RESEARCH ARTICLE

Evaluation of safety and *in-Vitro* Efficacy study of Anti-Fungal Cream

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ABSTRACT

Evaluation of Antifungal activity of herbo-mineral formulation and its ingredients were tested *In-vitro* against yeast *Candida albicans* by various methods using Miconazole as standard drug. Simultaneously safety study (skin irritation test) of herbal-mineral formulation was also performed according to OECD guidelines. Safety of the formulation was proved by skin irritation test and efficacy of the formulation was proved by *In-vitro* antifungal bio-assay.

Materials & Methods: The product was first checked for its solubility followed by anti fungal activity using both the Cup Method and Kirby Bauer Method. The Skin irritation test was carried out on Healthy adult male Wistar rats weighing 250-300g. All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: KBIPER/IAEC/2012/353) of KBIPER (K.B. Institute of pharmaceutical education and research) and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.: 35/1999/CPCSEA), Ministry of Social Justice and Empowerment, Government of India

Results & Discussion: The study clearly shows that the herbo-mineral formulation is showing good *in-vitro* anti-fungal activity against *C. albicans* and no skin irritation or adverse effects were observed in animals during the entire study.

Keywords: Skin irritation, *Candida albicans*, anti-fungal, Cutis cream, Herbo-mineral formulation.

INTRODUCTION

Candida albicans and other related species are known to cause infections in humans. There are a wide range of infections such as skin infection, oral and genital infection. The major habitat of commensal *Candida* species is believed to be the gastro intestinal tract. It has been shown that, fungaemia and funguria is commonly caused by *C. albicans,* if present in sufficient higher numbers. [1]

The human skin is considered as the largest organ of the body. It is the outer covering of the body. As it interacts with the environment, skin plays a major role in protecting the body against pathogens like bacteria and fungus. Other functions of the skin are also important such as insulation, temperature regulation, sensation etc. Hence before applying any topical formulation one should completely be aware of the safety of the formulation. [2]

Epithelial cells (ECs) at mucosal surfaces provide first line of defense against fungal infections as they are being in constant contact with this fungus. Because of Increasing in number of the fungal invasion and recognition of antifungal resistance, there is need for clinically relevant antifungal susceptibility testing. [3-6]

The herbo-mineral formulation used for this study had been carefully designed to treat fungal skin infections. It contains different oils like *Azadirachta indica* (Neem) oil, *Pongamia pinnata* (Karanj) oil, Mahamarichyadi taila, *Cinamom camphora* (Karpoor) oil, *C. citratas* (Lemongrass) oil, and metals like Shuddha Tankan powder (purified boric acid), Shuddha Tuttha powder (Purified Copper sulphate) and Shuddha Gandhak powder (Purified sulphur).

MATERIALS AND METHODS

Sample Preparation:

The sample was first determined for its solubility properties. Different solvents were used in order to test the solubility. 0.5g of the sample was accurately weighed and dispensed in different test tubes

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containing the solvents to be checked for. The results are as tabulated in Table 1.

The Cream was dissolved in 5mL of DMSO solvent taken in sterile screw capped containers. The mixture was vortexed and sonicated to get a uniform suspension and stored for not more than 24hours at 4°C till use.

Media Preparation:

Saboraud's Dextrose Broth was used for determining the activity. Media was prepared according the Manufacturer's instructions. The pH of the same was adjusted as per requirement; a known amount of sterile corn oil was added in order to enhance the growth of Candida. The media was then autoclaved at 15lbs pressure for 20minutes. The media was then allowed to cool down to about 45°C and then poured in sterile petri plates. The plates were then allowed to solidify overnight. The plates were then kept at 4°C till use.

Plating:

Two methods were followed for plating: The Kirby Bauer Method and the cup method.

