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Susceptibility Patterns of *Trichophyton mentagrophyte* and *Trichophyton rubrum* on Selected Topical Antifugal and Agents without Specific Antifungal Properties

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Abstract: ***Investigations to ascertain the effects of different brands of topical antifungal and other selected agents without specific antimicrobial properties was carried out using punch hole diffusion method to determine the susceptibility of dermatophytes to different brands of topical antifungal and other substances that their antifungal potentials are unknown. The agents were subjected to Minimum Inhibitory Concentration and Minimum Fungicidal Concentration. Trichophyton mentagrophyte and Trichophyton rubrum were subjected to twelve brands of topical anti-fungal agents (Betrosil, Fungbact-A, Fluzec-NM, 3G, Miracute, Mycoten, Mycozoral, Nixoderm, Quadriclear, Skineal, Tribotan and Tydineal) and four other selected agents without specific antimicrobial functions namely Close up toothpaste, Dabur, Herbal toothpaste, hydraulic fluid and shea butter. There was statistically significant difference (P < 0.05) between Quadriclear, Miracute, Tydineal, Betrosil, Mycoten, Funbact-A, Fluzec-NM and Tribotan and their actions were fungicidal. There was no significant difference (P > 0.05) between Skineal, mycozoral, 3G and Nixoderm, though they were fungistatic. Statistically there was significant difference (P < 0.05) between Hydraulic fluid and other selected agents without specific antimicrobial functions. Hydraulic fluid showed fungicidal activity on these two dermatophytes. This work supports the use of topical antifungal creams in the treatment of dermatophytoses. Topical use of hydraulic fluid should be discouraged, because prolong use may be harmful though highly effective and fungicidal against dermatophytes.***

Keywords: *Trichophyton mentagrophyte, Trichophyton rubrum,* Antifungal, Dermatophytoses

1. Introduction

Dermatophytoses are fungal infections that involve only superficial keratinized tissues of the body: skin, hair and nails (Gupta *et al*., 1997). These cutaneous mycoses are the most common fungal infections of man and are popularly called Tinea or ringworm (Ochei and Kolhatkar, 2000). Dermatophytes are probably restricted to the non-viable skin because most are unable to growth at 37^{o}C or in the presence of serum (Jawetz*et al*., 2007; Barry and Hainer, 2003).

Dermatophytes are group of about 40 related fungi that belong to three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. Clinically, ringworm is often referred to as *Tinea* and the locations involved are usually the surface of the body (*Tineacorporis*), the scalp (*Tineacapitis*), the foot (*Tineapedis*, or athlete's foot) and the nail (*Tineaunguium*, or Onchomycosis) (Cheesbrough, 2000). The genus *Trichophyton* is capable of invading the hair, skin and nails, the genus *Microsporum* invades the hair and skin; and the genus *Epidermophyton*, the skin and nails (Ochei and Kolhatkar, 2000).

Dermatophytoses are among the most prevalent infections in the world. Although they can be persistent and troublesome, but not life – threatening yet huge amount of naira is expended annually in their treatment. Being superficial, dermatophytes (ringworm) infections have been recognized since antiquity (Jawetz*et al*., 2007).

Ringworm infections are acquired from active ringworm lesions on humans (anthropophilic), animals (zoophilic) or sometimes from soil (geophilic) (Cheesbrough, 2000). Some infections such as those caused by *Trichophyton rubrum* tend to be chronic and do not respond well to treatment (Martins and Koboyashi, 1999).

2. Materials and Methods

Sample Collection

Clinical isolates of *Trichophyton rubrum and Trichophyton mentagrophytes* were collected from Federal Medical Center, Makurdi. They were immediately transported to the Laboratory Department of Biological Sciences University of Agriculture Makurdi for analysis. Also a total of twelve topical antifungal cream such as Betrosil, Funbact-A, Fluzec-NM, 3G cream, Miracute, Mycoten, Mycozoral, Nixoderm, Qaudriclear, Skineal, Tribotan, Tydineal and four other agents without specific antimicrobial properties such as Close up tooth paste, Dabur herbal tooth paste, Hydraulic fluid and Shea butter were obtained from reputable shops and pharmaceutical stores in Makurdi.

