WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 8, 884-901. Review Article ISSN 2277–7105

TO PREPARE AND PERFORM QUALITY CONTROL TEST FOR ANTIFUNGAL AND WOUND HEALING CREAM FROM NATURAL HEARB

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Article Received on
02 March 2024,

Revised on 22 March 2024,
Accepted on 12 April 2024

DOI: 10.20959/wjpr20248-31928

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ABSTRACT

Herbal antifungal cream use for fungal infection is the disease cause by fungus MYCOSES. Most of peoples survive from fungal infection or disease due to unhygienic lifestyle. Herbal antifungal cream is totally make from herbal ingredients, inert and non-irritable material. The main purpose of our cream is minimizing the usage of heavy antibiotics which directly apply on the skin of patient body. Which give side effect or adverse effect on the skin like locally irritation with ability to create wound with burning sensation in some cases lose of hair in site of administration. Which overcome by using herbal antifungal cream. Which is totally make from herbal ingredients, inert and non-irritable material. For example – Alum as main active therapeutic agent, peppermint oil as second active agent as well as cooling agent and soothing agent, beeswax as base material, and other herbal additives.

1. INTRODUCTION

Fungal infection is the disease cause by fungus **MYCOSES.** Fungus is the eukaryotic microorganism which reproduces by sexually or asexually which is beneficial or harmful to body. The fungus mycoses commonly occur in environmental and physiological condition which is main cause of fungal infection. Most of peoples survive from fungal infection or disease mostly are those people which residence or leaving in rural and developing area due to unhygienic lifestyle. The fungal infection occur in various body part most likely skin, mouth, intestines, and vagina due to variation in the balance of microorganism.

Antifungal agents are those chemical entities which are used in the treatment of fungal infections or used to inhibit the growth and development of various fungal infecting agents (fungicides). Most of antifungal agent present in the market in the form of strong antibiotics. Consumption of strong antibiotics kills the fungicides as well as good bacteria of the body which leads to other disease conditions. Various antifungal dosage are available in the market in the form of creams, ointments, liniment, lotion, tablet, capsule, solution etc. most effective way of treatment of fungal infection is local, topical, because they directly act on site of action.

For the treatment of fungal infection we prepare the antifungal cream. Which is directly act or work on site of action. The main mechanism of action of cream is to penetrate the skin and inhibit the growth and development of fungal infection at the level of subcutaneous layer. The main ingredients used in the our preparation is ALUM. The alum is the well-known and commonly used ingredient which is used for different purposes like water cleaning agent, astringent, antiseptic agent, antimicrobial agents, antibacterial agents, etc.

The main purpose of our cream is minimizing the usage of heavy antibiotics which directly apply on the skin of patient body. Which give side effect or adverse effect on the skin like locally irritation with ability to create wound with burning sensation in some cases lose of hair in site of administration. Which overcome by using herbal antifungal cream. Which is totally make from herbal ingredients, inert and non-irritable material. For example – Alum as main active therapeutic agent, peppermint oil as second active agent as well as cooling agent and soothing agent, beeswax as base material, and other herbal additives.

2. LITERATURE REVIEW

1. **Arati D. Powar**, *et al. (*2022) A Review – Polyherbal Antifungal Cream, World Journal Of Pharmaceutical Research: 906-911 And 916-918 For antifungal activity of antibiotics and its different side effects and also for the detail information about growth and development of fungal infection and primary prevention and treatment of fungal disease and different marketed preparation used for the treatment of fungal infection.
2. **Puja Saha,** *et al*. (2021) Formulation and Evaluation of herbal cream for skin care using Extract of Krishna Tulsi as Active Ingredient with Antimicrobial Activity. Used for information about the preparation of herbal cream with herbal ingredient for the external use. With their evaluation tests.

