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FORMULATION AND ITS IN-VITRO ANTI-FUNGAL STUDY OF FAGONIA SCHWEINFURTHII HADIDI EXTRACT CREAM

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ABSTRACT: Aim: The aim of present work was to develop and evaluate antifungal topical preparation of aqueous extract of herbal plant *Fagoniaschweinfurthii*Hadidi. Materials and Method: An oil in water (O/W) emulsion-based cream was formulated by using extract and various ingredients like oil soluble components white petrolatum, cetyl alcohol and water soluble components sodium lauryl sulphate, methyl paraban and water. Prepared formulation was subjected to various physiochemical parameters like pH, spread ability viscosity and stability study. Antifungal activity of cream was determined by cup plate method against three fungal strains. Result and Discussion: pH, spreadability and viscosity was found to be 7.2± 0.1, 20.55±0.47 gm.cm/sec. and 11820±5 cp respectively. The values of diameters of zones of inhibitions (in mm) by prepared cream and marketed formulation was found to be 36.00 ± 1 and 35.66± 1.52, 31.56± 1.52 and 37.33 ±1.15, 32.66± 0.57 and 35.66± 0.57 respectively against *Candida albicans*, *Aspergillus niger*and *Aspergillus fumigates*. Results indicated that developed formulation has potent antifungal activity. Conclusion: On the basis of data of stability study and other evaluation parameters, it can be concluded that stable formulation was developed which has potent anti fungal activity and can be used in treatment of skin diseases caused by fungal infections.

KEYWORDS: *Fagonia schweinfurthii* Hadidi, Antifungal activity, *Candida albicans*, Skin disorders.

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1. INTRODUCTION

Fungal infections, both invasive and superficial have become increasingly problem over the past few decades. A person's skin can become infected by a variety of fungi. Reports shows that most of the medicinal plants possess antimicrobial, antioxidant, and anti-inflammatory properties, which can be used in the prevention of many infectious diseases, also have potential benefits for the society[1], [2]. Plants and plant extracts have been used for the treatment of skin disorders for centuries. Drugs are applied topically to the skin mainly for their local action[3]. Development of resistance to the currently available antifungals, it is important that novel antifungal agents be identified and developed[4].Traditionally, a large number of plants are used for treatment of skin diseases caused by fungal infections. In Asian and African countries *Fagoniaschweinfurthii*Hadidi (Family Zygophyllaceae) and the closely related herbs are traditionally used for treatment of inflammation, wound healing, antimicrobial, skin disorder, allergies, etc. [5-7]. Antimicrobial study of ethanol extract of *Fagoniaindica* leaves was carried out (25, 50 and 100mg/ml) against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus*. In the study the maximum diameter of zone of inhibition was observed against *Staphylococcus aureus*[8]. Antimicrobial activity of methanolic extract of whole plant of *Fagoniacretica* was also carried out and significant antimicrobial activity against *Bacillus subtilis, Shigellaflexneri, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,Trichophytonlongifusus, Candida albicans, Aspergillus flavus*and *Candida glabrata*was observed[9-11].Creams are homogeneous, semi-solid or viscous preparations that possess a relatively fluid consistency and are intended for external application to the skin or certain mucous membranes for protective, therapeutic or prophylactic purposes. In present work the antifungal topical preparation of aqueous extract of herbal plant *Fagonia schweinfurthii* hadidi (Family: Zygophyllaceae) was developed and evaluated. Prepared cream was evaluated for various physical parameters like pH, homogeneity, viscosity, spreadibility and stability. The *in-vitro* antifungal activity was carried out by cup-plate method. For the study three strains of fungi were used as test microorganism [12].

2. MATERIALS AND METHODS

Plant Material

The plant was collected from Jodhpur region of Rajasthan, India and was authentified from Boatanical Survey of India (BSI), Jodhpur, Rajasthan (India). Voucher specimens and herbarium sheet was kept in the institute for further references.