Microbiological Analysis of Isolates

Media Preparation
All media such as Sabouraud Dextrose Agar (SDA) Mueller Hinton Agar (MHA) Potatoes Dextrose Agar (PDA) were prepared according to manufacturer's standard.

Inoculation of Culture Plates
Using a sterile wire loop each of the fungal isolate was separately inoculated onto the bijou bottles prepared SDA**.** The inoculated slants were incubated at room temperature for 4-15 days.

Microscopy
A drop of 95% ethanol was placed on a clean grease free slide. Using a sterile inoculating needle a small portion of the fungal growth was removed midway between the colony center and the edge. With the aid of two disserting needle, the fungus was teased gently such that it thinly spread out in the mounting medium. A drop of lactose phenol cotton blue was added and covered with a cover slip. The preparation was examined microscopically for spores or conidia (Macro or Micro conidia) as well as hyphae (which may be septate or non-septate) (Ochei and Kolhatkar, 2000).

Biochemical Analysis of Isolates

Urea Hydrolysis
Few colonies of the fungal growth were inoculated on the entire surface of Christensen's urea slop. It was incubated at room temperature (25^0C) for 3 days and it was observed for red –pink colour which is a positive test. Absence of red to pink colour is a negative test (Ochei and Kolhatkar, 2000).

Susceptibility Test

Determination of the Activities of Topical Antifungal Agents
An optimized agar base punch hole diffusion method was employed to determine the susceptibility of dermatophytes to different brands of topical antifungal preparation and other agents commonly used by local inhabitants of Makurdi metropolis and its environs.*T. rubrum*and *T mentagrophyte* were sub cultured on Potato Dextrose Agar (PDA) at 30^oC for 4 to 15 days. Following growth of dermatophytes, conidia were harvested in saline, and using a hemocytometer, the conidia suspension was adjusted to 1.0×10^6 conidial /ml according to the method described by Nweze*et al.* (2010). They were incubated at room temperature (25^oC ± 2^0C) for 2 hours and the turbidity adjusted to McFarland standard 1 turbidity. Mueller –Hilton (MH) agar plates were streaked evenly with a swab dipped into the standard inoculum suspension of each isolates. The plates were kept in hot air oven at 37^0Cfor excess moisture to be absorbed into the agar. Stock solution of each agents was prepared by weighing 1g of each agents (except for hydraulic) using aluminum foil and dispensed into 10ml of distilled water to allow the active ingredients be in solution form. Using an agar borer 13 holes was bored and 500µl each of the topical antifungal agents was dispensed into the corresponding pre-labeled hole with the aid of micropipette. Exactly 500ul mixture of fluconazole and mycozoral) was dispensed in one of the hole as control. Plates were incubated at room temperature (25^oC ± 2^0C) for 3-7 days to allow for fungal growth. Inhibition zone diameters (IZD) were measured in millimeters using a transparent meter rule. Agents showing activity against particular organisms were subjected to Minimum Inhibitory Concentration (MIC) (Ochei and Kolhatkar, 2000).

Determination of Activities of Other Agents without Specific Antimicrobial Functions
The same procedure was used to determine the effects of other agents (hydraulic fluid, close up toothpaste, shea butter and Dabur herbal toothpaste). Using agar borer 5 holes were bored on MHA plate. Exactly 1g each of close up tooth paste, shea butter and Dabur herbal tooth paste were weighed with aluminum foil and dissolve in 10ml normal saline and 500ul of each preparation and hydraulic was pipetted and dispensed into respective holes with each hole having approximately equidistance from each other. Fluconazole and mycozoral 500ul mixture was used as control in the central hole with the satellite holes containing the other agents. The agar was incubated and zone of inhibition read within 14 days.