3. **Chauhan Lalita,** *et al*. (2020) Cream: A Review on Classification, Preparation Method and Evaluation and its Applications. Use for Detail information about cream including classification, composition, evaluation test and mechanism of topical absorption through skin.
4. **Amol Pimpale,** et *al*. (2018) Formulation and Evaluation of Antibacterial, Antifungal Cream of Garlic Oil, International Journal of Trend in Scientific Research and Development (IJTSRD) :849-851he prepared topical creams were inspected visually for their color, homogeneity, consistency, spreadability and phase separation.
5. **Akhtar Ali,** *et al.* (2017) Shibb-E-Yamani (Alum) A Unique Drug and Its Utilization In Unani Medicine A Physicochemical and Pharmacological Review Alum (Phitkari) used as a antiseptic, anti-itching, to treat burned cells or tissues, purification property, anti sweating agent and ulcer healing properties. and other information like physical properties, chemical property.
6. **Mariyam Roquiya,** *et al*. (2015) A Review On Medicinal Aspect Of Alum Is Unani Medicine And Scientific Studies. used for the information about alum including source and therapeutic action.
7. **OSUALA FI,** *et al*. (2009) Evaluation of efficacy and safety of potassium aluminiumtetraoxosulphate (ALUM) in the treatment of tuberculosis. European Journal of Biological Science. Use for alum antimicrobial and anti tuberculosis activity.

3. AIM AND OBJECTIVE

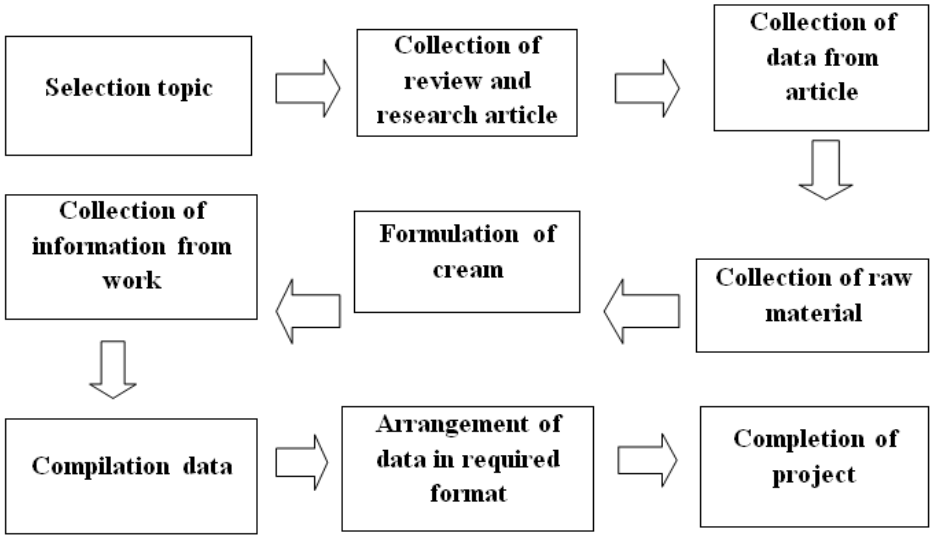
3.1: AIM

To prepare and perform quality control test for antifungal & wound healing cream from natural herb.

3.2: OBJECTIVES

1. It targets the site of fungal infection.
2. It reduces the risk of systemic circulation and lowering the risk of side effect.
3. It enhances the efficacy of treatment.
4. High patent compliance.
5. Economic and inexpensive

4. PLAN OF WORK



5. PHARMACOGNOSTIC ACCOUNT OF CRUDE DRUG

5.1: CRUDE DRUG: - ALUM

SYNONYMS

English= Alum, Marathi= Turti, Hindi/Urdu= Phitkari, etc.

BILOGICAL SOURCE

The ALUM is mineral origin crude drug naturally present in the Earth in the form of shells which is converted into salt form.

Fig. 1: Alum powder.

GEOGRAPHICAL SOURCE

It is primarily present with peroxide of iron in shilajeet or in alum earth of Nepal, Rajputana, Panjab, Cutch States, Bihar in the form of Shells. After the processing it will converted into salt and active form.