Test Microorganisms and Culture Media

Strains of fungi were obtained from MTCC (Microbial Type Culture Collection) Chandigarh, India. *Candida albicans* (MTCC 183), *Aspergillus niger* (MTCC 281) and *Aspergillus fumigatus* (MTCC 870) were selected for screening of Antimicrobial activity. *C. albicans, A. fumigatus* and *A. niger*

were grown in Sabouraud Dextrose Broth (Hi-Media) for determination of MIC and SabouraudDextrose Agar media for cup-plate method. The concentrations of all fungal suspensions were adjusted to 10^7 cells/mL before experiment.

Preparation of Aqueous extract

Fresh plant of *Fagoniaschweinfurthii*hadidiwas shade dried and grounded to prepare a moderately coarse powder. The extraction was carried out by decoction method with water at 40˚C. Extract was filtered through a 45 µm membrane filter, and the filtrate was dried with help of an evaporator. The crude extract was stored in desiccators [13-16].

Determination of Minimal Inhibitory Concentration (MIC) of Plant Extract

The minimal ınhibitory concentration was determined according to procedure given in Cooper and Gunn's Tutorial Pharmacy by J. S. Carter [17]. The turbidity in each test tube was observed after 24 hrs.

Formulation of Cream

Oil in water (O/W) cream (semisolid formulation) was formulated using various ingredients. Oil soluble components (White petrolatum, Cetyl alcohol) were dissolved in the oil phase (Part A) and heated to 75° C. The water soluble components (Extract, Sodium lauryl sulphate, Methyl paraban, and water) were dissolved in the aqueous phase (Part B) and heated to 75° C. The oily phase was added in portions to the aqueous phase with continuous stirring until semisolid uniform cream was formed. Flavor was added after cooling [18], [19]. The formulations were prepared using 3^2 factorial design, in which two components (SLS and cetyl alcohol) were selected at three levels. The formula for the cream is given in Table no 1.

Table 1: Formulations of cream using Factorial design

Sr. No.	Ingredients (in %w/w)	Formulation code								
		C1	C2	C3	C4	C5	C6	C7	C8	C9
1.	Aq. Extract	2	2	2	2	2	2	2	2	2
2.	SLS	0.5	1.5	2.5	0.5	1.5	2.5	0.5	1.5	2.5
3.	Cetylalcohol	4	4	4	7	7	7	10	10	10
4.	Propylene glycol	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4
5.	White Petroleum	31	31	31	31	31	31	31	31	31
6.	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7.	Flavouringagent	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	Water	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm	q.s. to 100 gm

Evaluation of the Cream

The cream was evaluated by the following parameters: [20-27]

Physical Properties

The physical properties of the cream were studied by visual appearance and characteristics like state, color, odor and appearance.

pH

The pH was measured by digital pH meter. About 0.5g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Viscosity

Viscosity of the formulation was determined by Brookfield Viscometer (model DV-E) at 10 rpm, using spindle no. 64.

Spreadability

Spreadability is a term expressed to denote the extent of area to which the topical application spreads on application to skin on the affected parts. The therapeutic efficiency of the formulation also depends upon its spreading value. Hence, determination of spreadability is very important in evaluating topical application characteristics. For the determination of spreadability, excess of sample (3g) was applied in between two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 minute. Thereafter weight (50g) was added and the top slide was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves the lower plate to cover a distance of 10 cm was noted. A shorter interval indicates better spreadability. The spreadability (S) was calculated using the formula

$$S = m.l/t$$

Where, S= spreadability, m=Weight tied to upper glass slide, l=length moved on glass slide and T= time.

Stability studies [28]

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. The stability study was carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 40 ± 2 °C / 75 ± 5 % RH for three months. At the end of studies, sample was analyzed for the physical appearance, pH and viscosity.

