



FORMULATION AND EVALUATION OF POLYHERBAL ANTIFUNGAL CREAM USING GUAVA LEAVES EXTRACT AND OCIMUM SANCTUM OIL

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Abstract:

Herbs are very useful to treat skin related diseases. Fungal infection is one of the major health concern in today's world. *Psidium Guajava* and *Ocimum sanctum* leaves are well known by its antifungal activity and its uses in skin diseases. The use of polyherbal antifungal creams incorporating *Psidium Guajava* leaves contain flavonoids and *Ocimum sanctum* leaves contain camphor, eugenol and eucalyptol have gained attention due to their potential antifungal properties. These plant extracts were selected for their known antifungal properties and traditional use in treating fungal infections. The cream was carefully formulated to optimize the concentration of the extracts while ensuring stability and sensory attributes. Physicochemical tests were conducted to evaluate the cream's properties, and in vitro and in vivo studies were performed to assess its antifungal activity. Our findings demonstrate the potential of this polyherbal cream as an effective and safe treatment option for fungal infections.

Keywords: Polyherbal cream, *Psidium guajava*, *Ocimum Sanctum*.

1. Introduction

1.1 Skin:

Nowadays, these herbal skincare creams are becoming more popular and prevalent among people due to their mindset and concerns about synthetic or chemical substances that may lead to adverse effects.

Skin is the largest organ of the human body which has been outlined to have more than 20 vital physiological functions. Skin acts as a protective barrier against pressure and trauma. It also acts as a barrier for external environments including pollution, sunlight, radiation, harmful microbes, and chemical. Skincare products are medicinal formulations skincare prepared to be used on external parts of the human body to produce therapeutic topical effects and shield the deteriorated skin.

Herbal skin care creams have been widely used by many generations for centuries for the purpose of skincare.

The main factors that lead to the usage of herbal ingredients in creams are their outside antioxidant, antimicrobial, and tyrosine inhibition properties. The studies on the potential pharmacologically active herbs for skincare creams allow us to understand their importance. [3]

Physiology of the normal skin

The skin is composed of three layers:

- Epidermis
- Dermis
- Hypodermis

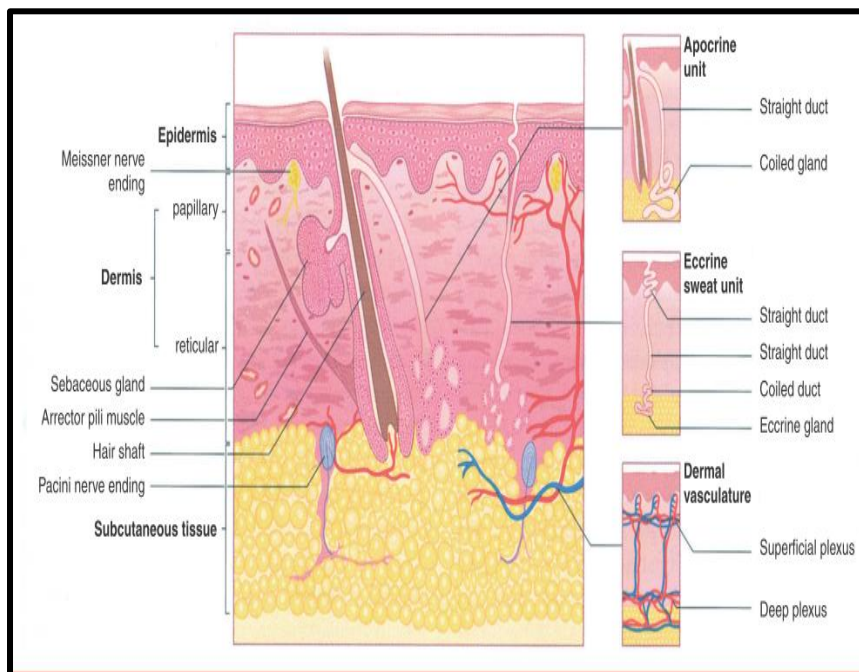


Fig. 1 Skin Structure

1.2 Cosmetic:

Cosmetic is defined under section of the Drugs and Cosmetics Act, 1940 as, any article intended to be rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to, the human body or any part for cleansing, beautifying, promoting attractiveness or altering the appearance, and includes any article intended for use as a component of cosmetic. [3]

