



Evaluation of Formulated Anti-dermatophyte Creams from Ethanol Extract of *Mitracarpus villosus* Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author ABF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FOO managed the analyses of the study. Author ABF managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Microsporium, *Trichophyton*, and *Epidermophyton* are asexual fungi usually called dermatophytes. They are known to cause a kind of skin diseases called dermatophytosis. There have been reported cases of resistance by the dermatophytes with prolonged usage of synthetic antifungals on the skin. The aim of this study was to formulate herbal antifungal cream containing extract of *Mitracarpus villosus* as an anti-dermatophytic preparation and evaluate its physicochemical properties, stability and efficacy of the product. The formulated creams containing 0.5, 1 and 2% w/w of extract were subjected to stability tests using temperature variation method at -10, 4, 30, 37 and 45°C. Freeze-thaw test, Centrifuge test, pH and exposure to UV light test were also carried out using standard method. Efficacy of the cream formulations were determined using Wistar rats as experimental animals.

The percentage yield of the extract was (2.1%). Percentage ethanol phytochemical composition indicated that for alkaloid it is 1.06±0.04%, saponins (0.96±0.07%), flavonoids (0.06±0.02%) and tannins (0.04±0.01%). The emulsion produced was an oil-in-water emulsion and had a white colour with pH of 7.02, spread of emulsion, rubbing-in effect and stability to centrifugation was very high. The antifungal results showed the activity against the dermatophytes to be in the increasing order

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Epidermophyton floccosum (9 mm) <*Microsporum audouinii* (12 mm) <*Trichophyton mentagrophytes* (13 mm) <*M. furfur* (12 mm). Temperature stability and Centrifuge testing indicated that the formulations were stable. Light testing indicated no change in the colour of the products. Animal studies evaluation of the formulations of the cream indicated that their efficacy against the dermatophytes is concentration dependent and is in the increasing order *M. audouinii* (34 µm) = *E. floccosum* (34 µm) <*T. mentagrophyte* (35 µm) <*M. furfur* (45 µm) which shows that 2% *Mitracarpus villosus* cream was statistically significant (P<0.05) against all the test microorganisms.

Keywords: *Dermatophytosis; antifungals; Mitracarpus villosus; alkaloid; cream.*

1. INTRODUCTION

The plant *Mitracarpus villosus* is of Rubiaceae family. It is widely distributed in tropical countries like Senegal, Gambia, Mali, Nigeria and Liberia. It is called Irawo-ile in Yoruba language of the south-western Nigeria where it used traditionally for skin infections. It has been reported to have antifungal [1] and antibacterial properties [2,3]. Bisignano et al. [2] examined the antimicrobial activity of methanol extract of *Mitracarpus villosus* against some pathogens found on the skin. They reported that the extract possesses both antibacterial and antimycotic activities. Gallic acid, 3, 4, 5-trimethoxybenzoic acid, 4-Methoxyacetophenone, 3,4,5-trimethoxyacetophenone, kaempferol-3-O-rutinoside, rutin and psoralen were some of the phytochemicals isolated from the plant. Okogun and his co-workers in 2005 also tested the bactericidal properties of 95% ethanolic crude extracts of *Mitracarpus villosus* against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus feacalis* and they were all inhibited. They reported that the minimum inhibitory concentrations (MIC) of the extract were in the range of 0.06-8.0 mg/mL, and minimum bactericidal concentrations (MBC) of 0.06-32.0 mg/mL.

Aboh et al. [1], investigated the antifungal activities of phyto compounds from *Mitracarpus villosus* against clinical isolates of *Candida albicans*, *Candida krusei*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus* and *Aspergillus niger*. In their results, they found that crude tannin extract produced the strongest antifungal activity against the yeasts with diameter zones of inhibition ranging from 21.67–23.67 mm while the crude saponin extract exhibited the strongest antifungal activity against the moulds with diameter zones of inhibition ranging from 21.00–24.67 mm. They therefore concluded that the plant has good potentials for development of new antifungal drugs.

Naturally occurring substances of plant origin have been reported to inhibit the growth of microorganisms. Plants extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities [4]. The development of resistance to antibiotics is an almost inevitable consequence of their application [5]. The speed of resistance depends on the respective class of antibiotics and their product use. For many years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instance, these are carbon copies of chemical identified in plants. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food or paste [6].