Minimum Inhibitory Concentration (MIC) Using Agar Dilution Test
Minimum Inhibitory Concentration was carried out for those agents that showed activity as described by Ochei and Kolhatkar (2000). From the stock solutions, dilutions were made in bottles to obtain the following concentrations 1000mg, 500mg, 250mg and 125 mg respectively. Exactly 20ml of molten MHA cooled to about 45^0C was dispensed in all the bottles and mixed by gentle shaking. These preparations were placed at an angle 45^0C to form a slant and standard conidial suspension was inoculated on the slant surface of respective bottles and incubated at room temperature (25^oC). The least concentration showing no visible growth after 3 weeks of incubation was the MIC.

Minimum Fungicidal Concentration (MFC)
Using a sterile swab stick the surface of all the bottles showing no visible fungal growth were swabbed and inoculated on to a fresh SDA plate devoid of antifungal agent. The plates were incubated at room temperature (25^oC ± 2^0C) for 3 weeks. The least concentration showing no visible growth is the Minimum Fungicidal Concentration (MFC); (Ochei and Kolhatkar, 2000).

3. Results and Discussion

Table 1: Effects of Twelve Selected Brands of Topical Antifungal Creams on *Trichophyton mentagrophyte*

Antifungal Cream	MIZD (mm)	IZDR (mm)	MIC (mg)	MFC (mg)
Betrosil	8.00±1.63	6-10	500	0
Funbact-A	10.50±3.42	6-14	250	500
Fluzec-NM	7.50±3.42	4-12	250	0
3G	0.00	0	0	0
Miracute	10.00±7.12	0-16	250	500
Mycoten	8.50±3.42	4-12	250	0
Mycozoral	8.25±3.86	3-12	250	0
Nixoderm	0.00	0	0	0
Qaudriclear	12.00±5.42	4-16	250	250
Skineal	1.00±2.00	0-4	500	0
Tribotan	5.00±4.16	0-10	500	0
Tydineal	9.75±4.03	5-12	250	500
Control	18..00±1.63	16-20	125	125
LSD (0.05)	5.26			

Key: MIZD: Mean Inhibition zone diameter,
IZDR: Inhibition Zone Diameter Range.
MIC: Minimum Inhibitory Concentration.
MFC: Minimum Fungicidal Concentration.

Table 2: Effects of Twelve Selected Brands of Topical Antifungal Creams on*Trichophyton rubrum*

Antifungal Creams	**MIZD (mm)**	**IZDR (mm)**	**MIC (mg)**	**MFC (mg)**
Betrosil	9.50±7.00	2-18	500	0
Funbact-A	10.00±4.32	4-14	500	0
Fluzec-NM	7.75±3.09	5-12	250	500
3G	1.75±2.10	0-4	500	0
Miracute	11.75±3.77	5-15	250	500
Mycoten	5.00±4.16	0-10	250	0
Mycozoral	7.25±2.50	4-10	500	0
Nixoderm	0.00	0	0	0
Quadriclear	11.70±3.30	7-14	125	250
Skineal	3.00±3.83	0-8	500	0
Tribotan	6.50±4.42	0-10	500	0
Tydineal	8.50±6.19	0-14	250	500
Control	18..00±1.63	16-20	125	125
LSD (0.05)	5.12			

Key: MIZD: Mean Inhibition zone diameter,
IZDR: Inhibition Zone Diameter Range.
MIC: Minimum Inhibitory Concentration.
MFC: Minimum Fungicidal Concentration.

