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Research Article

Formulation and Evaluation of Topical Antifungal Herbal Gels Containing Hydroalcoholic Extract of *Catharanthus roseus* and *Aloe vera*

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ABSTRACT

The present study deals with formulation and evaluation of herbal gel for the management of In Vitro Antifungal activity. Antifungal activity of extracts against *Candida albicans* was evaluated as it causes 90% of skin infection. In this study we had formulated the Antifungal herbal gel by using hydroalocoholic extract of *Catharanthus roseus* and *Aloe vera* in different concentration (0.2%, 0.4% and 0.6%, coding them as S1, S2 & S3 respectively) with carbapol 940 and other excipient. Thus formulation formulated was evaluated for its physiochemical parameters, In –Vitro antifungal activity and Stability study of the formulation. All the herbal gel formulation showed positive results for physiochemical parameters with pH .8. The result of anti fungal activity of the of *Catharanthus roseus* and *Aloe vera* gel topical formulations shows dose dependent zone of inhibitions in exponential manner as compared to the standard (FLUCONAZOLE) i.e. S3 formulation shows 189 ± 0.11mm zone of inhibition which greater than the remaining two formulations 2% shows153 ±0.23 mm and 4% shows 153 ±0.23 mm and the near about to standard Fluconazole showed the 194 ±0.01 mm zone of inhibition.

Keywords: Herbal gel, of *Catharanthus roseus*, *Aloe vera*, *Candida albicans*, Antifungal activity.

INTRODUCTION

The last two decades have witnessed an increase in fungal infection.Fungal infections are emergent diseases in hospital institutions. Bacteraemia and fungaemia are among the most frequent hospital-acquired infections. Increase on immunosuppressive diseases and conditions have been influencing the epidemiological pattern of mycoses in hospitalized patients the epidemiology of invasive fungal infections is currently at a crucial stage[1].

In recent years there is great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, efficacy and better therapeutic results, ease of availability, higher safety margin than the synthetic drugs and also due to its economic pricing compared to synthetic or allopathic drugs, which have several therapeutic complications. So, by performing this project based purely on natural drugs which contribute a newer, safer and efficient drug therapy against one of the most infectious disease i.e. Fungal disease This is increasing in very high ratio due to certain reason including opportunistic infection[2-3].

Fungal infection caused by *Candida* has become more prevalent than *Escherichia coli* and *Pseudomonas* sp., *Asprgellosis* sp. and other sp. And is now the fourth most common fatal infection in the world. *Candidiasis is* also directly associated with the severity of illness. There are many host factors that predispose patients to fungal infections. These include: immobility; mucositis; use of antibiotics; radiation therapy or certain immunosuppressive agents; intensive care unit (ICU)[4].

Candidiasis is most commonly encountered Opportunistic mycosis fungal infection worldwide. *Candida albicans* is the most common species in the genus which has been implicated in *Candidiasis.* The infections range from superficial of the skin to systemic diseases. *C.albicans, C. tropicalis, C. glabrata and C. parapsilosis* are part of the normal flora of human and can be isolated from oral cavity, vaginal and other parts of body sites from normal healthy people. Under certain circumstances, these organisms may gain access to many organ systems such as lung, spleen, kidney, liver, heart, brain, eye, skin and others. Lesions may occur in patients who have disseminated infections. Current Treatment available for *Candidiasis* are as**:** Topical antifungal: Ketoconazole, Miconazole, Nystatin**,** for Systemic: Amphotericin B, Fluconazole, and Itraconazole and for Chronic mucocutaneous: Amphotericin B, Fluconazole, Itraconazole but with these synthetics drugs there are certain side-effects[5].

Catharanthus roseus is dired whole plant belonging to family *Apocynaceae*. It is mostly found in tropical and subtropical area of south Africa, USA, Europe, Australia. In India it is mostly found in south india and north eastern states of india It is cultivated in. *Catharanthus roseus* grows all over india up to 500 metres. except the higly

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Table 1: Master Formula For 100gm Gel Formulation.

S.no	Ingredients	Quantity
1	Extract (*Catharanthus roseus* & *Aloe vera*	1gm.
2	Carbopol	30gm.
3	Sodium carboxy methyl cellulose	5gm.
4	Poly ethylene glycol	10gm.
5	Sodium benzoate	0.5gm.
6	Distilled water	q.s.

Table 2: Formula For 30 Gm 0.2%Gel Formulation.