Dermatophytes are classified as anthropophilic (humans), zoophilic (animals) or geophilic (soil) according to their normal habitat. These anamorphic (asexual or imperfect fungi) genera are: *Microsporum*, *Epidermophyton* and *Trichophyton*. Dermatophytosis have a variety of appearances; most easily identifiable are the enlarging raised red rings with a central area of clearing (ringworm). The same appearances of ringworm may also occur on the scalp (tinea capitis), beard area (tinea barbae) or the groin (tinea cruris, known as jock itch). Tinea corporis is the infection of dermatophytes on the arm and legs. The edge of the rash appears elevated and is scaly to touch. Sometimes the skin surrounding the rash may be dry and flaky. Almost invariably, there will be hair loss in areas of the infection [7]. In their own report, James et al. [8] observed that these dermatophytes normally live on the superficial skin surface, and when the opportunity is right, can induce a rash or infection. Individuals at high risk of acquiring ringworm include those who: Live in crowded, humid conditions, Sweat excessively, Participate in close contact sports like soccer, rugby, or

wrestling, Wear tight, constrictive clothing with poor aeration and those who have a weakened immune system (e.g., those infected with HIV or taking Immunosuppressive drugs).

Dermatophytes are transmitted by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in clothing, combs, hair brushes, theatre seats, caps, furniture, bed linens, shoes, socks, towels, hotel rugs, sauna, bathhouse, and locker room floors [8]. Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns, excessive temperature and humidity. Adaptation to growth on humans by most geophilic species resulted in diminished loss of sporulation, sexuality, and other soil-associated characteristics [9].

2. MATERIALS AND METHODS

Plant materials: Leaves of *Mitracarpus villosus* were collected at the Botanical Gardens of the University of Ibadan. It was identified and sample deposited with herbarium number MPNH/2017/1256 at Medicinal Plants of Nigeria Herbarium of NNMDA. The plants were air dried in the shade and pulverized to fine-sized particles for solvent extraction processes.

Preparation of plant extracts: 200 g of the pulverized plant sample was extracted with ethanol using soxhlet extraction method.

Determination of the Phytochemical constituents: The extract was evaluated for the presence of Tannins, flavonoids, saponins and alkaloids using simple qualitative and quantitative methods of Trease and Evans [10] and Sofowora [11].

Micro-organisms: Clinical isolates of *Microsporium aoudininn*, *Epidermophyton floccosum*, *Trychophyton mentagrophyte* and *Malassezia furfur* were obtained from Spectralab Medical and Diagnostic Services, Sagamu, Ogun State.

Materials for Emulsion Formulation: All oil soluble substances were placed in a stainless steel container and heated to between 70–75°C. All water-soluble substances were placed in another stainless steel container and heated to the same temperature. The oil phase was then

added to the aqueous phase slowly with stirring. Heating was continued at the same temperature for about 10-15 minutes. The coarse emulsion formed was then cooled to about 35°C gradually. The emulsion was allowed to stay at room temperature for twelve hours and then homogenized with the aid of a mechanical stirrer. Three different formularies containing 0.5%, 1% and 2%, of *Mitracarpus villosus* ethanolic extracts were produced. The prepared herbal emulsions were then vigorously homogenized.

Microbiological Assay: Zones of inhibition was determined using the method as described by Irobi et al. [4]. The zones of inhibition were measured in mm and recorded.

Stability Tests for the formulated emulsion: Stability tests were carried out on the emulsions following the methods as described by Cannel (1992) whereby the temperature variation tests include storing the samples at -10°C, 4°C, 30°C, 37°C and 45°C. All observations including pH, colour and odour were noted and recorded. The formulated creams were subjected to Freeze thaw cycles testing which involves making the samples pass through three cycles of temperature at -10°C for 24 hours and then at room temperature for 24 hours. The creams were subjected to centrifuge testing whereby the samples were heated to 50°C and then centrifuged for thirty minutes at 2000, 2500, 3000 and 4000 rpm. They were inspected for signs to determine if the dispersed phase of the emulsion has separated and risen to the top. Light testing was also carried out whereby the creams were placed in test tubes and also in the actual package and then put in the window where direct sun rays fell on them. This method is used to determine the sensitivity of the emulsions to the Ultra Violet radiation.