Determination of *In vitro* antifungal activity by cup plate method (Agar well diffusion method)

Cup plate method was used as an antimicrobial[29-32]. Sterile Sabouraud Dextrose Agar at 43-45ºC and poured into the petri plates (7 cm diameter). Then the agar was allowed to solidify for 1 h. 0.1 mL of different fungal culture inoculum applied of each plate. Inoculum was evenly spread on agar using a glass L- rod spreader. For agar well diffusion method, a well was prepared in the plates with

the help of a cup-borer (0.8cm). Standard formulations (2000µg/ml was prepared by dissolving in DMSO) were placed in the wells. The plates were incubated for 24 hrs. at 28° C. At the end of the period, the zones of inhibitions formed in the medium were measured. Same procedure applied for marketed antifungal cream and results were compared. All experiments were done in three replicates.

3. RESULTS AND DISCUSSION

Minimum inhibitory concentrations (MIC) of extract

Minimum inhibitory concentrations (in µg/ml) of Aq. extract against three fungal strains are given in table no. 2. The results shown that extract is more active against *Candida albicans*compare to *Aspergillus niger*and *Aspergillus fumigates.*

Table 2: MIC of extracts against different microbial species

Sr. No.	Microbial Strains	MIC of Aqueous Extract (µg/ml)
1.	*Candida albicans*	100
2.	*Aspergillus niger*	125
3.	*Aspergillus fumigatus*	125

Physical Evaluation

Nine formulations were prepared and evaluated for various physical parameters like state, color, odor, appearance and phase separation. These evaluation parameters are given in table no 3, 4 and 5. The result shown that the physical state of creams were found to be liquid to highly viscous and all are off-white in color. All formulations were homogenous in appearance except C1 and C2. The phase separation was occurring at different time for all the formulation but C5, C6, C8 and C9 were stable for throughout stability study. So formulations C1, C2, C3, C4, C7, C8 and C9 were discarded on the basis of state, appearance and phase separation.Formulations C5 and C6 were selected for the further evaluation. These formulations were compared with marketed cream for their viscosity and spreadability. The results shown that values of formulation C5 was more near to values of marketed formulation, therefore C5 was selected as optimized formulation.

Table 3: Physical Evaluation of Cream

Formulation code	State	Color	Odor	Appearance	Phase separation
C1	Liquid	-	-	Not Homogenous	Occurs
C2	Liquid	-	-	Not Homogenous	Occurs
C3	Low viscosity	Off- white	Strawberry	Homogenous	Occurs after 15 days
C4	Semisolid	Off- white	Strawberry	Homogenous	Occurs after 21 days
C5	Semisolid	Off- white	Strawberry	Homogenous	Not Occur
C6	Semisolid	Off- white	Strawberry	Homogenous	Not Occur

C7	Semisolid	Off- white	Strawberry	Homogenous	Occurs after 1 Month
C8	Viscous	Off- white	Strawberry	Homogenous	Not Occur
C9	Highly Viscous	Off- white	Strawberry	Homogenous	Not Occur

Table 4: Evaluation of Cream and Marketed product

Parameters	**Formulation code**		**Marketed Product**
	C5	**C6**	
Viscosity(in cp)	11820±5	14457±6.4	11753±3
% Torque(Newton meter)	19.7	21.2	19.3
Spreadability(gm.cm/sec.)	20.55±0.47	13.27±0.84	23.84±0.68

Table 5: Evaluation of optimized Cream formulation (C5)

S. No.	**Parameters**	**Values for optimized formulation (C5)**
1.	State	Semisolid
2.	Color	Off- white
3.	Odor	Strawberry
4.	pH	7.2± 0.1
5.	Viscosity(in cp)	11820±5
6.	%Torque (Newton meter)	19.7
7.	Spreadability (gm.cm/sec.)	20.55±0.47

Antifungal activity

Antifungal activity in term of diameters of inhibition zones are reported in Table no. 6 and bar graph is given in figure no.1.