0.1mL of the freshly revived culture (24h old culture) was pipette out and evenly spread with the help of a sterile cotton bud saturated with the culture. The plate was swirled at 45° angle and evenly spread throughout. The plate was kept aside for 5 minutes and allowed to dry.

a) Disk diffusion method: Sterile disks of about 6mm were dipped in the sample and gently placed on the agar plate with the help of sterile forceps. The disk was then gently touched with the forceps to ensure its contact with the media. The plates were incubated at 25°C for 4 to 5 days. The Zone of inhibition was then measured with the help of a standard antibiotic zone reader and recorded. [7]

b) Well diffusion method: After swabbing the culture, wells were made in the middle of the plate with the help of a sterile cork borer. 0.1mL of the sample was then filled in the well to the brim. The plates were then incubated at 25°C for 4 to 5 days. The Zone of inhibition was then measured with the help of a standard antibiotic zone reader and recorded. The samples were done in triplicates and after which the SD was calculated. [8] The results of both the methods are as tabulated as in Table 2

Skin Irritation test of Cutis Cream:

Animals: All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: KBIPER/IAEC/2012/353) of KBIPER (K.B. Institute of pharmaceutical education and research) and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.: 35/1999/CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult male Wistar rats weighing 250-300g were used. Rats were housed in polypropylene cages, maintained under standardized condition (12Nil hour light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to palleted 'Sabardan' diet and purified drinking water. [8-12]

Procedure: Skin irritation test was performed in 6 albino rats (either sex) were taken for the study. Procedure for this test was followed according to the OECD TG 404, 2002 guideline.

Rats were divided into two groups, (group A & group B), three rats in each group.

Rats were anesthetized and 1cm^2 area on the back was shaved.

Group A: Considered as a control group

Group B: 0.5 g of Cutis cream was applied to the shaved area on the back of rats.

After that continuous visual inspection of the rats of both the group were done at 2 hr, 4 hr. 6 hr, 24 hr and up to 48 hr.

Rats were observed for the production of any irritant response such as erythema, edema, and irritation after a single topical application.

The results were as tabulated in Table 3

RESULTS:

Table 1: Solubility Test of Cutis Cream.

S. No	Observation Time	Solubility in different solvents					
		Water	DMSO	Hexane	Chloroform	Acetone	Methanol
1	2 min	++	+++	+	+	+	++
2	10 min	++	+++	+	+	+	++

Keys: + (weakly soluble), ++ (Partially soluble), +++ (soluble), DMSO (Di Methyl Sulphoxide),

Based on the above data DMSO was chosen as the solvent for test.