In vivo Antidermatophytic Activity:

Laboratory Animals: Wistar rats weighing between 150-200 g were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine of the University of Ibadan. They were kept in well ventilated rat cages with free access to water and feed and were left in this environment for two (2) weeks to acclimatize [12].

Selection and grouping of animals: The animals were allocated to six (6) groups. The rats were then inoculated with the dermatophytes. One week after inoculation of the animals with

the dermatophytes, the inoculated skin area of 2cm² were treated with the plants extracts and the formulated creams with plants extracts for seven days. At the end of seven days, the animals were euthanized by cervical dislocation.

Histopathological studies: 2 cm² skin areas were cut and put in 10% formalin for histopathological analysis. Skin biopsy samples were examined for presence of fungal hyphae, hair follicles, sebaceous gland, inflammation and tissue destruction using light microscope.

Statistical Analysis of Data: Data generated were subjected to statistical analysis using one way ANOVA followed by tukeys post hoc analysis. It is reported as mean \pm SEM. $P \leq 0.05$ was considered significant. Also epidermal thickness and keratin layer were obtained with the aid of calibrated Toupview® software.

FTIR Analysis: The cream base sample and the products were qualitatively characterized by the FT-IR technique.

3. RESULTS AND DISCUSSION

Percentage yield of extracts was 2.1%. The Phytochemical Composition of the Plant was alkaloid (1.06 \pm 0.04%), Saponin (0.96 \pm 0.07%), Flavonoid (0.06 \pm 0.02%) and Tannin (0.04 \pm 0.01%).

3.1 Determination of Microbial Activity against the Dermatophytes

In Table 1, 0.5% formulation was most active against *Malassezia furfur* > *Trychophyton mentagrophyte* > *Microsporium aoudininn* > *Epidermophyton floccosum*. 1.0% formulation was most active against *Malassezia furfur* = *Trychophyton mentagrophyte* > *Epidermophyton floccosum* > *Microsporium aoudininn*. However, 2.0% formulation was most active against *Malassezia furfur* > *Trychophyton mentagrophyte* > *Microsporium aoudininn* > *Epidermophyton floccosum*. From the results

above, it is evident that the formulations are very active and most active against *Malassezia furfur*. Also the result shows that the activities of the formulations are concentration dependent.

3.2 Temperature Stability Testing

As shown in Table 2, there was no change in pH and colour of the formulations from the date of production through 16th week of accelerated stability test. This could be due to the fact that at the low temperature of -10°C, there is probability of restrictions on the movement of ions within the molecular system of the formulations. The same observations were made for samples stored at 4°C (Table 3). At 30°C, there was slight decrease in the pH of 0.5% formulations from 7.15 at production to 7.11 at the end of 16th week. The same observation was made for both 1% and 2% formulations with decrease in pH from 7.17 to 7.10 and 7.20 to 7.17 respectively (Table 4). What was observed for samples stored at 37 and 45°C were not different from what was observed for 37°C (Tables 5 and 6). There was slight decrease in the pH as the number of test weeks increases but the colour remain stable. These results show that the formulations were very stable at the various test temperatures.

3.3 Freeze-thaw Testing

There was no change observed in all the test samples during the cycle testing (Table 7). This shows that even when the products are subjected to extreme temperature swing, they will remain stable.

3.4 Centrifuge Testing

When the samples were subjected to centrifugation test, no phase separation was detected at 2000, 2500 and 3000 and 4,000 rpm as indicated in Table 8. This shows that the dispersed phase is properly particulated in the continuous phase thereby making the formulations to be stable.

