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Antidermatophytic Activities of *Musa sapientum* Methanol Leaf Extract *in-vitro*

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Authors' contributions

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

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ABSTRACT

Aim: The *in vitro* antidermatophytic activity of methanol leaf extract of *Musa sapientum* (MSL) against *Microsporum canis, Trichophyton tonsurans and Trichophyton rubrum* was investigated.
Methodology: The methanol leaf extract *M. sapientum* was obtained by cold extraction. The antifungal activity of the extract was assayed using agar well diffusion techniques. The time-kill antibacterial kinetics of the extract was assessed against one strain of *M. canis* and two strains of *T. tonsurans* by plate count technique.
Results: The extract had minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) values ranging from 0.0625 mg/ml to 0.5 mg/ml. The least MIC and MFC value was 0.0625 mg/ml against *M. canis* while *T. tonsurans* showed the highest MIC and MFC value of 0.5 mg/ml. The extract had no inhibitory or fungicidal effect against *T. rubrum*. Time-kill kinetic study indicated that the extract exhibited bacteriostatic and bacteriocidal effects against *Microsporum canis and Trichophyton tonsurans.*
Conclusion: The results obtained suggest that the methanol leaf extract of *Musa sapientum* possesses antidermatophytic properties.

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Keywords: Musa sapientium; antidermatophyte; antimicrobial; in vitro activity.

1. INTRODUCTION

Plants and herbs have been reported to produce a variety of bioactive molecules thus making it a rich source of different types of medicine. It has been observed that the efficacy of a wide variety of antibiotic and antimicrobial agents produced is reduced due to the emergence of pathogens that resist the activity of these drugs [1]. Moreover, the effective life span of classical antifungals is in fact limited due to their frequent use as antifungals and immunosuppressive drugs as well as in organ transplantation, lymphomas and HIV secondary infections [2].

There is therefore a need for continued research into antimicrobial and antifungal agents that are capable of combating these drug resistant pathogens. It has been observed that plant extracts represent a continuous effort to find new compounds against pathogens [3]. It has been reported that plant drugs still remain the principal source of pharmaceutical agents used in orthodox medicine [4]. The ability of plant to produce various types of phytochemicals such as alkaloid, flavonoid and saponin continues to attract the attention of natural products researcher [5].

Musa sapientum (Banana) is a large plant with a succulent pseudo-stem grown extensively throughout the tropical regions of the world. To date different parts of this plant have been reported to possess antidiabetic amongst others [6-12]. This study investigated the antidermatophytic activities of methanol leaf extract of *Musa sapientum* (MSL) *in vitro.*

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

Methanol leaf extract of *Musa sapientum* (MSL) was obtained as previously described by Adewoye and Ige [11]. The extracts obtained were stored at 4℃ until use.

2.2 Microorganisms Used

The following isolates of dermatophytes were subjected to biochemical tests to confirm their identity [13] and thereafter used in this study: Two strains of Microsporum canis (G01 and H14), two strains of Trichophyton tonsurans (H10 and H19), and one strain of Trichophyton rubrum (G04).

2.3 Antimicrobial Assay by Agar diffusion (Inhibition zones)

Initial determination of the antifungal activity of MSL was determined using the agar cup diffusion technique as described by Adeniyi et al. [14]. Briefly, the susceptibility screening of the test fungus to the extract and ketoconazole (used as control) Saboraud dextrose agar plates were seeded with the fungus. Using Pasteur pipette, 0.2 ml of the 10^{-1} dilution of the organism in Tryptone soy broth was placed on the surface of each SDA plate. This was uniformly spread with the aid of a sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37℃ for 20 mins. A standard cork borer of 8 mm in diameter was used to cut equidistant wells on the surface of the agar into which was added 0.2 ml solution of the extract at concentrations of 1, 0.5, 0.25, 0.125, 0.0625 mg/ml. The plates were incubated at room temperature for 72 hours after which diameters of inhibition were measured. All assays were carried out in triplicates.

2.4 Determination of Minimum Inhibitory Concentration (MIC)

This was carried out using the agar dilution method [15]. Different concentrations of the extract were prepared to final concentration in the range of 1 to 0.3125 mg/ml. A 2 ml of the extract was mixed with 18 mls of molten Saboraud dextrose agar and poured into sterile petri dish allowing the agar to set. The surface of the set agar was allowed to dry before streaking with overnight broth cultures of microorganisms. Plates were incubated at 25℃ for 48-72 h and examined for the presence or absence of growth. The lowest concentration preventing growth was taken as the MIC of the extract.