PHYSICAL PROPERTIES

Colour:- Colourless and transparent

Odour:- Odourless

pH:- 3 to 3.5 pH

Size & Shape:- Granular powder, Crystalline Shape

Taste:- Sweetish Astringent Taste

Solubility:- Soluble in Water and Organic solvent

CHEMICAL PROPERTIES

It is potassium aluminium sulphate which is organic salt

Molecular Formula:- K2SO4.Al2(SO4)3.24H2O

USES

- In rural area used as a water purifier.
- It is used as Haemostatic and wound healing agent.
- It is used as a expectorant.
- Also used as analgesic.

5.2: CRUDE DRUG:- PIPPERMINT OIL

SYNONYMS

Mentha oil, Aasmantara, OliumMenthapiperita, etc.

BIOLOGOCAL SOURCE

It is derived from steam distillation of fresh flowering tops of plant Mentha Piperita Linn.

FAMILY:-Labiatae

Fig. 2: Peppermint oil.

GEOGRAPHICAL SOURCE

It is cultivated and collected at global level, it is widely cultivated and collected in Japan, England, France, Italy, USA, Bulgaria and India. In India cultivated near Tarai region of Uttar Pradesh and Jammu.

PHYSICAL PROPERTIES

Colour:- Colourless to Yellow.

Odour:- Characteristic and Plasant.

Taste:- Pungent it followed by cooling sensation.

Solubility:- It is soluble in 70% Alcohol, ether and chloroform and insoluble in Water.

CHEMICAL COMPOSITION

It contain up to 70 percent menthol. And menthone, menthofuron, jasmine, menthylisovalerate and other terpenes including pinene, camphene, limonene, etc.

Uses

- It is used as flavouring agent, antiseptic, carminative, stimulant.
- It is also used in the preparation of creams like shaving cream, toothpaste, and different preparation dosage form.
- It is also used as pharmaceutical aid.
- It is also used for antitussive agent.

Source for Alum

Firstly we buy the alum from grocery shop which is in the state of rock form cause we convert into salt form which is easier for the our formulation.

Source of Oil and Additives

Peppermint oil and additives taken from pharmaceutics laboratory present in our college Shree Sant Gajanan Maharaj Collage of Pharmacy Buldana.

6. BENEFITS OF ALUM AND PEPPERMINT OIL

BENEFITS OF ALUM POWDER

- Aluminium Potassium Sulphate (Alum) act by astringent action of protein precipitation at the outer layer of cell, that result to decrease capillary permeability.

- Alum have sweat pores blocking activity which is very useful for antifungal action when it block the pores there are no secreation of sweat which result to decrease the moisture of site of action, which result to inhibition of growth of fungal infection and alum also have antifungal activity which also reduces the growth and development of fungal infection so alum reduces the fungal infection by double action.
- It also prevents the itching and alopecia.
- The alum is used in the treatment of numerous diseases because it is astringent, analgesic, hemostatic antipyretic, irritant property.
- In Nigeria Alum used as a Expectorant and antitussive agent.
- Alum also show emetic activity which is used as homemade remedy if any person swallows any toxic agent through oral route when alum solution was given to the person which induce vomiting and lower the risk of high toxicity.
- Different clinical studies show represents the antibacterial, ant tubercular activity.
- Alum is also used for their larvicidal activity.
- Alum also wound healing property.
- Alum also removes abnormal growth of tissue when, alum powder spray on tissue it removes necrosed tissue from the wounds and improve the healing property.
- The alum powder dissolves in water which prevents skin cracks in winter. It has ability to dry the skin which results to lower the acne problem because oily skin has more chances to cause pimples and acne.
- It also act as a anti-obesity agent in a pre-clinical trials study a wistar rats will take by scientist and high fat diet with alum powder will be given to the one group of wistar rat and high fat diet without alum powder will be given to the second group for one month the study found the first group serum triglycerides (TG) and total cholesterol (TC), high density lipoproteins (HDL) levels decreases as well as weight and obesity also decreases.