Table 1. Effect of formulations against the dermatophytes

Formulations in %	0.5	1.0	2.0
<i>Trychophyton mentagrophyte</i>	4	7	10
<i>Microsporium aoudininn</i>	3	4	6
<i>Epidermophyton floccosum</i>	2	5	5
<i>Malassezia furfur</i>	5	7	11

Zones of inhibition is in mm

Table 2. Effect of temperature (-10°C) on the formulations after production

Formulations	Day 1		2 wks		4 wks		8 wks		12 wks		16 wks	
	pH	Colour	pH	Colour	PH	Colour	pH	Colour	pH	Colour	pH	Colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
0.5%	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC
1.0%	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC
2.0%	7.20	NCC	7.20	NCC	7.20	NCC	7.20	NCC	7.20	NCC	7.20	NCC

NCC = No change in colour, CC = Change in colour

Table 3. Effect of temperature (4°C) on the formulations after production

Formulations	Day 1		2 wks		4 wks		8 wks		12 wks		16 wks	
	pH	Colour	pH	Colour	PH	Colour	pH	Colour	pH	Colour	pH	Colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
0.5%	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC	7.15	NCC
1.0%	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC
2.0%	7.20	NCC	7.20	NCC	7.20	NCC	7.20	NCC	7.20	NCC	7.20	NCC

NCC = No change in colour, CC = Change in colour

Table 4. Effect of temperature (30°C) on the formulations after production

Formulations	Day 1		2 wks		4 wks		8 wks		12 wks		16 wks	
	pH	Colour	pH	Colour	PH	Colour	pH	Colour	pH	Colour	pH	Colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
0.5%	7.15	NCC	7.14	NCC	7.14	NCC	7.13	NCC	7.11	NCC	7.11	NCC
1.0%	7.17	NCC	7.15	NCC	7.13	NCC	7.13	NCC	7.10	NCC	7.10	NCC
2.0%	7.20	NCC	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC	7.17	NCC

NCC = No change in colour, CC = Change in colour

Table 5. Effect of temperature (37°C) on the formulations after production

Formulations	Day 1		2 wks		4 wks		8 wks		12 wks		16 wks	
	pH	Colour	pH	Colour	PH	Colour	pH	Colour	pH	Colour	pH	Colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
0.5%	7.15	NCC	7.12	NCC	7.12	NCC	7.10	NCC	7.10	NCC	7.10	NCC
1.0%	7.17	NCC	7.11	NCC	7.10	NCC	7.09	NCC	7.09	NCC	7.09	NCC
2.0%	7.20	NCC	7.15	NCC	7.14	NCC	7.14	NCC	7.14	NCC	7.14	NCC

No change in colour = NCC, CC = Change in colour

Table 6. Effect of Temperature (45°C) on the formulations after production

Formulations	Day 1		2 wks		4 wks		8 wks		12 wks		16 wks	
	pH	Colour	pH	Colour	PH	Colour	pH	Colour	pH	Colour	pH	Colour
Control	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC	7.02	NCC
0.5%	7.15	NCC	7.10	NCC	7.09	NCC	7.09	NCC	7.07	NCC	7.07	NCC
1.0%	7.17	NCC	7.10	NCC	7.10	NCC	7.08	NCC	7.08	NCC	7.08	NCC
2.0%	7.20	NCC	7.15	NCC	7.12	NCC	7.12	NCC	7.12	NCC	7.12	NCC

NCC = No change in colour, CC = Change in colour

Table 7. Freeze thaw testing

Control	NPS
0.5%	NPS
1.0%	NPS
2.0%	NPS

NPS= No Phase separation, PS= Phase separation

Table 9. Light testing

Control	NCC
0.5%	NCC
1.0%	NCC
2.0%	NCC

NCC =No colour change, CC= colour change

Table 8. Centrifuge testing for the samples

Sample	2000 rpm	2500 rpm	3000 rpm	4000 rpm
Control	NPS	NPS	NPS	NPS
0.5% cream	NPS	NPS	NPS	NPS
1.0% cream	NPS	NPS	NPS	NPS
2.0% cream	NPS	NPS	NPS	NPS

NPS= No phase separation, PS = phase separation

3.6 Fourier Transform Infra-red Spectroscopy Analysis

The infra-red spectrum of the placebo (Fig. 1), show strong O-H stretch at 3373.00 cm⁻¹ of amines.N=H bending at 1640.00 cm⁻¹ indicating that the placebo contains an unsaturated amide group. O-H bending of phenyl group at 1409.00 cm⁻¹ indicated the presence of carboxylic acid group in the sample, S=O stretch at 1040.00 cm⁻¹ indicated presence of sulphoxide. Mv cream contains S-S stretch at 461.00cm⁻¹ indicating presence of polysulphides. Also spectrum of N=H bend at 1635.00 shows presence of amine group. The spectrum also

3.5 Light Testing

No changes were noticed for all the formulations. This shows that they were not sensitive to ultra violet light (Table 9).

