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HERB, CLIMBAZOLE + ZINC PYRITHIONE SYNERGY TO DEAL DRUG RESISTANCE

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ABSTRACT

Background: The present study deals with the importance of herbs in fortifying the anti-dandruff efficacy of Climbazole and Zinc Pyrithione (ZPTO) through a novel mechanism. The synergy between the herb and the synthetic anti-dandruff agents not only curb dandruff but also prevent drug resistance. The synergy may pose confusion to the pathogen to identify the target molecule and there by develop drug resistance. This is the first study to the best of our knowledge unravel the scientific mystery behind herb – synthetic molecule synergy for prognosis and drug resistance. **Method:** Zone of inhibition and minimum inhibitory concentration was studied for the individual test materials and market samples. Market samples also tested in different water types by zone of inhibition. A pilot scale study on market products was conducted in human volunteers to understand the aesthetics and hair properties after use. **Results:** All the studies prove the anti-dandruff activity of the Verdura anti-scaling scalp shampoo and other test samples. The stable efficacy of the Verdura was proven when studied in different waters. **Conclusion:** Verdura anti scaling scalp shampoo with two anti-dandruff agents Climbazole and Zinc pyrithione along with herbal extracts shows the better activity (at the tested concentrations) and also have superior conditioning benefits.

KEYWORDS: Anti-fungal drugs, Verdura anti-scaling scalp shampoo, Climbazole, Ketoconazole, Zinc pyrithione, Herbal anti-fungal drugs, Drug resistance.

INTRODUCTION

Dandruff and the fungal infection of the scalp caused by dermatophytes these days requires special medicaments than the conventional anti-fungal therapy. The reason being, etiology of dandruff and Tinea capitis are caused by the fungi that are mostly considered either as commensal flora of the scalp or they are strictly by an anthropophilic organism.[1,2] Further in both infections, the clinical manifestation often limits to the superficial layer of the epidermis i.e., stratum corneum, which is strictly a non-nucleated non-living cell deposition.[3]

Conventional anti-fungal therapy may be useful in treating the above infections but such treatment may lead to continuous and unnecessary exposure of the fungi to various anti-fungal drugs and such situation may also warrant anti-fungal drug resistance. Therefore, the treatment strategy of dandruff and Tinea capitis must be with a broad spectrum anti-fungal agent where the chance of fungus to isolate and identify the single ecological cause that act upon them may not be possible and thus the chance of fungus evolve drug resistance is minimal.

Indigenous system of medicine in India which is popularly referred as AYUSH has plenty of drugs for the management of fungal infections. Among them, preparations with *Cassia alata*, *Azadirachta indica*, *Wrightia tinctoria* etc., are considered to be more effective according to Siddha and Ayurveda.[4,5]

Recent studies have proved scientifically the anti-fungal effect of the above herbs and thereby validated the usefulness of the above herbal preparations for the treatment of various fungal infections.[6]

Our earlier study has proved that the combination of *Cassia alata* and *Azadirachta indica complex* besides exhibiting anti-fungal activity also prevent the fungal adhesion on the skin which was established by us by corneo-sulphometry.[7]

Based on our vast research experience and in-depth scientific knowledge in the field of Siddha research and anti-fungal therapy, we have employed the conjugation technology to formulate herbal + Climbazole + ZPTO fusion (Verdura anti-scaling scalp shampoo) to combat dandruff and other fungal scalp infections. Through the fusion technology we have attempted to create confusion

to the pathogen to identify the exact anti-fungal agent and thereby we have attempted to minimize the chance of anti-fungal resistance.

Commensal flora and or anthropophilic organism if develop drug resistance that might cause greater challenge to the medical fraternity due to human being as the most preferred and the only host/habitat. Therefore, the amalgamation of Siddha wisdom with conventional science is needed to combat such fungal diseases.

In the present study we have employed the fusion of goodness of siddha system with Climbazole and ZPTO to manage dandruff and the details are presented in the paper.

