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FORMULATION AND EVALUATION OF HERBAL ANTIBACTERIAL, ANTIFUNGAL CREAM.

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ABSTRACT

Sulphur (Gandhak) and Asadofoetida (Hing) is well known by antibacterial, antifungal activity and its uses is skin disease. The aim of this study was to evaluate the antibacterial, antifungal activity of sulphur (Gandhak) and Asadofoetida (Hing) against Staphylococcus aureus and E. coli species. Also to formulate effective stable herbal antibacterial and antifungal cream and evaluate its physical, antibacterial and antifungal properties. The prepared cream was evaluated for their physical, rheological and antifungal, antibacterial properties. The stability study was also done.

KEYWORD: Herbal antibacterial-antifungal cream, development, stability study.

1. INTRODUCTION

Therapy with herbal drugs is an old tradition all medicine plants have been used over the years for the treatment of numerous health problems including infectious and non-infectious skin disorders.

Gandhak is the most important drug and widely used for many diseases. It is an ingredient in many formulations prescribed in skin disorders such as Gandhakmalahar etc. Rasashastra classical texts have mentioned about Gandhak is a keetnashak, keetaghna, krumighna, pamari, kushtari, dadrughnas – It denotes its action on skin diseases. Staphylococcus aureus, E. coli, Aspergillosis candidiasis are the main pathogens that causes skin infections.

The aim of the present study was antifungal, antibacterial activity of cream to formulate a nature, safe antibacterial, antifungalcream and to evaluate its physiochemical properties and stability.

2. MATERIALS AND METHOD

2.1.Chemicals and solvent

Sulphur (Gandhak), Asadofoetida (Hing), Jatyadi oil, Cetostearyl alcohol, Stearic acid, Sodium lauryl ethyl sulphate (SLES), Water, Nutrient Broth, nutrient agar.

2.2.Test strains

Staphylococcus aureus, Escherchia coli.

Table: 1 Cream formulation

Sr. No.	Formula	30m
1	Sulphur (Gandhak)	0.1 gm
2	Asadofoetida (Hing)	0.05m
3	Jatyadi oil	0ml
4	Karanji oil	15ml
5	Stearic acid	1gm
6	Cetostearyl alcohol	0.6 gm
7	Sodium Lauryl Ethyl Sulphate	0.05m
8	Water	10 ml

Table: 2 Development of cream formulation

Sr. No.	Ingredient	Sample A	Sample B	Sample C
1	Sulphur (Gandhak)	0.1gm	0.1gm	0.1gm
2	Asadofoetida (Hing)	0.05gm	0.05gm	0.05m
3	Jatyadi oil	0ml	0ml	1ml
4	Karanji oil	15l	15l	15l
5	Stearic acid	2gm	2gm	2gm
6	Cetostearyl alcohol	0.6gm	0.9gm	1.2gm
7	Sodium Lauryl Ethyl Sulphate	0.05m	0.05m	0.05M

2.3. Procedure

1. Take two beakers A and B, wash and clean properly.
2. In beaker A take karanj oil and in beaker B Jatyadi oil, sulphur (gandhak), Asadofoetida (Hing)
3. Simultaneously, boil both the beaker A and B on water bath.
4. Mix beaker A and B together with constant mechanical stirring.
5. Melt cetostearyl alcohol and stearic acid and add to the above formulation.

6. Again with mechanical stirring, add sodium lauryl ethyl sulphate.
7. Add slowly in cool water with constant mechanical stirring.
8. Milky, white appearance is seen.
9. Then the formulation cool in ice bath with stirring.

2.4. Characterization of herbal antibacterial, antifungal cream

2.4.1. Physical evaluation

Physical parameters such as colour, odour and appearance were evaluated.

2.4.2. pH determination

The pH of various gel formulations were determined by using digital pH meter. 2.0gm of cream was accurately weighed and dispersed in 20 ml of distilled water and stored for two hours. The measurement of pH was carried out. The pH values are represented. The pH of dispersions was measured using pH meter.

2.4.3. Type of emulsion under dye test

Scarlet red dye mixed with cream. Take drop into cream-dye mixture and placed on microscopic slide and covered with cover slip. Eximined under microscope which type of emulsion.