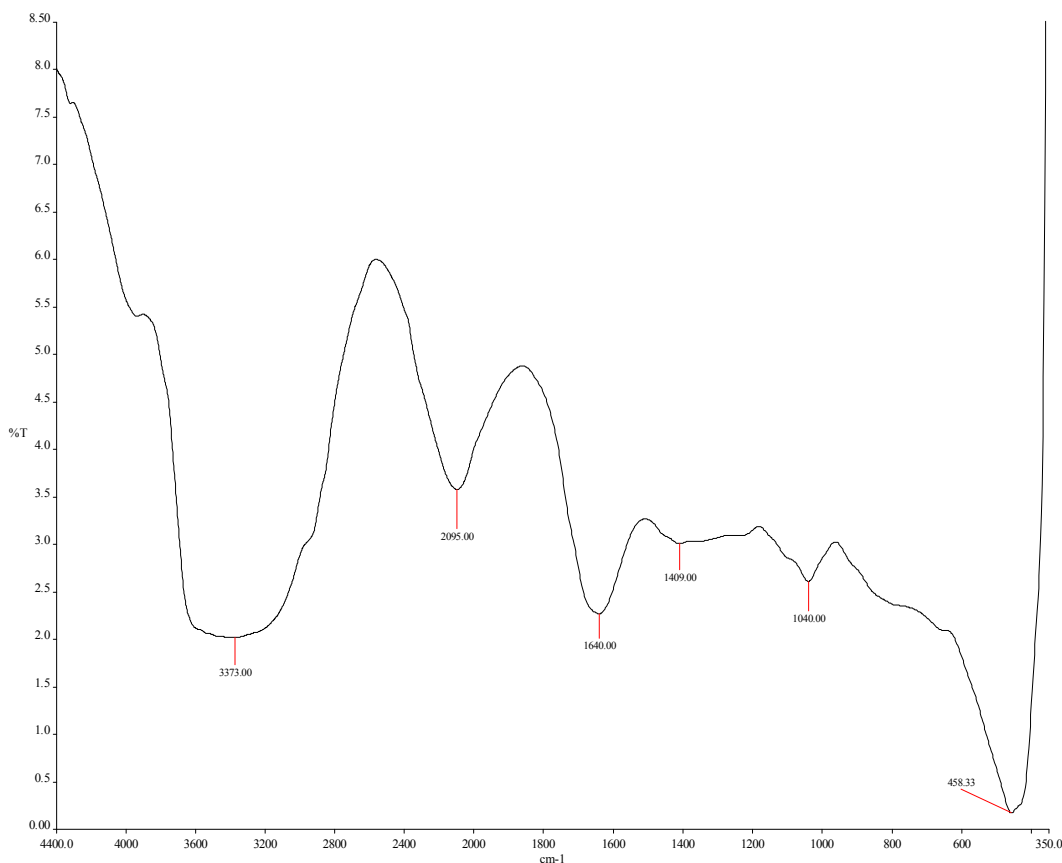


Fig. 1. FTIR spectrum of emulsion alone (Placebo)