Table 2: The Zone of Inhibition

Sr No	Name of the Sample	Concentration / Method	Method	Zone of Inhibition (mm)
STANDARD				
1*	Miconazole	30 µg/disk	Disk	16 mm
INDIVIDUAL INGREDIENTS OF FORMULATION				
1	0.5g Shuddh Tankan	5 mL of DMSO	Well	22 mm ± 0.71
2	0.5g Shuddh Tankan	5 mL of DMSO	Disk	24 mm ± 1.41
3	1.0g Shuddh Tankan	5 mL of DMSO	Well	34 mm ± 1.41
4	1.0g Shuddh Tankan	5 mL of DMSO	Disk	32 mm ± 0.71
5	0.5g Shuddh Tuttha	5 mL of DMSO	Well	40 mm ± 1.41
6	0.5g Shuddh Tuttha	5 mL of DMSO	Disk	40 mm ± 0.71
7	1.0g Shuddh Tuttha	5 mL of DMSO	Well	40 mm ± 1.41
8	1.0 g Shuddh Tuttha	5 mL of DMSO	Disk	40 mm ± 0.71
9	0.5 g Shuddh Gandhak	5 mL of DMSO	Well	25 mm ± 0.71
10	0.5 g Shuddh Gandhak	5 mL of DMSO	Disk	20 mm ± 0.71
11	1.0 g Shuddh Gandhak	5 mL of DMSO	Well	26 mm ± 1.41
12	1.0 g Shuddh Gandhak	5 mL of DMSO	Disk	24 mm ± 0.71
13	0.5 mL Lemongrass oil	5 mL of DMSO	Well	40 mm±0.71
14	1.0 mL Lemongrass oil	5 mL of DMSO	Disk	40 mm±0.71
15	0.5 mL Lemongrass oil	5 mL of DMSO	Well	40 mm±0.71
16	1.0 mL Lemongrass oil	5 mL of DMSO	Disk	40 mm±0.71
17	0.5 mL Karpoor oil	5 mL of DMSO	Well	15 mm ± 0.71
18	1.0 mL Karpoor oil	5 mL of DMSO	Disk	14 mm ±0.71
19	0.5 mL Karpoor oil	5 mL of DMSO	Well	20 mm ±0.71
20	1.0 mL Karpoor oil	5 mL of DMSO	Disk	22 mm ±1.41
21	1.5 mL Karanj oil	5 mL of DMSO	Well	13 mm±0.71
22	2.0 mL Karanj oil	5 mL of DMSO	Well	14 mm±0.71
23	1.5 mL Mahamarichyadi taila	5 mL of DMSO	Well	14 mm±0.71
24	2.0 mL Mahamarichyadi taila	5 mL of DMSO	Well	15 mm±0.71
Minero-Herbal formulation				
1	0.5 g Cutis cream	5 mL of DMSO	Well	15mm±1.41
2	1.0 g Cutis cream	5 mL of DMSO	Well	17mm±1.41
3	1.5 g Cutis cream	5 mL of DMSO	Well	18mm±1.41
4	2.0 g Cutis cream	5 mL of DMSO	Well	23mm±1.41

Comparison of formulation with standard.

Preparation of standard plate:

Miconazole patch having concentration of 30 µg was placed in the center of the plate.

Preparation of test plate:

To obtain 30 µg concentrations, 1g of Cutis cream was dissolved in 5 ml of DMSO, from that 0.15 µl of DMSO was subjected on nitrocellulose filter paper. And that filter paper was placed in the middle of the plate as described in the procedure.

Table 3: Skin irritation test of Antifungal Cutis Cream

After applying topical dose of 2.0 g of the preparations the rats were visually observed up to 48 hrs and there was no sign of any untoward response.

Group	Signs of skin irritation	Cutis Cream					
		Time in hour					
		2 hr	4hr	6hr	12hr	24hr	48hr
Group A	Erythema	Nil	Nil	Nil	Nil	Nil	Nil
	Edema	Nil	Nil	Nil	Nil	Nil	Nil
	Irritation	Nil	Nil	Nil	Nil	Nil	Nil
Group B	Erythema	Nil	Nil	Nil	Nil	Nil	Nil
	Edema	Nil	Nil	Nil	Nil	Nil	Nil
	Irritation	Nil	Nil	Nil	Nil	Nil	Nil

Fig.1: Standard miconazole (30 µg) showed inhibition of 16 mm.

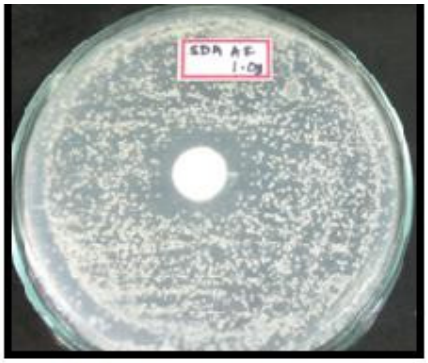

Fig.2: Cutis cream (30 µg) showed inhibition of 17 mm.

DISCUSSION

From the above compiled data the study clearly shows that the herbo-mineral formulation is showing good *in-vitro* anti-fungal activity against *C. albicans* and no skin irritation or adverse effects were observed in animals during the entire study.

CONCLUSION

Present study showed that the Herbo-mineral formulation can be employed as safe and effective remedy against fungal skin infections, especially of *Candida sp.*

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