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EVALUATION OF SAFETY AND IN-VITRO EFFICACY STUDY OF ANTI-FUNGAL DUSTING POWDER

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ABSTRACT
The herbo-mineral formulation and its ingredients were screened for their anti fungal activity against Candida albicans using Miconazole as standard and also checked for skin irritation test as per OECD guideline. Cutis Dusting Powder proved to be safe during skin irritation test. Study showed good anti fungal activity of individual ingredients and finished product thus it can be concluded that Cutis Dusting Powder can be employed as safe and effective remedy for fungal skin diseases.

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INTRODUCTION

Candida albicans is the most prevalent pathogenic Candida species for fungal infection in tropical countries like India. *C. albicans* possesses many putative virulence attributes that contribute to general survival, fitness and persistence within the host as well as specific factors associated with adhesion, invasion, cell damage and induction/evasion of host responses.[1]

The skin is important to us in many ways and is the largest and most visible organ in the body, with an average surface area of about two square yards. Many conditions affect the human integumentary system; the organ system covering the entire surface of the body and composed of skin, hair, nails, and related muscle and glands.[6]

Epithelial cells (ECs) at mucosal surfaces are in the unique position of being in constant contact with *C. albicans* and thereby constitute the first line of defense against the fungal infection. The need for reproducible, clinically relevant antifungal susceptibility testing has been prompted by the increasing number of invasive fungal infections, the expanding use of new and established antifungal agents, and recognition of antifungal resistance as an important clinical problem.[2-5]

A poly herbal formulation used for this study was carefully designed to treat fungal skin infections. It contains different powders like Pushpanjan (Purified zinc oxide) Powder, Shuddh Tankan (Purified boric acid) Powder, Shuddh Gandhak (Purified sulphar) Powder, *Azadirachta indica*

(Neem) Extract and *Trachyspermum ammi* (Ajwain) Oil along with the required excipients.

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

Different aliquotes of the sample were prepared as mentioned in Table 2. They were 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.

b) Well diffusion method: After swabbing the culture, well 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. The results of both the methods are as tabulated as in Table 3

SKIN IRRITATION TEST OF CUTIS DUSTING POWDER: [7-10]

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 250-300 g were used. Rats were housed in polypropylene cages, maintained under standardized condition (12Nilhour light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to palleted 'Sabardan' diet and purified drinking water.

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 dusting powder 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, oedema, irritation after a single topical application.

The results were as tabulated in Table 4

Table 1: Solubility Test **(Final Formulation of Cutis Dusting Powder)**

Sr 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: Aliquote Preparation

Sr No	Weight of Sample (g)	Solvent Used
Final Poly Herbal Formulation		
1	0.5g of the sample	Water
2	1.0g of the sample	Water
3	0.5g of the sample	DMSO
4	1.0g of the sample	DMSO
5	0.5g of the sample	Methanol
6	1.0g of the sample	Methanol
7	0.5g of the sample	Ethyl Acetate
8	1.0g of the sample	Ethyl Acetate

Table 3: The Zone of Inhibition

Sr No	Name of the Sample	Concentration / Method	Method	Zone of Inhibition (mm)
STANDARD				
1*	**Miconazole**	30mcg/disk	Disk	16mm
POLY HERBAL FORMULATION				
1	0.5g of sample in Water	5mL of Solvent	Well	-
2	1.0g of sample in Water	5mL of Solvent	Well	11mm
3	0.5g of sample in DMSO	5mL of Solvent	Well	18mm
4	1.0g of sample in DMSO	5mL of Solvent	Well	22mm
5	0.5g of sample in Methanol	5mL of Solvent	Well	15mm
6	1.0g of sample in Methanol	5mL of Solvent	Well	16mm
7	0.5g of sample in Ethyl Acetate	5mL of Solvent	Well	28mm
8	1.0g of sample in Ethyl Acetate	5mL of Solvent	Well	32mm
INDIVIDUAL INGREDIENTS OF FORMULATION				
9	0.5g Shuddh Tankan	5mL of DMSO	Well	22mm $\pm$ 0.71
10	0.5g Shuddh Tankan	5mL of DMSO	Disk	24mm $\pm$ 1.41
11	1.0g Shuddh Tankan	5mL of DMSO	Well	34mm $\pm$ 1.41
12	1.0g Shuddh Tankan	5mL of DMSO	Disk	32mm $\pm$ 0.71
13	0.5g Pushpanjan	5mL of DMSO	Well	16mm $\pm$ 1.41
14	0.5g Pushpanjan	5mL of DMSO	Disk	18mm $\pm$ 1.41
15	1.0g Pushpanjan	5mL of DMSO	Well	17mm $\pm$ 0.71

16	1.0g Pushpanjan	5mL of DMSO	Disk	18mm ± 0.71
17	0.5g Gandhak	5mL of DMSO	Well	25mm ± 0.71
18	0.5g Gandhak	5mL of DMSO	Disk	20mm ± 0.71
19	1.0g Gandhak	5mL of DMSO	Well	26mm ± 1.41
20	1.0g Gandhak	5mL of DMSO	Disk	24mm ± 0.71

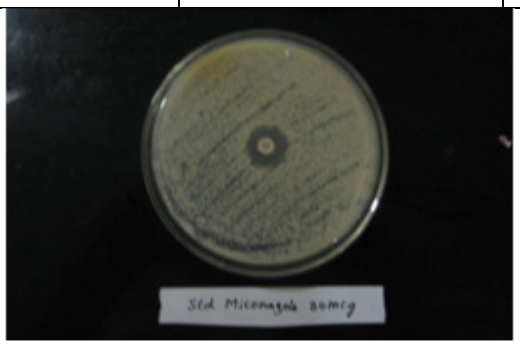

Figure 1: Std Miconazole 30mcg/disk showing inhibition of 16mm

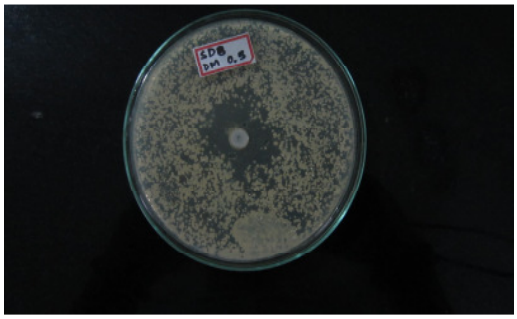

Figure 2: Cutis Dusting Powder showing the ZOI

Table 4: Skin irritation test of Antifungal cutis dusting powder

After applying topical dose of 0.5 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 Dusting powder**					
		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

DISCUSSION: From the above compiled data the study clearly shows that the poly-herbal 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 poly-herbal formulation can be employed as safe and effective remedy against fungal skin infections, especially of *Candida sp.*

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