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Effects of Some Selected Brands of Antifungal With and Without Specific Antifungal Activities on Microsporiumcanis and Epidermophytonfloccosum

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Abstract: *Effects of some selected brands of antifungal with and without specific antifungal activities on Microsporiumcanis and Epidermophytonfloccosumwas carried out to ascertain their potency. Punch hole diffusion method was used to determine the susceptibility of dermatophytes to different brands of topical antifungal with and without specific antifungal activities. Minimum Inhibitory Concentration and Minimum Fungicidal Concentration was determined to ascertain potency.EpidermophytonfloccosumandMicrosporum Canis were subjected to twelve brands of topical anti-fungal creams (Betrosil, Funbact-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 they were fungicidal. There was no significant difference (P > 0.05) between Skineal, mycozoral, 3G and Nixoderm and they werefungistatic. Statistically there was significant difference (P < 0.05) between Hydraulic fluids and other selected agents without specific antifungal activities. Hydraulic fluid showed fungicidal activity on the twodermatophytes tested Therefore antifungal creams should be used on dematophytes topically. Use of non-antifungal agents such as hydraulic fluid with antifungal properties should be prohibitedas this might constitute health hazard.*

Key Words: *Microsporiumcanis, Epidermophytonfloccosum,hydraulic fluid, Fungicidal, Fungistatic*

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I. Introduction

Fungal infections that involve only superficial keratinized tissues of the body like the skin, hair and nails are called dermatophytoses (Gupta et al., 1997). Ringworms are the most common fungal infections of man. (Ochei and Kolhatkar, 2000).A single species is able to cause more than one type of clinical infection, e.g.Tineacorporis may be caused by more than one dermatophytes species (Jawetzet al., 2007). Therapy for dermatophytoses consist of removal of infected and dead epithelium structures on application of topical antifungal cream, but relapse may occur for some dermatophytes species and primary resistance of Trichophytonrubrumstrains to terbinafine underscore the need for determination of their in vitro antifungal susceptibilities (Nwezeet al.,2010). The most prevalent infections in the world is dermatophytoses, they can be persistent and troublesome, but not life – threatening. (Jawetzet al., 2007).Laboratory diagnosis is made by microscopic and cultural examination of the specimen such as skin, nail or hair. The identification of the isolate depends on the colonial morphology and microscopic appearance of the isolated fungus (Ochei and Kolhatkar, 2000). DNA sequencing is the gold standard (Packueet al., 2014).

II. Materials and Methods

Sample Collection

Clinical isolates of *Microsporiumcanis andEpidermophytonfloccosum* were collected from Benue State University Teaching Hospital, Makurdi. Isolates were immediately transported to the Laboratory Department of Biological Sciences University of Agriculture Makurdi. 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 antifungal 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 for 3 days and observed for red –pink colour which is a positive test. Absence of red to pink colour indicates a negative test (Ochei and Kolhatkar, 2000).

Susceptibility Test

Determination of Activity of Topical Antifungal Creams

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 inhabitants of Makurdimetropolis.*Microsporiumcanis*and*Epidermophytonfloccosum*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, 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 ($25^oC \pm 2^0C$) for 2 hours and the turbidity adjusted to about 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 $37^0C$for 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 ($25^oC \pm 2^0C$) 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 Properties

Hydraulic fluid, close up toothpaste, shea butter and dabur herbal toothpaste were employed. 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. Exactly 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 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^{o}C). 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 onto a fresh SDA plate devoid of antifungal agent. The plates were incubated at room temperature (25^{o}C ± 2^{0}C) for 3 weeks. The least concentration showing no visible growth is the Minimum Fungicidal Concentration (MFC) (Ochei and Kolhatkar, 2000).

III. Results and Discussion

Table 1: Antifungal Activities of Twelve Selected Brands of Topical antifungal Creams on*Epidermophytonfloccosum*.