S.no	Ingredients	Quantity
1	Extract(*Catharanthus roseus* & *Aloe vera*)	200mg.
2	Carbopol	9gm.
3	Sodium carboxy methyl cellulose	1.5gm.
4	Poly ethylene glycol	3ml.
5	Sodium benzoate	0.15gm.
6	Distilled water	q.s.

Table 3: formula for 30 gm 0.4% gel formulation.

S.no	Ingredients	Quantity
1	Extract(*Catharanthus roseus* & *Aloe vera)*	400mg.
2	Carbopol	9gm.
3	Sodium carboxy methyl cellulose	1.5gm.
4	Poly ethylene glycol	3ml.
5	Sodium benzoate	0.15gm.
6	Distilled water	q.s.

Table 4: formula for 30 gm 0.6%gel formulation.

S.no	Ingredients	Quantity
1	Extract(*Catharanthus roseus* & *Aloe vera)*	600mg.
2	Carbopol	9gm.
3	Sodium carboxy methyl cellulose	1.5gm.
4	Poly ethylene glycol	3ml.
5	Sodium benzoate	0.15gm.
6	Distilled water	q.s.

Table 5: Potato dextrose agar medium: (ph: 6-7).

S.no	Ingredient	Quantity
1.	Pieces of potato	40.0 g
2.	Dextrose	4.0 g
3.	Yeast extract	0.02 g
4.	Distilled water	200.0 ml
5.	Agar	4.0 g

alkaline or water logged soil, *Catharanthus roseus* does not require any special canditions of soil it favourably grows in light sandy soil which is rich in humus. A large number of Indole alkaloids are present in *Catharanthus roseus*. Out of them, about 20 dimeric Indole-dihydroindole alkaloids possess Oncolytic activity, and among them, Vincristine and Vinblastine are most significant. it is used intravenously in the treatment of acute leukemia of children, Hodgkin diseases, Reticulum cell sarcoma, lymphosarcoma and myosarcoma, hypotension and antidiabetic[6,7].

The main objective of the present investigation was to prepare a herbal Antifungal gels is for treatment of human skin infected with fungus *Candida albicans* and try to improves the drawbacks of the available synthetic formulation in the market. The projected formulation is based upon the plant products having synergistic effect hence enhancing the antifungal activity with negligible side effects or toxicity.

MATERIAL AND METHOD

Collection of plant material

Fresh leaves of plants was collected from herbal garden of Acropolis Institute of Pharmaceutical Education, Indore then it was identified and authenticated on the basis of its morphological and microscopical characters.

Preparation of extracts

The fresh leaves of *Catharanthus roseus and Aloe vera* was washed, chopped into tiny fragments and sun-dried. The dried leaves were then grounded into powder with pestle and mortar. From the pulverized leaves, 250g powder was taken in dish and sufficient quantity of Hydro alcoholic (50: 50) solvent about 750 ml was added. This sample was vigorously shaken thrice daily, for 2 days. Then the mixture was filtered and the filtrate obtained was placed on a heating mental maintained at 40^{O}C.After evaporation of the solvent, the resulting extract was placed in a sealed bottle until ready for use[8].

Fungal strain

The fungal strain employed in the study was obtained from the Choithram Hospital Pathology, Indore.

Preparation of herbal gel formulations[9,10]

Different proportions of Sodium CMC and Carbopol 934 were dispersed in distilled water with a continuous stirring by the help of mechanical stirrer where as another beaker consist of weighed and required quantity of extracted drug powder which was then dissolved in polyethylene glycol and sonicated for 15 minutes. After sonication for 15 min this mixture was transferred to the first solution which was containing mixture of Sodium CMC and Carbopol 934 with continues stiring. On other hand 5 ml of distilled water was taken and required quantity of sodium benzoate was dissolved by heating on water bath. Solution was cooled and polyethylene glycol was added and mixed with above solution. Finally all ingredients were mixed properly to the Carbopol 934 with continuous stirring to obtain the gel in required consistency. By using this method 3 herbal gel formulations with three different concentrations of *Catharanthus roseus* and *Aloe vera* leaves extract i.e 0.2%, 0.4%and 0.6% respectively were prepared (Table 1, 2, 3 & 4).

Coding of gels are done as follows

S1 This contain 0.2% of each extracts (*Catharanthus roseus* and *Aloe vera*)

S2 This contain 0.4% of each extract (*Catharanthus roseus* and *Aloe vera*)

S3 This contain 0.6% of each extract (*Catharanthus roseus* and *Aloe vera*)

Table 5: Result of physiochemical parameters.