Table 6: Antifungal activity in term of diameters of inhibition zones

S. No.	**Fungal Strains**	**Diameter of zone of Inhibition (in mm)**	
		For prepared cream	**For marketed formulation**
1.	*Candida albicans*	36± 10	35± 1.52
2.	*Aspergillus niger*	31± 1.5	37 ±1.15
3.	*Aspergillus fumigatus*	32± 0.5	35± 0.57

n= 3

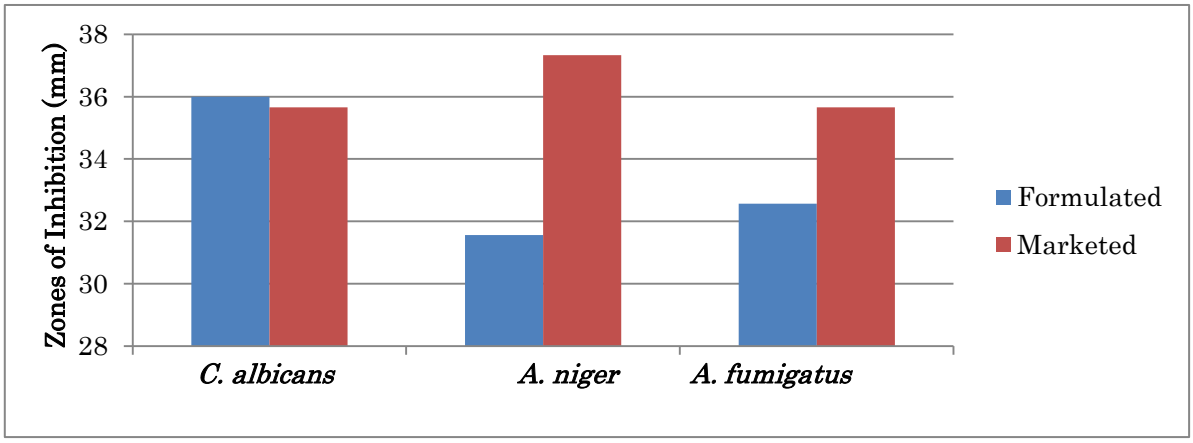

Figure 1: Bar graph Antifungal activity of prepared and marketed formulation

Diameters of zones of inhibition produce by prepared cream and marked formulation against three fungal strains were recorded. From the obtained results it can be developed formulation possess antifungal potential.

Stability study of formulation

After the three month samples were analyzed for the physical appearance, pH and viscosity. The values of these parameters at initial time and at completion of time period are given in table no. 7.

Table 7: Stability study of Cream formulation

Time (Month)	pH	Viscosity	Physical Appearance
Initial	7.2± 0.1	11820±5 cp	No phase separation
After 3 Month	7.4± 0.3	12105±6 cp	No phase separation
After 6 Month	7.4± 0.1	12450±6 cp	No phase separation

n= 3

The values of parameters shows that there were no significant changes were found after stability study. So the prepared formulation of cream is stable and passed stability study.

DISCUSSION

The various therapeutic effects of *Fagoniaschweinfurthii*hadidi has been reported which includes anti-inflammatory, *wound* healing activity, antioxidant, hepato-protective activity in allergies, skin diseases, skin eruptions and orally as blood purifier. The values of diameters of zones of inhibition (in mm) by prepared cream was found to be 36.00 ± 1, 31.56± 1.52 and 32.66± 0.57 respectively against *Candida albicans, Aspergillus niger*and *Aspergillus fumigates* and in case of marketed formulation the diameters of zones of inhibition (in mm) was found to be 35.66± 1.52, and 37.33 ±1.15, and 35.66± 0.57 respectively against *Candida albicans, Aspergillus niger*and *Aspergillus fumigates.* In result although the greater zones of inhibition (in mm) was found by marketed formulation but results also shows that prepared formulation has potent anti fungal activity and can be used in treatment of skin diseases caused by fungal infections.

4. CONCLUSION

Previous studies has shown that various extracts of *Fagonia have* antimicrobial activity. But, these studies have not considered the inherent antifungal activity of *Fagoniaschweinfurthii*hadidi and no one prepared any topical formulation by using the extract. From present study it was concluded that topical formulation cream, prepared by aqueous extract of herbal plant *Fagoniaschweinfurthii*hadidiwas shown potent anti fungal activity with acceptable physical property and passes stability study.

CONFLICT OF INTEREST

No conflict of interest exists.

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