Chromatography-Mass Spectrophotometry (GC-MS) technique used for further separation of the various crude extracts of *M. villosus* showed that each of the crude extract consist of tens of organic compounds. The data generated also show similarities in compounds as some of these constituents are common to all three crude extracts or among two, like hexadecenoic acid, undecanoic acid, ethanimidic acid ester, chloroformate etc These similarities in the chemical composition further reflects that the extract have one origin and the few variations could be as a result of the different solvents used for extraction since they do not have the same extraction ability. Oguntona *et al.*<sup>26</sup>, and Ezihe *et al.*<sup>24</sup> in their studies also showed a wide range of bioactive compounds such as hexadecanoic acid, heptadecanoic acid etc in the various extracts.

Fluconazole exhibits the most significant antifungal activity, especially against *T. rubrum* and *T. mentagrophyte* in this study. This indicates its strong efficacy and potential as a first-line treatment for infections caused by these dermatophytes. This differs with result from a study in Okelele Community in North Central Nigeria by Ekundayo *et al.*<sup>27</sup>, which stated that all isolates of *T. rubrum* were resistant to Fluconazole, Ketoconazole, Itraconazole and Griseofulvin. These variations could be due to the mode of collection and handling of isolates, time of carrying out the analysis or even different environmental factors. Ketoconazole shows excellent antifungal properties, particularly effective against *M. canis* and *E. floccosum*. This makes it a potent alternative to Fluconazole, especially for these specific fungi.

The methanolic extract demonstrates substantial antifungal activity against *E. floccosum*. This suggests that methanol is an effective solvent for extracting bioactive compounds from *M. villosus* that can inhibit infection caused by *E. floccosum*. The aqueous extract shows moderate inhibition zones, particularly effective against *M. canis* and *T. mentagrophyte*. This indicates that water-soluble compounds in *M. villosus* can inhibit these fungi, although to a less extent than methanolic extracts. Ethyl acetate extract displays the least antifungal activity, especially against *T. mentagrophyte*. This suggests that the bioactive compounds soluble in ethyl acetate might not be as potent or effective in inhibiting fungal growth.

Nystatin is effective against *E. floccosum* but less effective against other fungi. This selective inhibition implies that Nystatin's mode of action or its target site within the fungal cells is more specific to *E. floccosum*. Griseofulvin shows high effectiveness against *T. mentagrophyte* but has no inhibitory effect on *E. floccosum*. This specificity suggests that Griseofulvin targets unique aspects of *T. mentagrophyte's* cellular structure or metabolic processes. For infections caused by *T. rubrum* and *T. mentagrophyte*, Fluconazole should be the preferred treatment due to its highest zones of inhibition. Ketoconazole is highly recommended for *M. canis* and *E. floccosum* infections, providing a strong alternative to Fluconazole. The methanolic extract of *M. villosus* can be considered for *E. floccosum* infections offering a natural alternative to synthetic antifungals which have been reported to cause liver function disorders. The moderate efficacy of aqueous extracts against *M. canis* and *T. mentagrophyte* suggests potential for developing herbal treatments though they may require higher doses or combination with other extracts for enhanced effectiveness. The selective effectiveness of Nystatin and Griseofulvin highlights the need for targeted antifungal therapies based on the specific dermatophyte involved. This can optimize treatment outcomes and minimize side effects.

The MIC and MFC values across the three types of extracts in the study indicates that these extracts have similar inhibitory effects against the fungal isolates regardless of the solvent used for extraction. This agrees with the findings of Aboh *et al.*<sup>28</sup>, who reported that *M. villosus* extracts have similar MIC and MFC against fungal isolates.

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## 5. Conclusion

From the result of this study and data provided by literature it can be concluded that *M. villosus* has significant pharmacological potential, this confirms claims of traditional healers in the use of the plant. The level of phytochemicals identified and quantified in this study and the antifungal activity exhibited by the extracts with multiple identified therapeutic uses such as antioxidant, antimicrobial and anti-inflammatory properties present the extract as a very useful material that could be used for drug discovery in phytomedicine. This study supports the use of different *M. villosus* extract with each extraction method offering unique benefits based on its phytochemical profile.

### Recommendations

There is need for further investigation to establish the economic viability of exploiting *M. villosus* to address fungal infections.

The extracts of *M. villosus* are recommended for use as antifungal agent for dermatophytosis. Being found to be effective against dermatophytes.

Given the pharmacological activities of this plant, further clinical studies are recommended on its toxicity so as to determine its safety profile, hence promote its therapeutic uses.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of Ethical approval*

Ethical Clearance was obtained from Benue State Ministry of health and sampling consent was sought from Makurdi Local Government Education Authority and school administrator

### *Statement informed consent*

Informed consent was obtained from parents, guardians or caregivers of participating pupils in the study.

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