Parameters	Day-0			Day-15			Day-30		
Physical Parameters	*S1*	*S2*	S3	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*
Color	Pear green	Dark green	Pickle green	Pear Green	Dark green	Pickle green	Pear Green	Dark green	Pickle green
Consistency	Good	Good	Good	Good	Good	Good	Good	Good	Good
Odour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour
Greasiness	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy	Non greasy
Extrude ability	+++	+++	+++	+++	+++	+++	+++	+++	+++
Homogeneity	+++	+++	+++	+++	+++	+++	+++	+++	+++
pH	*6.8*	*6.8*	*6.8*	*6.8*	*6.8*	*6.8*	*6.8*	*6.8*	*6.8*
Skin irritation	Non-irritating	Non-irritating	Non-irritating	Non-irritating	Non-irritating	Non-irritating	Non-irritating	Non-irritating	Non-irritating
Spreadability	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 6: Result of anti-fungal activity of *catharanthus roseus and aloe vera* gel formulation.

S.no	Herbal gel formulations	Name of the cultured starin	Zone of inhibition in mm
1	S1 (0.2%)		146 ± 0.11mm
2	S2 (0.4%)		153 ±0.23 mm
3	S3 (0.6%)	*Candida Albican*	189 ± 0.11mm
4	Positive control (Fluconazole)		195±0.01 mm

*All values are expressed as mean ± of three experiments

Preparation potato dextrose agar medium

40.0 g of peeled potatoes were cut into small pieces and suspended in 200.0 ml of distilled water which was steamed for 30 min. Decanted the extract through muslin cloth and made the final volume to 200.0 ml to it then 4.0 g of dextrose, 0.02 g of yeast extract and 4.0 g of agar was added to prepare the culture media.

Minimum inhibition concentration of each extract using well diffusion method

The respective medium was sterilized by autoclaving at 121^0C ($15lb/ln^2$). for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification.15µl of inoculums of fungi *Candida albican* was transferred to respective Petri plate. Three wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 28^0C for 48 hours. The diameter of zone of inhibition surrounding each of the wells was recorded[11-12].

Evaluation of herbal gel[13-14]

Color

The color of the formulations was checked out against white & black backgrounds.

Odour

The odour of the gels was checked by mixing a little amount of gel in water and by taking smell

Consistency

The consistency was checked by applying the gel on to the skin.

Greasiness

The greasiness of the formulations was observed by the applying the gel on to the skin.

Homogeneity

All the formulated gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their manifestation and occurrence of any aggregate.

pH

The pH of various ointment formulations will be determined by using digital pH meter. One gram of ointment will be dissolve in 100 ml distilled water and store for two hours. The measurement of pH of each formulation will be done in triplicate and average values will be calculated.

Extrudability study

Extrudability was measured on the basis the quantity in percentage of gels on application of finger pressure. More quantity extruded better was extrudability[15].

Non irritancy test

Herbal gels prepared was applied to the skin of human being and effect was observed visually[16].

Stability studies

Stability studies was carried on all the three formulations in during different timing that is on day-0, 15th day and on 30th day. On these time intervals all the evaluation studies and skin irritation studies were performed to check the stability of the topical herbal gel formulations[17].

RESULT AND DISCUSSION

The prepared herbal topical gels of different concentration (0.2%. 0.4% & 0.6%) was evaluated immediately after the preparation of gels at three different time intervals i. e on day-0, 15th day and 30th day. All the gels of different concentrations were evaluated for their above mentioned parameters. Physical parameters revealed that the color of the formulations was founds to be light green with no odour and sour taste. The result of consistency and greasiness was found good and non greasy where as homogenisity, extrudability and spreadibilty was also found to be good. The pH for all the formulations of different concentration was 6.8 and the results of skin irritancy studies indicated that the prepared gels were free from dermatological reaction. The Antifungal activity against *Candida albican* of Herbal gel formulations was shown in a dose dependent zone of inhibition in exponential manner ie. S3 formulation (0.6% of Hydroalcoholic extract of *Catharanthus roseus* and *Aloe vera* shows 189 ± 0.11mm zone of inhibition which greater than the remaining two formulations 2% shows153 ±0.23 mm and 4% shows 153 ±0.23 mm and the standard Fluconazole showed the 195±0.01 mm zone of inhibition.

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