MATERIALS AND METHODS

I. Details of the products tested

Verdura anti-scaling scalp shampoo: A unique combination of herbs and 1% each of Climbazole and ZPTO from Dr.JRK's Research and Pharmaceuticals Pvt Ltd. indicated for dandruff and scalp scales.

Scalpe: Shampoo formulated with 2% of Ketoconazole and 1% ZPTO from Glenmark indicated for Dandruff.

Ketomark: Contains 2% of Ketoconazole from Elixer, indicated for Dandruff.

II. List of test samples and preparation: 1 gm of the test sample was taken in 100 ml of water to obtain 1% concentration. For combination samples i.e., ZPTO+ Climbazole (1%) and ZPTO + Ketoconazole (1%) etc., 1 gm of each compound was taken in 200 ml of sterile distilled water to obtain 1% solution respectively. (Table-A).

Table A: List of test samples.

S. No	Test samples
1	Blank (Saline)
2	SLES (1%)
3	Climbazole (1%)
4	Ketoconazole (1%)
5	Zinc pyrithione (1%)
6	Zpto+ climbazole (1%)
7	Zpto + ketoconazole (1%)
8	*Wrightia tinctoria(1%)*
9	*Cassia alata(1%)*
10	*Aloe vera(1%)*
11	Herbs(Each 1%) + Climbazole(1%) +ZPTO(1%)
12	Climbazole + ZPTO (2%+1%)
13	Ketoconazole + ZPTO (1% + 1%)
14	Ketoconazole + ZPTO (2% + 1%)
15	Ketoconazole + ZPTO (1% + 2%)
16	Climbazole + ZPTO (2%+1%)
17	Verdura anti-scaling scalp shampoo (1%)
18	Ketomark (1%)
19	Scalpe (1%)

III. Preparation of extracts

Hydro-alcoholic extracts of individual herbs were used for the present study. Maceration method was followed and filtered after incubation for 24 hours. The filtrate was distilled to evaporate to get the extract and used for the study.

IV. Zone of inhibition[8,9]

Inoculum preparation: A loop-full of organism was added in 100 ml of sterile distilled water (Stock). *P.ovale* and *C.albicans* suspension adjusted to achieve 10^3 CFU/ml was used as inoculum. Coconut oil supplementation was used for studying *P.ovale*.

Preparation of test samples: 1 gm of the test sample was taken in 100 ml of water to obtain 1% concentration.

For combination samples i.e., ZPTO+ Climbazole (1%) and ZPTO + Ketoconazole (1%), 1 gm of each compound was taken in 200 ml of sterile distilled water to obtain 1% solution.

0.1 ml of inoculam is taken and evenly spread on the sabouraud dextrose agar medium with chloramphenicol. For promoting growth of *P.ovale* coconut oil was added. 5 mm well was made in saboraud's dextrose agar medium using well-cutter and to it 50 µl of test sample was added. All the plates were incubated at room temperature for 3-5 days.

Post incubation period plates were observed for zone of inhibition and diameter of each zone was measured in mm.

V. Determination of MIC[8,9]

Inoculum preparation: A loop-full of organism was added in 100 ml of sterile distilled water (Stock). *P.ovale* and *C.albicans* suspension adjusted to achieve 10^3 CFU/ml was used as inoculum. Coconut oil supplementation was used for studying *P.ovale*.

Preparation of test samples: 1 gm of the test samples each was taken in 100 ml of sterile distilled water. From that serial dilution of the stock solution was done to obtain various concentrations as follows i.e., 0.75%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.031% and 0.015%.

Sabouraud dextrose agar medium was used for MIC evaluation. 2 ml of test sample from each of the above mentioned concentration was added to sterile petri plates to which sterile Sabouraud dextrose agar medium (20 ml) was added and mixed thoroughly. Plates were allowed to set. From the stock solution of *Candida* 0.1 ml was spread plated using L-rod. Similarly *P.ovale* culture was inoculated along with coconut oil.