2.5 Determination of the Minimum Fungicidal Concentration (MFC)

This was determined by a modification of the method of Adeniyi et al. [16]. To a 0.5 ml at different concentrations as used in the MIC assay that shows no visible growth on the agar plate was added 0.5 ml of test organism in tubes. These were incubated at 25℃ for 24-48 hrs. Samples were streaked out from the tubes on to Sabouraud dextrose agar to determine the minimum concentration of the extract required to kill the organism. These concentrations were

indicated by failure of the organism to grow on transfer to these media plates. The lowest concentration that prevented fungal growth after days of incubation was recorded as the Minimum fungicidal concentration. The entire tests were performed in duplicates to ensure accuracy. Agar plates without extract and another without organism were also incubated to serve as positive and negative control.

2.6 Determination of Mechanisms of Antibiosis (Fungicidal or Fungistatic)

The mechanism of antibiosis of the extracts was calculated using the ratio of MFC/MIC or MIC index as described by Shanmughapriya et al. [17].

2.7 Time-Kill Kinetics Assay

The 0.5 McFarland standard was used in this experiment and corresponds to bacteria density of $1.5x10^8$ cfu/ml. Assays for the rate of killing bacteria by the methanol extract were assessed using the modified pour plate technique of Adeniyi et al. [16]. Extract were incorporated into 18ml Sabouraud dextrose agar (SDA) in plate. Two controls, one Sabouraud dextrose agar without extract inoculated with rest organisms and SDA incorporated with the extracts at the test concentrations without test organisms, were included. The plates were inoculated at 0 sec, 30 mins, 60 mins, 90 mins, 120 mins, 150 mins, 180 mins, 210 mins, 240 mins, and 24 h intervals and incubated for 24-36 hrs. Colonies were counted by the plate count technique. The values obtained were then used to construct curves to demonstrate changes in colony counts with time as a function of exposure to the antimicrobials. Results were plotted as log.10 of observed cfu / ml against time. The percentage reduction and log reduction from initial microbial population for each time point was calculated to express the change (reduction or increase) of the microbial population relative to a starting inoculums. The change was determined as follows:

Percentage reduction (%) [18] =

$$\frac{\text{Initial count} - \text{Count at x time interval}}{\text{Initial count}} \times 100$$

The Log reduction [19] was calculated as follows:
Log10 reduction = Log10 (initial count) – (Log10 at x time interval)

3. RESULTS

3.1 Microorganisms

Standard biochemical analysis of the microbial isolates confirmed the presence of two (2) strains of *Microsporum canis*, three (3) strains of *Trichophyton tonsurans* and ones (1) strain of *trichophyton rubrum* (Table 1).

3.2 Antifungal Activity

The antifungal susceptibility assay represents a means of predicting therapeutic concentration of antifungal agents. The results obtained from this study shows that the extract showed antifungal activity at concentrations that range between 0.5 mg/ml and 0.0625 mg/ml against the first strain of *Microsporum canis* (G01), the two strains of *Trichophyton tonsurans* (H10, H19) and had no effect against the second strain of M.canis (H14) and *Trichophyton rubrum* (Table 2).

3.3 Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and Antibiosis

The results showed that the extract had the same MIC and MFC values of 0.0625 mg/ml, 0.25 mg/ml and 0.50 mg/ml against G01, H10 and H19 respectively (Table 3). The MFC: MIC ratio (antibiosis) observed in this study was of 1:1 (Table 3).

3.4 Time-Kill Kinetics

The Time-Kill kinetics study is often carried out when assessing the spectrum and kill rate of an antibacterial agent. Values observed obtained indicates that the average log reductions in viable cell counts for the extract was 2.32 log10 cfu/ml for *Microsporum canis* and 2.33 log10 cfu/ml for the two strains of *Trichophyton tonsurans* after four (4) hours. Post exposure to the extract at 24 hours showed complete elimination of the test organisms (Fig. 1). Table 4 shows the percentage reduction and log reductions from the initial microbial population for each time. The values obtained show a consistent decline in the microbial population as the time increased with values obtained at 4 hour post treatment ranging between 86.6 – 89.6% (Table 4).