Antifungal Cream	MIZD (mm)	IZDR (mm)	MIC (mg)	MFC (mg)
Betrosil	6.00±1.63	4-8	500	0
Funbact-A	10.00±4.32	6-16	250	0
Fluzec-NM	7.50±1.91	6-10	125	250
3G	10.50±7.18	0-16	250	500
Miracute	11.00±2.58	8-14	125	250
Mycoten	3.50±3.00	0-8	250	0
Mycozoral	2.50±3.00	0-6	250	0
Nixoderm	8.50±6.19	0-12	500	0
Qaudriclear	12.50±1.73	10-14	125	250
Skineal	5.00±5.03	0-14	250	0
Tribotan	6.00±3.42	0-8	250	0
Tydineal	7.00±2.58	4-10	125	0
Control	19.00±2.58	16-22	125	125
LSD (0.05)	5.34			

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

Table2:Antifungal Activities of Twelve Selected Brands of Topical Antifungal Creams on *Microsporumcanis*

Antifungal Cream	MIZD (mm)	IZDR (mm)	MIC (mg)	MFC (mg)
Betrosil	6.00±1.63	4-8	500	0
Funbact-A	9.50±2.52	6-12	250	500
Fluzec-NM	2.50±3.00	0-6	250	500
3G	6.00±0.00	0-6	500	0
Miracute	10.50±2.52	6-16	250	500
Mycoten	5.75±4.03	0-9	500	1000
Mycozoral	7.00±2.58	4-10	250	500
Nixoderm	0.00	0	0	0
Qaudriclear	8.00±3.26	4-12	125	250
Skineal	0.00	0	0	0
Tribotan	8.50±1.91	6-10	500	125
Tydineal	6.50±1.73	5-9	250	250
Control	17..50±3.42	14-22	125	125
LSD (0.05)	4.11			

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

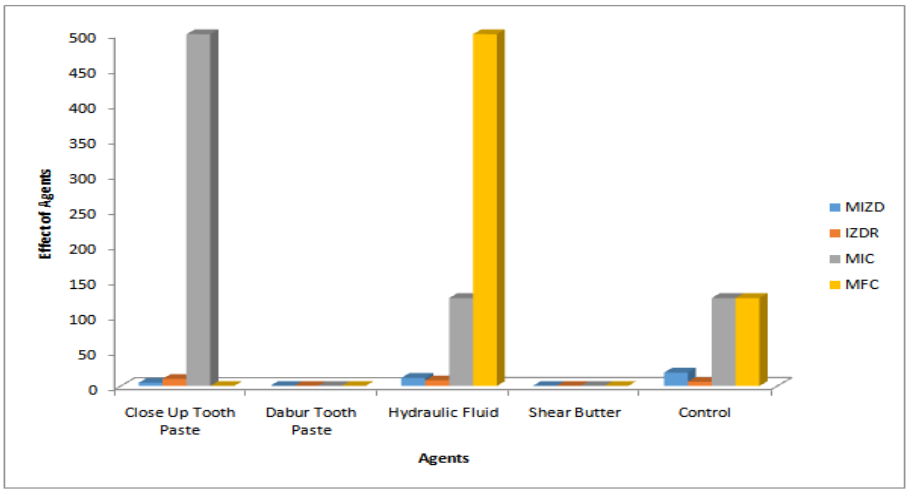

Figure 1: Four Selected Agents without Specific Antifungal Properties and Control on *Epidermophytonfloccosum*