7. MATERIAL AND METHOD OF PREPRATION

a. Preparation of extracts

Sr. No.	Ingredients	Chemical Constituents	Process of extraction/Collection or raw material	Image
1.	Alum	Potassium Aluminium Sulphate	It was purchased from buldana market.	

2.	Pippermint Oil	menthol. and menthone, menthofuron, jasmine, menthylisovalerate and other terpenes including pinene, camphene, limonene,etc .	It is issue from our college laboratory SSGMCOP Buldana	
3.	Bees Wax	Hydrocarbons (12%-16%) with predominant chain length of C27-C33, mainly heptacosane, hentriacontane, pentacosane and tricosane.	It is issue from our college laboratory SSGMCOP Buldana	

b. Method for the preparation of Antifungal Cream

1. Add the required quantity of Borax in sufficient amount of water and prepare a solution by heating on water bath.
2. The above solution, add the required quantity of peppermint oil. [Solution 1]
3. Weight accurately Alum Powder and add into Chloroform.
4. The above solution of Alum Powder is added into beeswax contained in china dish, melt to prepare the solution. [Solution 2]
5. Add [Solution 1] drop wise into [Solution 2] when both phases get mixed properly, add methyl paraben as preservative and add rose oil as a perfume for good fragrance.
6. Fill the formulated cream into the container and labeled it properly.
7. The formulated Antifungal Cream was kept a side for about an hour in cool and dry place indirect to sunlight till it sets completely and this cream was used after 48 hours after keeping at room temperature for stability and analytical testing and also perform the evaluation study.

Table 1: Composition of antifungal cream.

Sr.No	Ingredients	Quantity Taken	Role
1	Alum	10 gm	Antifungal, Antibacterial
2	Peppermint oil	5 ml	Antifungal Antioxidant
3	Bees Wax	3.2 gm	Humectant
4	Chloroform	5 ml	Organic Solvent
5	Borax	0.16 gm	Emulsifier
6	Methyl paraben	0.02 gm	Preservative
7	Water	q.s	Vehicle
8	Perfume (Rose Water)	q.s	Fragrance

Table 2: Formulation Table.

Sr.No	Ingredients	F1HC	F2HC
1	Alum	02gm	04gm
2	Peppermint oil	5 ml	4 ml
3	Bees Wax	3.2 gm	3.1 gm
4	Chloroform	5 ml	4.9 ml
5	Borax	0.16 gm	0.14 gm
6	Methyl paraben	0.02 gm	0.03 gm
7	Water	q.s	q.s
8	Perfume (Rose Water)	q.s	q.s