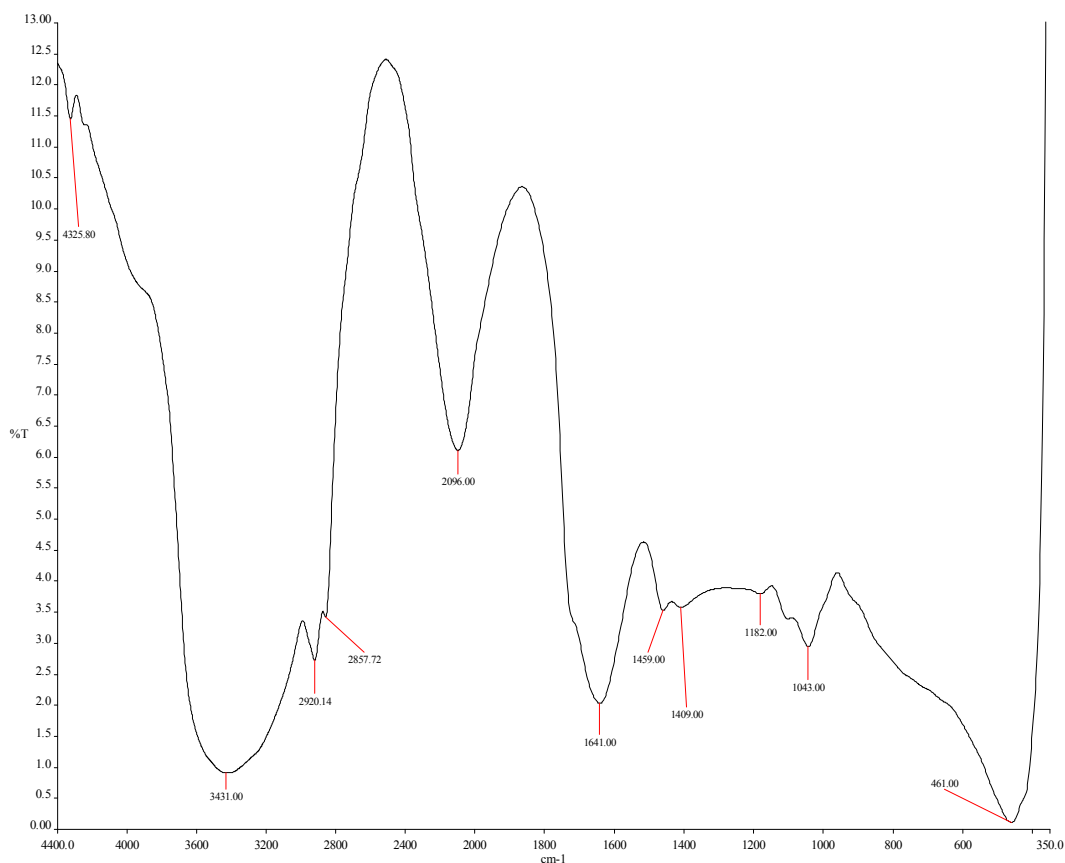


Fig. 2. FTIR Spectrum of *Mitracarpus villosus* cosmeutical

shows NH stretch of aliphatic amine at 3431.00cm^{-1} . The spectrum also shows presence of S=O stretch at 1043.00cm^{-1} , C-N stretch of pyridines at 1182.00cm^{-1} and also polymeric O-H stretch at 3158.00cm^{-1} . CH_2 of ketones at 1409.00cm^{-1} was also observed for. Spectrum of C=C stretch of aromatic ring at 1641.00cm^{-1} was detected. These peaks confirm the presence of aromatic structure. An aromatic C-N stretch peak at 1182.00cm^{-1} was also observed. The peak of S=O stretch observed at 1043.00cm^{-1} indicated presence of sulphoxide and peak of S-S stretch of Aryl observed at 461.00cm^{-1} . Spectrum of 3372.00cm^{-1} indicated the presence of dimeric O-H. There is a spectrum of C-N stretch which peaks at 1182.00cm^{-1} (Fig. 2). Some of these functional groups found in the herbal cream formulations which are not in the cream base maybe responsible for the plant effectiveness in the management of dermatophytosis.

3.7 Histopathology of Skin Tissues

Tissues were examined for the presence of fungal elements; inflammation, Fungal hyphae,

loss of hair follicles, absence of sebaceous gland and discontinuity/Tissue destruction.