Table 1. Biochemical and phenotypic test of microorganisms

Organisms	Culture Characteristics	Microscopy	Catalase Test	Urease Test	Suspected Organism
G01	White golden yellow border with fluffy center.	Thin walls of about 3-6 cells with rounded ends.	Negative	Positive	*Microsporum canis*
H10	Yellow/colorless folds.	Thin smooth walls that are irregular in shape.	Positive	Positive	*Trichophyton tonsurans*.
H19	Yellowish, raised edges.	Irregular shaped walls	Positive	Positive	*T. tonsurans*.
H15	Golden yellow and a raised fold at the edge.	Smooth wall with irregular shape.	Positive	Negative	*T. tonsurans*
H14	White fluffy center with golden yellow border.	Many spiny thin wall with 3-6 cells with rounded ends.	Positive	Positive	*M. canis*
G04	Whitish, shinning raised edges	Numerous cells appearing on parallel sides.	Positive	Positive	*Trichophyton. rubrum*

Table 2. Antifungal susceptibility assay

Organism	1mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml	Control (ket)
G01	15±0.1	13±0.1	10±0.1	10±0.1	10±0.1	16±0.2
H10	15±0.1	14±0.1	-	-	-	14±0.1
H19	15±0.1	12±0.1	-	-	-	15±0.2
H14	-	-	-	-	-	13±0.1
G04	-	-	-	-	-	12±0.1

G01 = Microsporum canis; H10 = Trichophyton tonsurans; H19 = Trichophyton tonsurans; H14 = M. canis; G04 = Trichophyton rubrum

Table 3. Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and Antibiosis of methanol extract of *Musa sapientum* leaves

Organism	MIC	MFC	Antibiosis ratio
G01	0.0625	0.0625	1:1
H10	0.25	0.25	1:1
H19	0.50	0.50	1:1

G01 = Microsporum canis; H10 = Trichophyton tonsurans; H19 = Trichophyton tonsurans

4. DISCUSSION

Dermatophytes are fungi that require keratin for growth [20]. These fungi can cause superficial infections of the skin, hair, and nails. Dermatophytes are spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites [20].

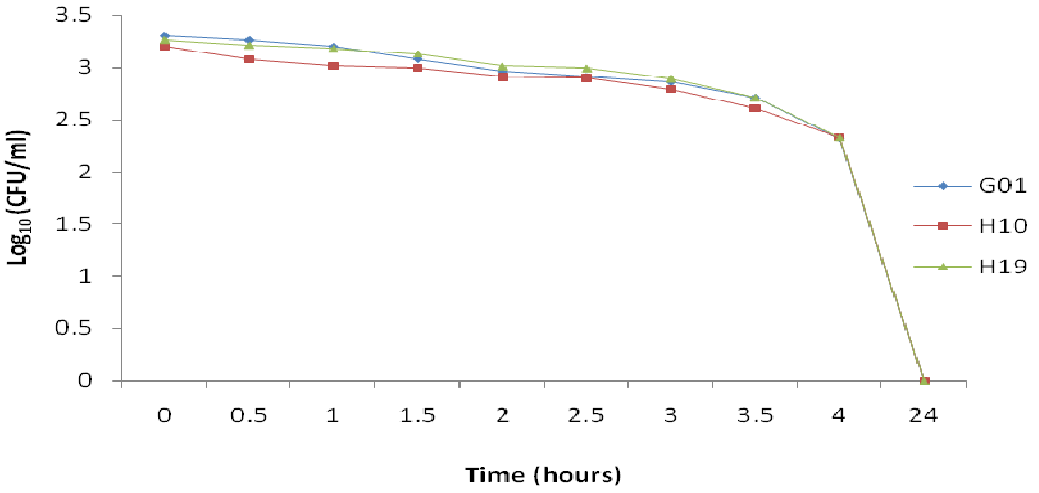

Fig. 1. Time-Kill kinetics Assay of *M. sapientum* on exposure to microorganisms. Results are expressed as logarithm to base 10 of observed colony forming units per ml against time.
G01 = Microsporum canis; H10 = Trichophyton tonsurans; H19 = Trichophyton tonsurans