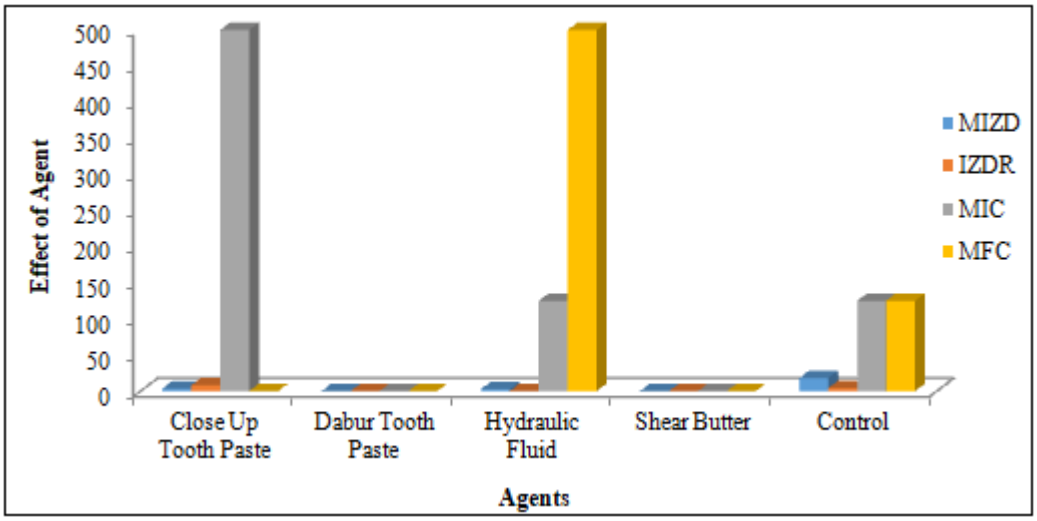

Figure 1: Activities of Four Selected Agents without Specific Antimicrobial Functions and control on *Trichophyton mentagrophyte*

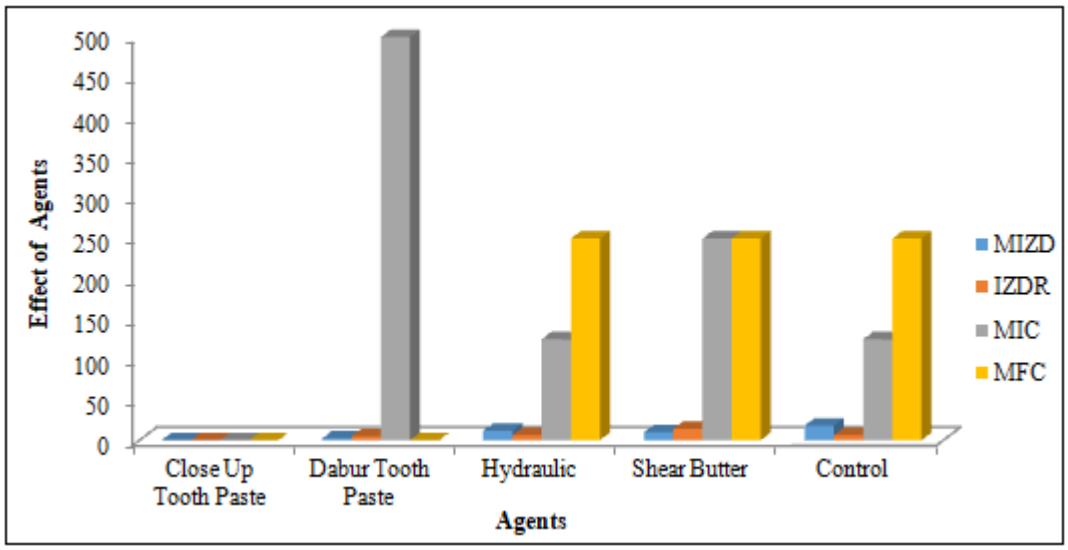

Figure 2: Activities of Four Selected Agents without Specific Antimicrobial Functions and Control on *Trichophyton rubrum*