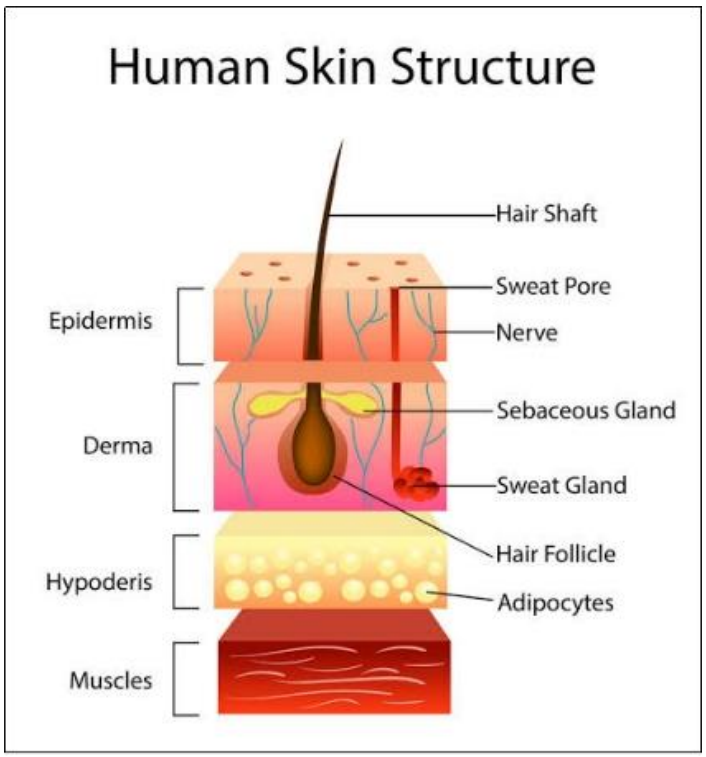


Fig. 3: Human skin structure.

Physiology of normal skin

The skin is composed of three layers

- Epidermis (50–100 μm)
- Dermis (1–2 mm)
- Hypodermis (1–2 mm)

The barrier to percutaneous absorption lies within the stratum corneum, the most superficial layer of the epidermis. The function of the stratum corneum is to reduce water loss, provide protection against abrasive action and microorganisms, and generally act as a permeability barrier to the environment.

The stratum corneum is a 10–20 μm thick, multilayer stratum of flat, polyhedral-shaped, 2 to 3 μm thick, non-nucleated cells named corneocytes. Corneocytes are composed primarily of insoluble bundled keratins surrounded by a cell envelope stabilized by cross-linked proteins and covalently bound lipids. Corneodesmosomes are membrane junctions interconnecting corneocytes and contributing to stratum corneum cohesion. The intercellular space between corneocytes is composed of lipids primarily generated from the exocytosis of lamellar bodies during the terminal differentiation of the keratinocytes. These lipids are required for a competent skin barrier function.

The epidermis is composed of 10–20 layers of cells. This pluristratified epithelium also contains melanocytes involved in skin pigmentation, and Langerhans' cells, involved in antigen presentation and immune responses. The epidermis, as for any epithelium, obtains its nutrients from the dermal vascular network The epidermis is a dynamic structure and the renewal of the stratum corneum is controlled by complex regulatory systems of cellular differentiation. Current knowledge of the function of the stratum corneum has come from studies of the epidermal responses to perturbation of the skin barrier such as:

(i) Extraction of skin lipids with apolar solvents

(ii) Physical stripping of the stratum corneum using adhesive tape

(iii) Chemically-induced irritation.

Fungi

"Fungi are a kingdom of usually multicellular eukaryotic organism that are heterotrops and have important role in nutrient cycling in an ecosystem".

Characteristics of fungi

Some fungi are single-celled, while others are multicellular. single-celled fungi are called yeast. some fungi alternate between single celled yeast and multicellular forms depending on what stage of the life cycle they are in. Fungi cells have a nucleus and organelles, like plant and animal cellsdo. The cell walls of fungi contain chitin, which is hard substance also found in the exoskeletons of insects and arthropods such as crustaceans. They do not contain cellulose, which commonly makes up plant cell walls. Multicellular fungi have many hyphae, which are braching filaments. Hyphae have tubular shape and are split into cell-like compartments by walls that are known as septa. These cells can have more than one nucleus, and nuclei and other organelles can move in between them. A fungus network of hyphae is calles a mycelium.

Types of Fungi

- Chytridiomycota
- Zygomycota
- Glomeromycota
- Ascomycota

Fungal Infection

Definition: - An inflammatory condition caused by a fungus. mycosis. zymosis - (medicine) the development and spread of an infectious disease (especially one caused by a fungus) blastomycosis - any of several infections of the skin or mucous membrane caused by Blastomycosis.

Fungal infections are common throught much of natural world. In humans, fungal infection occur when an invading fungas takes over an area of the body and is too much for the immune system to handle. Fungi can live in the air, soil, water and plants. There are also some fungi that live naturally in the human body.

Type of fungal infection

1. Superficial: Affect skin – mucous membrane. e.g. tineaversicolordermatophytes: Fungi that affect keratin layer of skin, hair, nail. e.g. tineapedis, ring worm infection Candidiasis: Yeast- like, oral thrush, vulvo-vaginitis, nail infections.