Table 4. Time-Kill kinetic - percentage and Log_{10} reductions of microbial population after *M. sapientum* treatment

Micoorganism time (hours)	G01		H10		H19	
	% reduction	Log_{10} reduction	% reduction	Log_{10} reduction	% reduction	Log_{10} reduction
0	0	0	0	0	0	0
0.5	10	0.04	25	0.12	10.2	0.05
1	19.5	0.1	34.3	0.18	15.9	0.08
1.5	39.7	0.22	43.6	0.2	25.9	0.13
2	55	0.34	49.3	0.29	43.3	0.25
2.5	59.2	0.39	50.6	0.3	45.2	0.27
3	63.9	0.44	61.1	0.41	57.0	0.37
3.5	74.4	0.59	74.8	0.59	71.6	0.55
4	89.6	0.98	86.6	0.87	88.2	0.93
24	-	-	-	-	-	-

G01 = Microsporum canis; H10 = Trichophyton tonsurans; H19 = Trichophyton tonsurans

In this study the presence of *Microsporum canis*, *Trychophyton tonsurans* and *Tricophyton rubrum* was confirmed in the microbial isolates used. *Microsporum canis* is a known fungus which has been reported to cause *tinea capitis*, a cutaneous fungal infection of the scalp, in humans. *Trichophyton tonsurans* is also a fungus which belongs to the *arthrodermataceae* family and has been observed to cause ringworm infection of the scalp. *Trichophyton rubrum* is a dermatophytic fungus that colonizes the upper layers of dead skin resulting in *tinea pedis, tinea cruris* and fungal infection of the nails.

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration of an antimicrobial and antifungal agent is defined as the lowest concentration of that substance which will inhibit the visible growth of a microorganism and fungus after an overnight incubation period. The MIC is used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints.

The nature of the antibacterial effect of the extract with respect to inhibition of the tested bacteria is important. Hence the MFC/MIC ratio is used to specify the nature of the antimicrobial effect against that pathogen. When the MFC/MIC ratio of a pathogen ranges between 1:1 and 2:1 the chemical substance is considered as fungicidal against the pathogen. If however the

ratio is >2:1 the mode of antimicrobial action is more likely to be fungistatic. This study shows an MFC: MIC ratio of 1:1 (Table 3) of the extract (*M. sapientum*) against the microorganisms used thus suggesting that the extract has fungicidal properties.

The Time-Kill kinetics study has been reported to form the basis of *in vitro* investigations for pharmacodynamic drug interaction [19]. In this study the extract exhibited bacteriostatic and bacteriocidal effects against the bacterium used. The result obtained showed that the extract exhibited significant ($p<0.05$) bacteriocidal activity at 4hours post treatment and complete clearance of microbes at 24 hours post treatment.

Musa sapientum is plant various parts of which has been reported to exert different pharmacological and therapeutic effects which include anti-ulcer, hypolipidemic and anti-diabetic, antihypertensive, antioxidant wound healing, anti-allergic and diuretic properties [21,22,6-12,23]. Its antimicrobial effect against staphylococcus and pseudomonas species has also been reported [24].

Its biological activity has been attributed its phytochemical constituents which include catecholamines [25], tryptophan, indole compounds, tannin [26], several triterpenes [27] and carbohydrates [28]. Adewoye et al. [10] reported the presence of alkaloids, cardenolides and saponins while Sahaa et al. [29] reported the presence of flavonoids in the methanol leaf extract of *Musa sapientum*. These substances have all been reported to exert potent antimicrobial activities. Therefore it is not unlikely that the antidermatophytic (fungistatic and fungicidal) effects of *M. sapientium* observed in this study may be due to its phytochemical constituents which include alkaloids, cardenolides, saponins and flavonoids.

5. CONCLUSION

In conclusion, this study has shown that the methanol leaf extract of *Musa sapientum* possesses antidermatophytic effects against Microsporum canis and Trichophyton tonsurans. The minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) values of the extract against these microbes ranged from 0.0625 mg/ml to 0.5 mg/ml. The Time-kill kinetic studies indicated that the extract exhibited both fungistatic and fungicidal effects against *Microsporum canis and Trichophyton tonsurans.* This preliminary study therefore suggests *Musa sapientum* may be a good source of antidermatophytic agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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