2.4.4. Irritancy test

An area (1cm^2) on the dorsal left hand surface was marked. The cream was applied on this marked surface. Then irritancy, erythema, edema were checked for regular time interval up to 24 hrs and the time was noted and reported.

2.4.5. Spread ability

Formulation placed between two glass slides and 100gm weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness. Weight 50gm was added to the pan. The time in seconds require to separate the two slides was taken as measure of spreadability.

2.4.6. Viscosity measurement

Viscosity of cream was determined by using Brookfield rotational viscometer at 5,10,20,30 and50 rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

2.4.7. Microbial test

Nutrient agar, nutrient broth media was used in microbial growth study. In this method the blank and sample petriplates were used and cream sample were aseptically transferred on to the sample plates in a cross pattern, the microbial growth was observed. Antimicrobial activity was assessed against staphylococcus aureus, E. coli strain after 24hrs, 48hrs and 72hrs, found to exhibit significant antimicrobial activity.

2.4.8. Stability study

For *in vitro* evaluation of herbal antibacterial, antifungal cream was placed at different temperatures i.e., at 8, 25 and 40˚C and 40˚C at 75% RH (relative humidity) in stability chambers for 28 days. Any change in color, liquefaction, phase separation, conductivity and pH was observe and record.

3. RESULT AND DISCUSSION

3.1. Physical evaluation

Colour, Odour and appearance of the cream is checked and is as mentioned below in Table 3.

3.2. pH determination

The pH of cream formulations was determined by using digital pH meter. 2.5gm of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH was carried out. The pH values are represented in Table 3.

3.3. Type of emulsion

Scarlet red dye mixed with cream. Take drop into cream-dye mixture and placed on microscopic slide and covered with cover slip. Eximined under microscope which type of emulsion. and the result was noted and reported in table 3.

3.4. Irritancy

An area (1cm^2) on the dorsal left hand surface was marked. The cream was applied on this marked surface. Then irritancy, erythema, edema were checked for regular time interval up to 24hrs and the result was noted and reported in table 3.

3.5. Spreadability

Formulation placed between two glass slides and 100gm weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness. Weight 50 gm was

added to the pan. The time in seconds require to separate the two slides was taken as measure of spreadability (Table 3.).

Table: 3. Characterization of herbal antibacterial, antifungal cream

Sr. No	Test	Result
1	Physical evaluation	
	Color	Yellowish white
	Odour	Characteristics
	Appearance	Semisolid with smooth texture
2	pH determination	5.6 (acidic)
3	Type of emulsion under dye test	Oil – Water type (O/W)
4	Irritancy test	No irritation on skin
5	Spreadability test	17.21 gm/cm/sec.

Microbial test

Nutrient agar media was used in microbial growth study. In this method the blank and sample petriplates were used and gel sample were aseptically transferred on to the sample plates in a cross pattern, the microbial growth was observed (Table 4. And Fig.3).

Fig: 1. Spreadability test

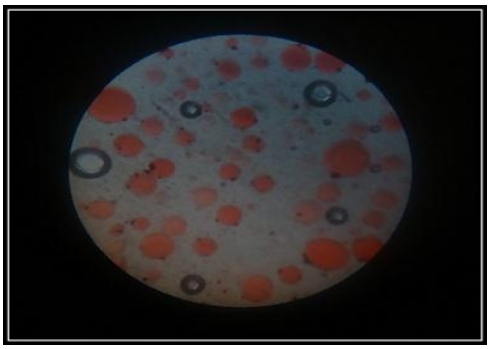
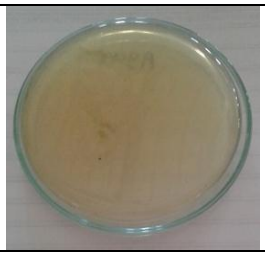
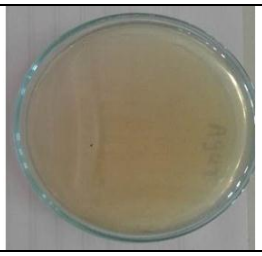
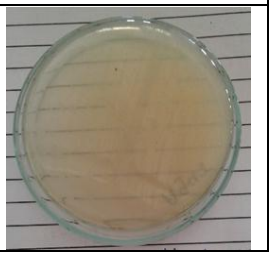