4. Discussion

A wide variety of topical antifungal agents is available in creams, gel, lotion, and shampoo formulations. In this study antifungal creams used were analysed for its potency on *Trichophyton mentagrophyte* and *Trichophyton rubrum*. There was no significant difference ($P > 0.05$) between Skineal and Tribotan on *Trichophyton mentagrophyte*. There was statistically significant difference ($P < 0.05$) between Betrosil, Funbact-A, Fluzec-NM, Miracute, Mycoten, Mycozoral, Qaudriclear and Tydineal on *Trichophyton mentagrophyte*. Miracute and Quadriclear were higher in activity when compared to other creams on *Trichophyton mentagrophyte*. Nixoderm and 3G had no effect on *Trichophyton mentagrophyte* used for this study. Eight (66.7%) of the agents were fungistatic while four (33.3%) were fungicidal. Table 2 showed that 3G, Mycoten and Skineal had no significant difference ($P > 0.05$) on *Trichophyton rubrum*. Nixoderm also had no effect on this organism. This could be due to resistance over time as *Trichophyton rubrum* is the most common dermatophyte isolated from human. This agrees with Cheesbrough (2000) that the most common dermatophyte isolate is *Trichophyton rubrum*. Among the four selected agent without specific antimicrobial functions on dermatophytes, hydraulic fluid was fungicidal on the two dermatophytes used in this study; the effectiveness of hydraulic fluid could be due to its harsh compositions. *Trichophyton mentagrophyte and Trichophyton rubrum*, showed varying degree of resistant to Dabur toothpaste, shea butter and close up toothpaste. This result proves that these products were not effective as people use it except for hydraulic fluid. Generally all the brands of topical antifungal agents were significantly effective at varying degrees with the exception of quadriclear that showed high activity against these two organisms tested. Qaudriclearis one of the newest preparations and it has 100% activity against *T.mentagrophyte* and *T.rubrum*, this could be due to the fact that since it is new in the market a lot of people may not have use or abuse it.

5. Conclusion

It has been concluded from the analysis of this study that Qaudriclear was the most effective antifungal agent on *Trichophyton mentagrophyte and Trichophyton rubrum*, as compared to other antifungal agents used. Hydraulic fluid from the non-antifungal agents without specific antimicrobial function showed highest activity on the test organisms as compared to close-up toothpaste, Dabur herbal tooth paste and shea butter. The use of hydraulic fluid for topical treatment of fungal infection though effective should not be encouraged

References

[1] Barry, L and Hainer, M.D. (2003): Dermatophyte infections, *America family physician* (6): 101-108 Cheesbrough, M. (2000): District Laboratory Practice in Tropical Countries. *Cambridge University press. Inc., New York*. Pp 602-615.

[2] Gupta, A.k., Jail, H.C., Lynde, C.W., Watteel, G.N and Summerbell, R.C. (1997): Prevalence and epidemiology of unsuspected Onchomycosis in patients visiting dermatologist's office in Ontario, Canada-a multicentered survey of 2, 001 patients *International Journal of Dermatology*, (360): 783-7

[3] Jawetz, E., Melnik, J and Land Adelberg, E.A. (2007): Medical Microbiology.24thEdition, *Lange Medical publications* Los Angeles California page 262 – 269.

[4] Martin, A.C and Koboyashi, G.S. (1999): Superficial fungal infection: Dermatophytosis, *Tinea nigra, piedra*. Dermatology in General Medicine. 25thEdition.*New McGraw Hill*.Pp 2337-2357.

[5] Nweze, E.I; Mukherjeev, P.K and Ghannoum, M.A. (2010): Agar Based Disk Diffusion Assay for Susceptibility Testing of dermatophytes. *Journal of Clinical Microbiology*.(48): 3750 – 3752

[6] Ochei, J and Kolhatkar, A.A. (2000): Medical Laboratory Science Theory and Practice. *Tata MacCraw- Hill Publishing Companies New Delhi*. PP 1007 – 1107