All the plates were incubated at room temperature for 3-5 days. Post incubation period plates were observed for growth.

VI. Activity evaluation in Different waters

Preparation of test samples – Zone of Inhibition

1. 1 gm of the test sample was taken in 500 ml of different kinds of water such as Distilled water, Brackish water and Water with high TDS.

Zone of inhibition

Two species of fungus i.e *Pityrosporum ovale* and *Candida albicans* were taken from the 1-week old plate. The organisms were sub cultured and taken for the study.

Loop full of organism was taken in 100 ml of sterile distilled water (Stock solution). From stock 0.1 ml is taken and evenly spread on the Sabouraud dextrose agar medium with chloramphenicol. For promoting growth of *P.ovale* coconut oil was added.

Well was cut using well-cutter and to it 50 µl of test sample was added. All the plates were incubated at room temperature for 3-5 days.

Post incubation period plates were observed for zone of inhibition and diameter of each zone was measured in mm.

Clinical evaluation

6 volunteers were chosen for the study who are diagnosed with dandruff. Age group is 20 – 40 years. Three brands were taken for the study and for each shampoo two volunteers were allotted and the volunteers were asked to wash the scalp and hair with the shampoo provided to them thrice weekly. And at the end of week volunteers were asked for their feedback on the following parameters such as itching, removal of scales and reduction in hair fall if any.

RESULTS

Zone of Inhibition

In the zone of inhibition assay, ZPTO showed marginally better anti-fungal activity against *P.ovale* and *Candida* than Climbazole or Ketoconazole. We have used distilled water as solvent to solubilize the anti-fungal agents. The combination of ZPTO and Ketoconazole and ZPTO and Climbazole showed comparable zone of inhibition suggesting both combinations may possess similar activity.

Two anti-dandruff formulations that are available in the market such as Scalpe, Ketomark were tested along with Verdura anti-scaling scalp shampoo. All the three products showed comparable anti-fungal activity. (Table-1).

Table 1: Zone of inhibition.

S. No.	Test	Zone of inhibition (Diameter in mm) 20 µl of 1% sample used for testing	
		P.ovale	Candida
1	Blank (Saline)	-	-
2	SLES (1%)	-	-
3	Climbazole (1%)	32	22
4	Ketoconazole (1%)	26	20
5	Zinc pyrithione (1%)	38	40
6	Zpto+ climbazole (1%)	30	32
7	Zpto + ketoconazole (1%)	32	36
8	Scalpe (1%)	32	27
9	Ketomark (1%)	27	33
10	Verdura shampoo (1%)	32	32

Determination of MIC

The MIC of Climbazole, Ketoconazole and ZPTO when tested individually although showed a slight variation in the MIC value but the anti-fungal activity of all the three ingredients were comparable. *Candida albicans* showed greater susceptibility to Ketoconazole than to Climbazole and ZPTO was the most effective than other two ingredients.

The combination of Climbazole with ZPTO and Ketoconazole with ZPTO at two different concentrations also showed comparable anti-fungal activity. All the three shampoos when tested for MIC showed comparable result. The formulation of all the three shampoos are different and hence the possible weight variability of different ingredients in each formulation cannot be accurately measured during testing. Therefore, the MIC value of all the three shampoo needs to be harmonized than looked at with the respective minimal variability. (table-2).

Table 2: Determination of MIC.

Test	MIC (mg/ml)	
	P.ovale	Candida
Climbazole	0.75	0.75
Ketoconazole	0.75	0.5
Zinc pyrithione	0.125	0.125
Climbazole + ZPTO (1%+1%)	0.015	0.031
Climbazole + ZPTO (1%+2%)	0.015	0.031
Climbazole + ZPTO (2%+1%)	0.015	0.015
Ketoconazole + ZPTO (1% + 1%)	0.015	0.015
Ketoconazole + ZPTO (2% + 1%)	0.015	0.015
Ketoconazole + ZPTO (1% + 2%)	0.015	0.015
Scalpe (K 2+ ZPTO 1)	0.125 (0.05K+0.025 Z) 0.075	0.125
Verdura shampoo(C1+ZPTO 1)	0.5(0.025C+0.025Z) 0.05	0.5
Ketomark (K2)	0.75(0.05K) 0.05	0.5