Fig: 2. Type of emulsion under dye test

Table: 4. Microbial test

Sr.No.	Test strain	24 hrs	48 hrs	72 hrs
1	Nutrient agar media + Cream	No growth	No growth	No growth
2	Nutrient agar media + Staphylococcus aureas + cream	No growth	No growth	No growth
3	Nutrient agar media + E. coli + cream	No growth	No growth	No growth

	24 hrs	48 hrs	72 hrs
Nutrient agar media + Cream			

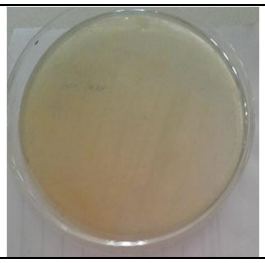
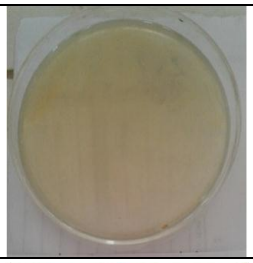
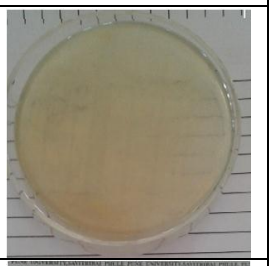
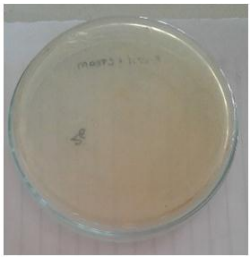
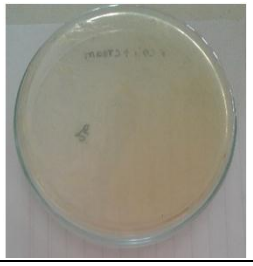
Nutrient agar media + Staphylococcus aureas + cream			
Nutrient agar media + E. coli + crea			

Figure: 1. Images of Microbial Test

1.7. Stability study

For *in vitro* evaluation of herbal antibacterial, antifungal cream was placed at different temperatures i.e., at 8, 25 and 40˚C and 40˚C at 75% RH (relative humidity) in stability chambers for 28 days (Table 5). No change in color, liquefaction and phase separation was observed; furthermore, the electrical conductivity test was also negative for each sample of creams.andthe pH of herbal antibacterial, antifungal cream at different storage conditions of temperature and humidity were in the range of normal skin pH. The pH of freshly prepared formulation was 5.6, respectively. The samples of formulation showed gradual decrease in pH from after 3rd day to 28thday study period. At the end of study (on 28th day) pH of base samples decreased, respectively,

Table: 5. Stability characteristics of herbal antibacterial, antifungal cream

		Fresh	After 24 h	After 3 days	After 7 days	After 14 days	After 21 days	After 28 days
Color	A	WY	WY	WY	WY	WY	WY	WY
	B	WY	WY	WY	WY	WY	WY	WY
	C	WY	WY	WY	WY	WY	WY	WY
	D	WY	WY	WY	WY	WY	WY	WY
Liquefaction	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	+ve
	D	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Phase separation	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Conductivity	A	N	N	N	N	N	N	N
	B	N	N	N	N	N	N	N
	C	N	N	N	N	N	N	N
	D	N	N	N	N	N	N	N
PH	A	5.6	5.6	5.6	5.49	5.36	5.10	5.01
	B	5.6	5.6	5.57	5.54	5.27	5.13	5.02
	C	5.6	5.6	5.45	5.32	5.15	5.07	4.94
	D	5.6	5.6	5.45	5.36	5.12	5.10	4.96

2. CONCLUSSION

The purpose of the study was to prepare antibacterial, antifungal cream by using easily available herbal drugs. The study proved that the cream prepared from Gandhak, Jatyadi oil, Karanji oil and Hing reduces skin infections. Further stability and clinical studies are needed to validate therapeutic potential of this herbal antibacterial, antifungal cream against all skin disorders.

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