1.3 Herbal cosmetics:

Herbal cosmetics are defined as beauty products containing herbal ingredients that have desired physiological activities such as healing, smoothing appearance, enhancing, and conditioning qualities. The use of herbs in cosmeceutical production has greatly risen in recent years in the personal care system and there is a high demand for herbal cosmetics. Cosmetics are substances that are applied to the human body with the purpose of cleansing, beautifying, increasing attractiveness, and changing appearance without harming the body's structure or functions. [4]

1.4 Topical Route:

Topical route is most suitable route for skin infections. The development of topical drug delivery systems designed to have systemic effects appears to be beneficial for a number of drugs on account of the several advantages over conventional routes of drug administration. ^[6]

1.5 Cream:

Cream is defined as semisolid emulsions which are (o/w) or (w/o) type and these semisolid emulsions are intended for external application. Cream is classified as oil in water and water in oil emulsion. It is applied on outer part or superficial part of the skin and its main ability is to remain for a longer period of time at the site of application. The function of a skin cream is to protect the skin against different environmental condition, weather and gives soothing effect to the skin. There are different types of creams like cleansing, cold, foundation, vanishing, night, massage, hand and body creams. ^[8]

1.6 Herbal Cream:

Herbal Cosmetics are the cosmetics which are prepared using plant products having cosmetic actions. In cosmetics, both natural and Phytoingredients are used. Natural products include oils, extracts, secretions etc. Cream is defined as semisolid emulsions which are oil in water (o/w) or water in oil (w/o) type and these semisolid emulsions are intended for external application.

1.7 Classification of creams:

All the skin creams can be classified on different basis:

1. According to function, e.g. foundation, massage ,cleasing etc.
2. According to characteristics properties, e.g. cold creams, vanishing creams, etc.
3. According to the nature or type of emulsion.

Types of creams according to function, characteristic properties and type of emulsion:

1. Make-up cream (o/w emulsion): a) Vanishing creams. b) Foundation creams.
2. Cleansing cream, cleansing milk, cleansing lotion (w/o emulsion).
3. Winter cream (w/o emulsion): a) Cold cream or moisturizing creams.
4. All-purpose cream and general creams.
5. Skin protective cream.
6. Hand and body creams.
7. Night cream and massage creams. ^[9]

1.8 Types of Fungi:

Chytridiomycota: Chytrids, the organisms found in Chytridiomycota, are usually asexual, and produce spores that no around using flagella, small tail like appendages. It can cause fungal infection in frogs by burrowing under their skin.

Zygomycota: These are mainly terrestrial. They cause problem by growing on human few sources.

Ex:Rhizopus stolonifer a bread mold.

Glomeromycota: They are found in soil. The fungi obtain sugar from plant and in return, dissolves, minerals in the soil to provide the plant with nutrients. This fungi also reproduce asexually.

Ascomycota: These are the pathogens of plant and animals, including humans in which they are responsible for infection like Athlete's Foot, Ringworm, and ergotism, which causes vomiting, convulsions, hallucination and sometimes even death. Dissolves, minerals in the soil to provide the plant with nutrients. This fungi also reproduce asexually.

1.9 Fungal Infection:

Definition: -

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

Fungal infections are common thought much of natural world. In humans, fungal infection occur when an invading fungus 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.

1.9.1 Types of fungal infection:

1. Superficial: Affect skin – mucous membrane. E.g. tinea versicolor dermatophytes: Fungi that affect keratin layer of skin, hair, nail. E.g. tinea Pedi's, ring worm infection Candidiasis: Yeast- like, oral thrush, Volvo-vaginitis, nail infections.

2. Deep infections: Affect internal organs as: lungs, heart, brain leading to pneumonia, Endocarditis, meningitis.^[10]



Fig. 2 Fungal Infection

2. Plant profile :

2.1 Guava



Fig.No.3: Psidium Guajava

Scientific name: Psidium Guajava

Family: Myrtaceae

Chemical constituents:

- Uses:**
- 1) Help to cure cancerous cells and prevent skin aging.
 - 2) Used in treatment of diarrhea.
 - 3) Used in treatment of hypertension, diabetes and pain relief.
 - 4) Have Fungistatic and bacteriostatic.

2.2 Tulsi (Basil):



Fig.No.:4 Osimum Sanctum

- **Scientific name:** Ocimum Sanctum
- **Family:** Lamiaceae (tribe ocimeae)
- **Chemical constituents:**
- **Uses:** 1) It has Antimicrobial, antibacterial, antiviral, antifungal properties.
2) Used as anti-pyretic, anti-allergic.
3) Used as memory enhancement, anti-asthmatic, anti-thyroid etc.
4) Used as anthelmintic, mosquito repellent, anti-diarrheal agent.