Effect of SLES on anti-fungal activity

In-order to understand the influence of SLES on the anti-fungal effect of various ingredients, the present study was conducted. The anti-fungal activity of the combination of Climbazole and ZPTO was unaffected by SLES, wherein a proportionate increase of either Climbazole or ZPTO, one step decrease in the MIC concentration was observed. On the contrary the effect of combination of ketoconazole and ZPTO was slightly modified by SLES because the proportionate increase of ketoconazole or ZPTO did not show greater MIC activity (Table-3).

Table 3: Effect of SLES on anti-fungal activity.

Test	MIC (mg/ml) in SLES	
	P.ovale	*Candida*
Climbazole + ZPTO (1%+1%)	0.125	0.75
Climbazole + ZPTO (1%+2%)	0.0625	0.0625
Climbazole + ZPTO (2%+1%)	0.0625	0.0625
Ketoconazole + ZPTO (1% + 1%)	0.0625	0.0625
Ketoconazole + ZPTO (1% + 2%)	0.0625	0.0625
Ketoconazole + ZPTO (2% + 1%)	0.0625	0.0625

Effect of quality of water on the anti-fungal activity

Influence of the quality of water on the anti-fungal activity of three shampoos were tested by incorporating 1 gm of shampoo in 500 ml of three types of water prepared in our laboratory such as water with distilled water, water with high TDS and salt water. Sterilized water was used to prepare the above samples.

Zone of inhibition assay was done and result show that all the three shampoos showed comparable efficacy. (Table-4).

Table 4: Effect of quality of water on the anti-fungal activity.

S. No	Types of water	Distilled water		Water with high TDS		Salt water	
	Test (1:500)	Zone of inhibition (Diameter in mm)		Zone of inhibition (Diameter in mm)		Zone of inhibition (Diameter in mm)	
		P.ovale	*Candida*	*P.ovale*	*Candida*	*P.ovale*	*Candida*
1	Scalpe	30	25	28	24	28	25
2	Ketomark	26	31	24	30	25	29
3	Control	-	-	-	-	-	-
4	Verdura	28	28	28	28	30	30

In-house evaluation in Dandruff sufferers

Clinically and mycologically diagnosed dandruff volunteers were recruited for the present study. The age extent of infection, duration of infection etc., among all the volunteers were matched. For each shampoo two volunteers were allotted and the volunteers were asked to wash the scalp and hair with the shampoo provided to them thrice weekly. And at the end of third wash volunteers were asked for their feedback on the following parameters such as itching, removal of scales and reduction in hair fall if any.

The two volunteers who used Ketomark reported itching of scalp as well as dryness. Both the volunteers who used Scalpe shampoo although reported scalp dryness but did not complain of itching. The volunteers who used Verdura shampoo reported positive feedback about the shampoo and also soft feel of the hair. (Table-5).

Table 5: Feedback on the efficacy of test products.

No. of patients	Shampoo	No. of Wash	Reduction in Itching	Reduction in Scales	Reduction in Hair fall	Hair texture post use
2	Ketomark	3	-	-	-	Dry and stiff
2	Scalpe	3	+	+	-	Dry
2	Verdura shampoo	3	+	+	+	Soft

+ →Reduction - →No reduction

Table 6: Role of herbs.