3.8 Comparative Efficacy of *Mitracarpus villosus* Cream Formulations against the Dermatophytes

In the biopsies of the skin of the animals infected but not treated, there were marked presence of fungal hyphae on the skin with marked tissue destruction (Fig. 3). Inflammation of the skin was marked (Fig. 4). There were no sebaceous gland seen and few or no hair follicles were seen which indicated that the infection in the untreated group was successful (Table 10). In the group treated with 1% clotrimazole cream (Standard drug), there were few or no presence of fungal hyphae, moderate hair follicle and abundant sebaceous glands were observed. Also, there were no inflammation and there were absence of tissue destruction. This implies that the reference drug is effective in the management of dermatophytosis. Results obtained for animals treated with formulated emulsion alone (Placebo) indicated that there were marked presence of

fungal hyphae (Fig. 5), few hair follicles and sebaceous glands were seen. There were moderate inflammation but marked tissue destruction was observed. This indicated that emulsion alone has no effect on the

dermatophytic infection but was able to reduce the attack on the hair follicles and sebaceous glands due to the presence of alternative oil in the emulsion which the dermatophytes attack (Table 10).



Fig. 3. Lymphocytic exocytosis. H& E stain, x100

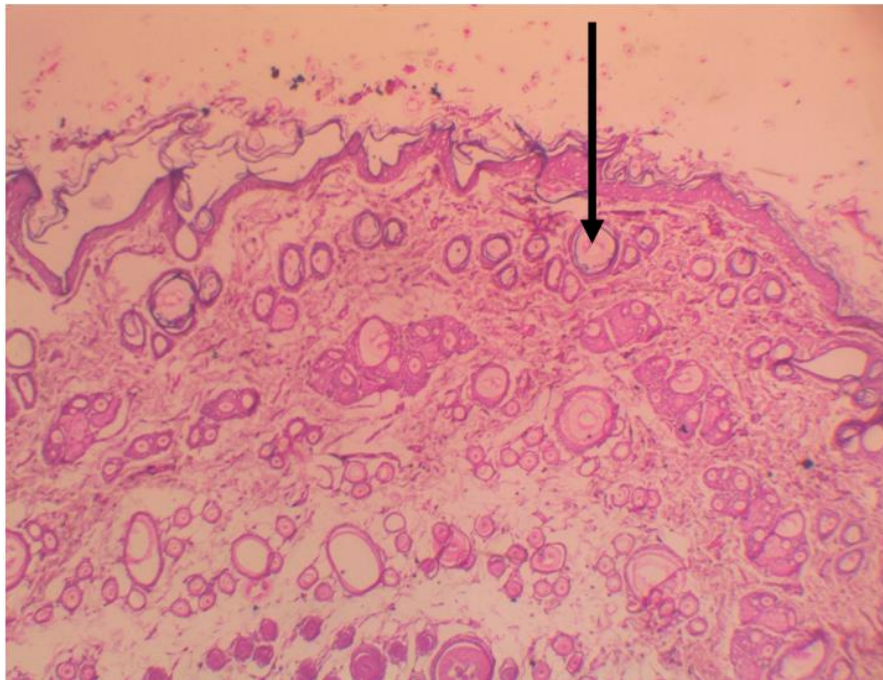


Fig. 4. Dense dermal infiltrate of mononuclear cells, H & E stain x100

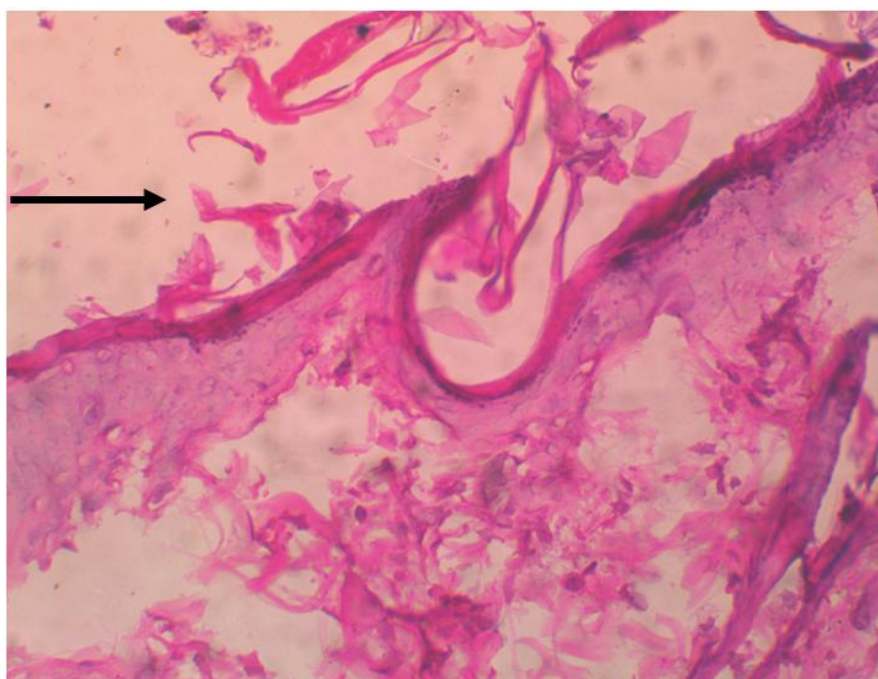


Fig. 5. Formation of a mycelia mass in the epidermis H & E stain x100

Table 10. Mycological efficacy of *Mitracarpus villosus* formulations against the dermatophytes

Group	Fungal hyphae	Hair follicles	Sebaceous gland	Inflammation	Continuity/Tissue destruction
Negative control	+++	-	-	+++	+++
Positive control	-	++	+++	-	-
Emulsion alone	+++	+	+	++	+++
0.5% formulation	++	+	+	++	++
1.0% formulation	++	+	+	++	++
2.0% formulation	++	++	++	+	+

- = Absent, Mild = +, Moderate = ++, Marked = +++

The ability of 0.5% cream formulation to reduce dekeratinisation was most effective against *Malassezia furfur* ($32.04 \pm 0.30 \mu\text{m}$) > *Epidermophyton floccosum* ($28.07 \pm 0.22 \mu\text{m}$) > *Microsporium aoudinin* ($27.50 \pm 0.14 \mu\text{m}$) > *Trichophyton mentagrophytes* ($25.31 \pm 0.12 \mu\text{m}$). The 1.0% formulation efficacy was against *Malassezia furfur* ($37.12 \pm 0.24 \mu\text{m}$) > *Microsporium aoudinin* ($33.40 \pm 0.16 \mu\text{m}$) > *Trichophyton mentagrophytes* ($32.21 \pm 0.08 \mu\text{m}$) > *Epidermophyton floccosum* ($30.14 \pm 0.32 \mu\text{m}$). However, 2.0% formulation was most effective against *Malassezia furfur* ($45.07 \pm 0.26 \mu\text{m}$) > *Trichophyton mentagrophytes* ($36.02 \pm 0.16 \mu\text{m}$) > *Microsporium aoudinin* ($35.20 \pm 0.11 \mu\text{m}$) = *Epidermophyton floccosum* ($35.24 \pm 0.28 \mu\text{m}$). The results show that the efficacy of the cream formulations is concentration dependent,

and that the most effective is the 2.0% formulation. This result agree with the observation made by Aboh et al. [1] who in their work concluded that the antifungal activities could be ascribed to the phytochemicals present in the plant. Also, previous data obtained by Anchisi et al. [13] proved that functional agents present in medicinal plants can be harnessed in managing diseases. However, in their own work, Aghel et al. [14], highlighted the resistance of many pathogens to synthetic drug therapies but Moghimipour et al. [6], reported that toxicity associated with long-term treatment with conventional antifungal drugs can be avoided using natural products.

In general the results from the various tests carried out showed that the medicinal plant

extract is effective and that the formulated creams released the phytoconstituents unto the surface of the skin. It was also observed that though all the formulations were effective but the efficacy is concentration dependent. The formulations were stable to centrifugation and the pH was within the acceptable limit. Efforts should be geared towards harnessing our bio-resources in the management of various diseases plaguing man as it has been reported by various scientists the adverse effect associated with prolonged use of synthetic chemicals.

4. CONCLUSION

In conclusion, the cream formulations were stable and very effective against the dermatophytes, but the 2% cream formulation effectiveness compared favourably with 1% Clotrimazole which is the standard drug. The results justified the use of this plant in the management of dermatophytosis by the local traditional healers.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The experimental procedures carried out in this study were in compliance with University of Ibadan ethics committee for the care and use of laboratory animals in line with approval number UI-ACUREC/App/12/2016/06.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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