2. Deep infections: Affect internal organs as lung, heart, brain leading to pneumonia, endocarditis, meningitis.

Overview of Fungal Skin Infections

Fungi usually make their homes in moist areas of the body where skin surfaces meet: between the toes, in the genital area, and under the breasts. Common fungal skin infections are caused by yeasts (such as Candida or Malassezia furfur) or dermatophytes, such as Epidermophyton, Microsporum, and Trichophyton. Many such fungi live only in the topmost layer of the epidermis (stratum corneum) and do not penetrate deeper. Obese people are more likely to get these infections because they have excessive skinfolds, especially if the skin within a skinfold becomes irritated and broken down (intertrigo). People with diabetes tend to be more susceptible to fungal infections as well. Strangely, fungal infections on one part of the body can cause rashes on other parts of the body that are not infected. For example, a fungal infection on the foot may cause an itchy, bumpy rash on the fingers.

These eruptions (dermatophytids, or identity or id reactions) are allergic reactions to the fungus. They do not result from touching the infected area.

Fig. 4: Fungal infection.

Symptoms

- Skin changes, including red and possibly cracking or peeling skin.
- Itching.
- Histaminergic response.

Causes of fungal skin infection: Imbalance of bacteria is due to following reasons:

- Due to use of antibiotics
- Hormone imbalance
- Poor eating habbits
- Unhygienic lifestyle

8. BENEFITS OF HERBAL ANTI FUNGAL CREAM

Cream

Definition- "Cream is semisolid preparation of a medication for topical use (on the skin) that contains a water base. Essentially, it is a preapation of oil (often lanolin or petrolatum) in water. "An ointment is preparation for topical use".

Antifungal cream: "Cream which is used for destroying fungi or inhibiting their growth"

Advantages of Cream

It is totally made up of herbal ingreidiants which lower the chances of any side effect and adverse effect compare to commercial and other zole derivative antifungal cream.

It is economic compare to other antibic antifungal cream because the antibiotics purchasing cost is very high cuase the antibiotic creams are expensive to formulate if manufacturing cost is high as well as retail price also high in the case of herbal cream the ingreidiant price also cheap so manufacturing cost also economic which directly effect on MRP of product.

It lower the risk of candidiasis.

Alum and pipermint oil antifungal cream also used for wound healing purpose.

- Able to calm inflammation.
- Promote skin tone
- Keep wrinkles and acne away.
- Increase cell metabolism and blood circulation
- Easily water washable. Easy to wipe away

Disadvantages of Cream

- Stability is not as good as ointment
- They are less hygroscopic than other semi-solid preparation, so risk of contamination is high than other.
- Less viscous than other semi-solid preparation

9. Quality Control test of Antifungal & Wound healing Cream

1. Physical Properties: The Cream was observed for colour, odour and appearance.

Table: Physical Properties.

Sr. No	Properties	F1C	F2C
1	Colour	White	White
2	Odour	Characteristic	Characteristic
3	Appearance	Semi-Solid	Semi-Solid
4	Texture	Smooth	Smooth

2. Stability studies- Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 30 ± 2°C/ 65 ± 5% RH and 40 ± 2°C / 75 ± 5%

RH for a month. At the end of studies, samples were analyzed for the physical properties and viscosity.

Table: Stability Test.

Test	After one month
Physical appearance	Semi-solid
Texture	Smooth and creamy
Color	White
Odour	Characteristic
pH value	6.1 to 6.6
Thermal stability	Stable
Degradation of product	No

3. Determination of pH: 0. 5 ± 0.01g of the Cream was weighed accurately in a 10ml test tube. 4.5ml of water was added & dispersed the Cream in it. The pH of the suspension was determined at 27^{o}C using the pH meter.

Table: PH Test.