S. No	Test	Zone of inhibition (Diameter in mm) 20 µl of 1% sample used for testing		MIC (mg/ml)	
		P.ovale	Candida	P.ovale	Candida
1	Blank (Saline)	-	-	-	-
2	SLES (1%)	-	-	-	-
3	Climbazole (1%)	32	22	0.75	0.75
4	Ketoconazole (1%)	26	20	0.75	0.5
5	Zinc pyrithione (1%)	38	40	0.125	0.125
6	ZPTO+ Climbazole (1%)	30	32	0.015	0.031
7	ZPTO + Ketoconazole (1%)	32	36	0.015	0.015
8	*Wrightia tinctoria*	15	18	15	12
9	*Cassia alata*	10	9	18	15
10	*Aloe vera*	2	3	-	-
11	Herbs+ Climbazole +ZPTO	45	49	0.0075	0.0075

DISCUSSION

The present investigation has revealed the importance of conjugation of ancient wisdom with conventional science to manage various infectious diseases and to prevent possible drug resistance. Wherever possible, especially to treat the infections caused by either commensal flora or by strictly an anthrapophilic organism, conjugation therapy of herb + conventional method may be adopted.

The drug resistance is more often due to the frequent exposure of the pathogen to multi-various anti-fungal/anti-bacterial drugs.[10] On constant / in frequent exposure of the organism to the drug may facilitate the evolution of drug resistance in them to thrive in the ecosystem. Whenever treatment is specific and targeted, identification and isolation of the target drug by the pathogen is easier for the organism than when treatment is through conjugation technology. The conjugation technology may act as 'ambush hunt' on the organism leaving least to no chance for the organism to identify the drug molecule and develop resistance.

Herbal drugs by itself may not effectively control the pathogens because the herbal extract in toto is comprised of several chemical molecules that are known to exhibit

synergistic activity. The herbal synergism is less explained in the scientific plethora due to the lack of concerted studies. The ancient traditional wisdom of healing and herbal science can offer lots of drugs to humanity. However, the herbal preparations may not act as effectively like the well identified synthetic single drug molecule. But the herbal preparations can aid in the better delivery of the synthetic molecule and can confuse or camouflage the pathogen from identifying the exact nature of the drug molecule.

It is scientifically proven that urea coated with neem oil is more effective for agriculture by enhancing sustained release of urea and produce less eco-toxicity than use of urea without neem oil coating.[11]

In our present study we found that the combination of *Wrightia tinctoria*+ *Cassia alata* + *Aloe vera*+ ZPTO+ Climbazole showed superior anti-fungal activity against *P.ovale* and *Candida albicans* than the combination of Climbazole+ ZPTO. Initially we presumed that the combination of *Wrightia tinctoria*+ *Cassia alata* + *Aloe vera*+ ZPTO+ Climbazole may be less effective due to the presence of each herb in the final quantity of the sample that was used for testing because the total weight of the above sample in all likely hood would represent only less proportion of ZPTO and Climbazole than the sample with only ZPTO + Climbazole. But surprisingly we found that despite the slight decrease in the concentration of ZPTO and Climbazole we have got significant anti-fungal activity. This possibility clearly points towards the existence of synergistic effect and that might be the reason for the superior anti-fungal activity of the above combination.

Due to the rampant use of Climbazole, Ketoconzole and ZPTO in most of the anti-dandruff products, the chance of the fungus to develop resistance against the above agents is quite high. However, when the above ingredients are presented to the pathogen along with anti-fungal herbs (although the anti-fungal activity of the herbs need not be great), the fungus may find it difficult to identify the multilayered attack therefore the fungus may not able to evolve effectively towards such combination and become drug resistant.

Treatment of diseases should also include the evolutionary aspect and adaptation possibilities of the pathogen if the pathogen involved in the disease is commensal flora or anthrapophilic organism. We are the first to device such path breaking treatment strategy for dandruff and associated scalp diseases. The comparison of anti-fungal activity and the associated superiority of the formulation may not give complete solution to the problem of Dandruff but the strategy of ‘ambush hunt’ by integrating the ancient wisdom of siddha system of healing along with the conventional treatment alone would solve many infectious diseases.

Our study highlights the scientific fact that an effective anti-dandruff product is not defined by the mere presence of either Ketoconazole, Climbazole or ZPTO but the combination of herbal drug along with proven anti-dandruff agent(s) alone may offer better prognosis and least drug resistance.

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