2.3 Excipients profile:

1. Petroleum jelly
2. Cetyl alcohol
3. Glyceryl monosterate
4. Methylparaben
5. Propylparaben
6. Rose water

3. Materials and Method:

3.1 Materials

Psidium guajava leaves were collected from located farms at MP India and Ocimum Sanctum oil is also purchased from the near market and remaining chemicals from the laboratory.

3.2 Methods

A) Preparation of leaves powder

First we collected the leaves and washed them and dried the leaves under the shade for 30days and subjected for grinding using mixer. Prepared powder was passed from sieve number 120 and used for extraction.

B) Formulation preparation

The formulation components used were listed in the Table below. Oil in water emulsion of 20 and 60% of drugs were formulated. The emulsifier (glycerol monostearate) and other oil soluble components (petroleum jelly, Cetyl alcohol and tulsi oil) were dissolved in the oil phase (Part A) and heated up to 80° C. Extract and water soluble components (Methyl paraben, Propyl paraben and guava leaves extract) were dissolved in (Part B) and heated up to 80°C. After heating, the aqueous phase was added in portions to the oil phase with constant stirring until cream is formed, And cream was formulated having superb color i.e. pale green.

S. No.	Ingredients	F1	F2	F3	F4
1	Guvava extract	1.5ml	2.5ml	1.5ml	1ml
2	Tulsi oil	6ml	5ml	6ml	6ml
3	Petrolium jelly	6 gm	5gm	4gm	7.4gm
4	Cetyl alcohol	1.7gm	1.8gm	2.0gm	2.1gm
5	Glyceryl monostearate	1.3gm	1.7gm	1.6gm	1.0gm
6	Propylparaben	0.9gm	0.9gm	0.9gm	1.2gm
7	Methylparaben	1.2gm	1.2gm	1.2gm	1.0gm
8	Rose water	7.9 ml	7.4 ml	7.3ml	7.3 ml

4. EVALUATION OF CREAM:

4.1 Organoleptic Properties:

This refers to the sensory properties of the cream, such as its appearance, color, odor, and texture. Evaluating these properties can give an idea of the cream's overall quality and user experience.. The formulations are visually inspected for its clarity and presence of any foreign particles.

4.2 Determination of pH :

The pH meter was calibrated using standard buffer. About 0.50g of the cream was weighed and dissolved in 50ml of distilled water and the pH was measured by using a digital pH meter.

4.3 Safety:

There should not be any adverse reaction or side effects associated with the cream.

4.4 Homogeneity:

The formulations were tested for the homogeneity, visual inspection and touch.

4.5 Phase Separation :

The prepared cream was transferred in a suitable wide mouth container. Then stored the cream for visualization after 24 hours ,We will visualise the oil phase and aqueous phase separation .

4.6 Irritancy:

The cream was applied on the skin and leave for few minutes and the effect was studied.

4.7 Washability:

Formulation was applied on the skin and then ease extends of washing with water was checked. It should be easily washed and should not remain in our hands after washing.

4.8 Antifungal activity:

A) Sterilization of glassware and media:

In started the antifungal activity firstly sterilized the whole area in room by fumigation, pipettes, test tubes, loop, conical flask, measuring cylinder are washed and wrapped in brown paper and sterilized at 160°C for one hour. Agar media, Saline solution were aput in maintain at pH 5.4, autoclave at 121°C, 15 lbs. pressure for 20 minutes and use to culturing.

B) Preparation of media:

Sabouraud Dextrose Agar (SDA Solid Medium)

Composition	SDA (g/ml)
Dextrose	40
Peptone	10
Agar	15
Water	1L

Final pH	(25 C) 5.6+0.2
Direction	65 grams in 1L distilled water. Heat to boiling to dissolve the medium completely.
Sterile Condition	15 lbs pressure (121 C) for 15 min.

Table 04: Ingredients of media

C) Maintenance of fungal strains

The sample of strain of *Candida Albicans* fungus culture was maintained and available with the Department of Microbiology, Choithram hospital, Indore. Culture were maintain by streaking a loopful of microbes on agar slant and incubate at 25 °c (48 tur.) for all species slant were kept in refrigerator .this Procedure was repeated every week to maintain culture in pure state.