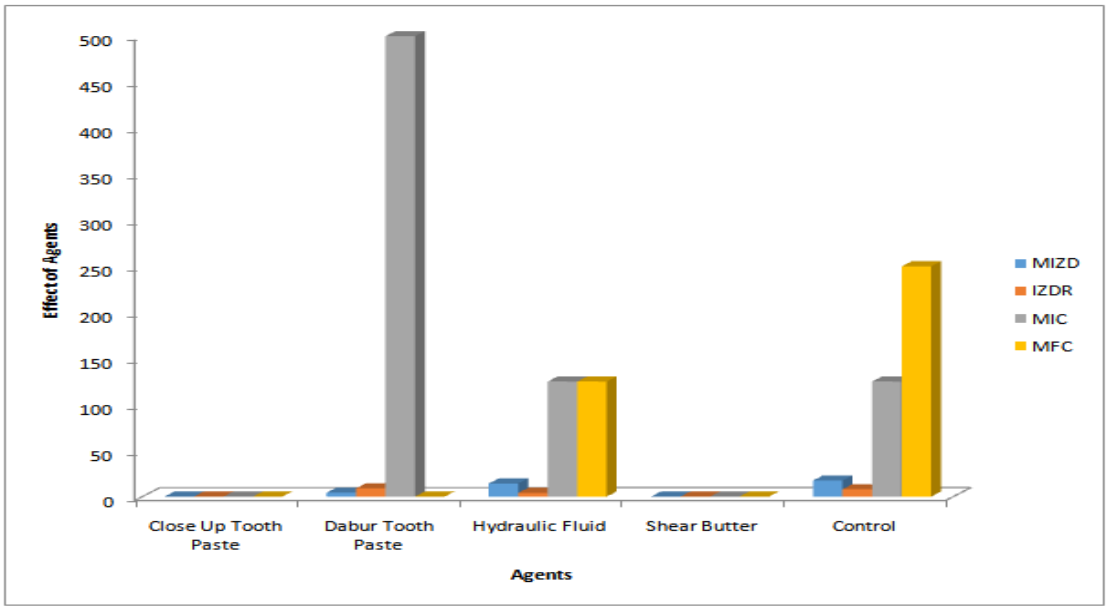

Figure 2: Four Selected Agents without Specific Antifungal Properties and Control on *Microsporumcanis*

IV. Discussion

Dermatophytes have become resistant to treatments globally, this could be reasons for the use of agents without antifungal properties as a remedy for treatment. Based on the analysis from findings of this study there was significant difference ($P < 0.05$) between Betrosil, Funbact-A, Fluzec-NM, 3G, Miracute, Nixoderm, Qaudriclear, Tribotan and Tydineal (75%) of these creams on *Epidermophytonfloccosum* as compared with (25%) that had no significant difference ($P > 0.05$).Quadriclear and Miracute are more effective than Mycoten and Mycozoral as compared to the rest creams used. Quadriclear, Miracute and Fluzec-NM were fungicidal at a concentration of 250ml and cure rate will be faster when used. This agrees with Raghu *et al*. (2014) who said that fungicidal drugs are preferred over fungistatic drugs for superficial dermatophyte infections because higher cure rates are achieved in shorter treatment times, thus increasing the likelihood of patients adhering and decreasing the incidence of recurrence. There is statistically significant difference ($P < 0.05$) between all the antifungal creams on *Microsporumcanis*except for Fluzec-NM with no significant difference ($P > 0.05$) Nixoderm and Skineal. Miracute and FunbactA showed a higher degree of activity against *Microsporumcanis.* Nixoderm and Skineal had no effect and this could be due to abuse of these products prompting the organisms to become resistant. Among the four selected agents without specific antifungal functions on dermatophytes, hydraulic fluid was fungicidal on the two dermatophytes used for this study, the effectiveness of hydraulic fluid could be due to some of its strong ingredients on these organisms.*Epidermophytonfloccosum andMicrosporumcanis*showed varying degree of resistant to close up anddabur toothpaste. The use of agents without antifungal properties by some people could be due to beliefs and sentiments and not because they are effective except hydraulic fluids which proved to be effective.

Qaudriclear was the most potent of all the antifungal creams on *Epidermophytonfloccosum andMicrosporumcanis*. This could be due to the fact that people are not aware of its potency, and hence, its abuse is minimal.

V. Conclusion

It was established from the findings of this study that Qaudriclearis the most potent antifungal cream on *Epidermophytonfloccosum and Microsporumcanis*as compared to the other antifungal creams used. Hydraulic fluid without antifungal properties showed highest activity on the test organisms as compared to other agents without specific antifungal agents. Hydraulic fluid should not be used on the human skin because of its harsh compositions.

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