Sr. No.	Formulation	pH
1	F1C	6.2
2	F2C	6.3

4. Spread ability Test

Take two slides add small amount (1gm) of cream over both slides, then spread the cream over the slide. Then observe the slide time in second.

The spreadability was then calculated from the following formula:

$$\textbf{Spread ability} = m \times l / t$$

Where, m = weight tied to the upper slide (1g),

l =length of glass slide (5cm),

t =time taken in seconds

Table: Spreadibility Test.

Sr.No	Formulation	Time (sec)	Spreadability (gcm/sec)
1.	F1C	7	3
2	F2C	8	2.17

5. Test for microbial growth in formulated creams: The formulated creams were inoculated on the plates of agar media by streak plate method and a control was prepared by omitting the cream. The plates were placed in to the incubator and are incubated at 37^{o}C for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

Fig. 5: M+icrobial growth.

6. Wash ability

Formulation was applied on the skin and then ease extends of washing with water was checked. Wash ability test was carried out by applying a small amount of cream on the hand and then washing it with help of tap water. All three formulations were easily washable.

Table: Wash ability test.

Sr.No	Formulation	Wash Ability
1.	F1C	Easily Washable
2	F2C	Easily Washable

7. Phase separation

Prepared cream is kept in tightly closed container at room temperature away from sunlight and observed for 24 hours for phase. Phase separation The prepared cream was transferred in a suitable wide mouth container. Set aside for storage, the oil phase and aqueous phase separation were visualizing after 24hr.

Sr.No	Formulation	Phase Separation
1.	F1C	No Phase Separation
2	F2C	No Phase Separation

8. Non-irritancy test

Herbal cream formulation was evaluated for the non-irritancy test. Observation of the sites was done for 24 h.Mark the area (1 cm sq.) on the left hand dorsal surface. Then the cream was applied to the area and the time noted. After interval up to 24 hr. it is checked for irritant effect, erythema and edema if any than reported.

Patch Test – About 1-3gm of material to be tested was placed on a piece of fabric or funnel and applied to the sensitive part of the skin e.g. skin behind ears. The cosmetic to be tested

was applied to an area of 1sq.m. of the skin. Control patches were also applied. The site of patch is inspected after 24 hrs

Result- No any inflammation or irritation to the skin.

Table: Non-irritancy test.

Sr.No	Formulation	Irritancy effect	Erythema	Edema
1.	F1C	Nil	Nil	Nil
2	F2C	Nil	Nil	Nil

10. Result

Sr.No	Evolution	Result
1	Physical Evaluation	
	Color	White
	Odour	Characteristic
	State	Semisolid
	Consistency	Smooth
	pH	6.2
2	Spredability	3g.cm /Sec
3	Wash Ability	Easily Washable
4	Non-irritancy Test	Non-irritant
5	Viscosity	-
6	Phase Separation	No phase Separation

DISCUSSION

The present research formulation mainly focused on preparation and quality contol test of herbal antifungal and wound healing cream by using herbal ingredient. This cream formulation is o/w emulsion; hence this formulation was easily washed by normal water after application and has good spread ability. Viscosity and pH of this cream found in standard range. The herbal antifungal cream was non-greasy and easily removable. The formulation was inert and safe for skin. The phase separation of formulated cream is not occur.

11. CONCLUSION

The mineral origin crude drug alum (Potassium alluminium sulphate) and Peppermint oil have antifungal, antiseptic, astringent, wound healing, larvicidal, antitubercular and analgesic activity. Formulation of antifungal and wound healing cream was done by fusion method and evaluated by various quality control test like physical property pH, Spread ability, wash ability, irritancy test, viscosity test and phase separation test gives standard result. The present experimental studies showed that it is possible to develop antifungal and wound healing cream by herbal ingredients which give potent result.

ACKNOWLEDGEMENT

The author are thankful to our guide Prof. Shaikh S.J. Sir and Shri Sant Gajanan Maharaj College of Pharmacy, Buldana, for providing the facilities required for carrying out this research work.

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