D. Cup plate method:

Sabouraud's Dextrose agar Plates was used to culture media for fungi. 20 ml ot sterile molten agar was poured into sterile Petripiates to form a layer them made four welis on the plate to allow a solidify and 0.1 ml of organism Spread on the plate, then test first wells, 1 M of Garlic extract, third well in the Liposomal suspension and last well into the liposomal gel throughout the experiment .After pouring the plated was refrigerator for 2 hour for diffusion. Then kept plates for 48 hours at 27°C the zone of J inhibition was recorded for fungis.

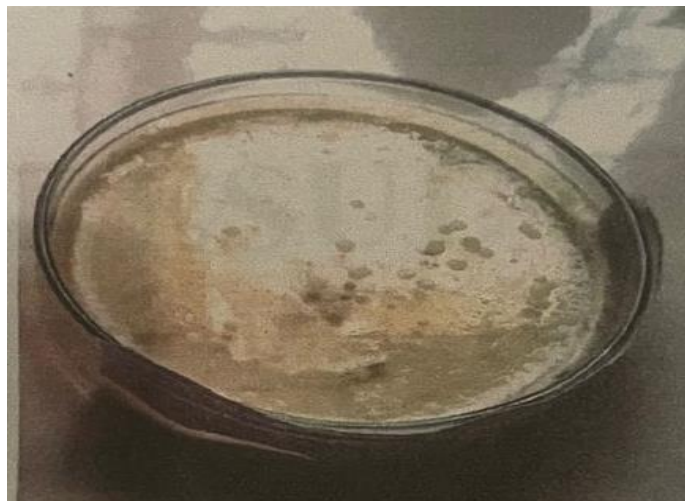


Fig.No.05: Zone of inhibiton: Agar Plate contain Fungus.

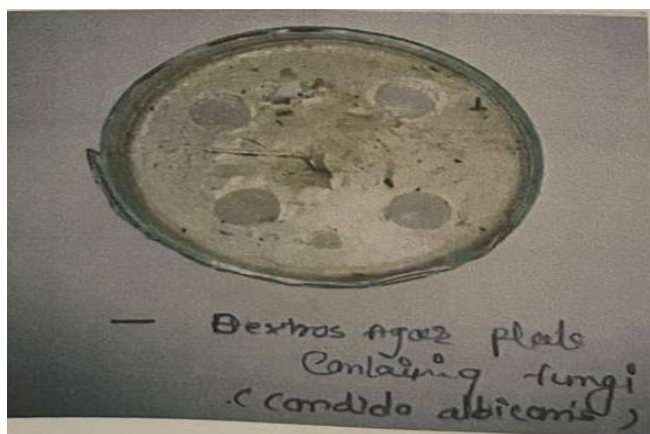


Fig.No.06: Zone of inhibition: Inhibit growth of Fungus.

5. RESULT AND DISCUSSION:

5.1 Organoleptic Evaluation:

S.NO.	Parameters	Observations			
		F1	F2	F3	F4
1	Color	Light green	Light green	Light green	Light green
2	Odor	Pleasant	Pleasant	Pleasant	Pleasant
3	Texture	Smooth	Smooth	Smooth	Smooth
4	State	Semisolid	Semisolid	Semisolid	Semisolid
5.	Appearance	Creamy	Creamy	Creamy	Creamy

Table no.04: Organoleptic evaluation result

5.2 Washability, pH and Irritancy results:

S.NO.	Parameters	Observations			
		F1	F2	F3	F4
1	washability	Easily washable	Easily washable	Easily washable	Easily washable
2	Irritancy	Nil	Nil	Nil	Nil
3	pH	5.5	5.7	5.6	5.5

Table no.05: Washability, pH and Irritancy results**5.3 Phase separation and Greasiness results:**

S.NO.	Parameters	Observations			
		F1	F2	F3	F4
1	Phase separation	No phase separation	No phase separation	No phase separation	No phase separation
2	Greasiness	Non-greasy	Non-greasy	Non-greasy	Non-greasy

Table no.06: Phase separation and Greasiness results**6. CONCLUSION**

In the present work, it was decided to extract and formulate polyherbal antifungal cream from Psidium Guajava and Ocimum Sactum leaves. The antifungal cream was o/w type emulsion, hence can be easily washed with plane water that is better customer compliance. The use of herbal cosmetics is increasing day by day because of less sideeffects . The extracts exhibited good anti-fungal activity. The prepared formulation has no evidence of phase separation and good consistency during the study period. Stability parameters like appearance, nature and odour of the formulations showed that there was no significant variation during the study period the formulation has PH range of approximately 5.5 which is not irritant to skin and suitable for skin, it conforms the compatibility of the formulations to skin secretions. The cream is expected to produce protection to the skin from fungal infections.

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