Formulation and Evaluation of Herbal Antifungal Cream

[1]Nickee Chandravanshi, [2]Dr. Atul Bisen, [3]Dr. Surendra Pardhi
[1]Research Scholar of Institute of Pharmaceutical Science & Research, Balaghat (MP)
[2]Asso. Professor of Institute of Pharmaceutical Science & Research, Balaghat (MP)
[3]Professor of Institute of Pharmaceutical Science & Research, Balaghat (MP)

ABSTRACT

Need: For skin infections, the topical approach is the best option. The last few decades have witnessed an increase in fungal infection. Because of the numerous advantages over traditional routes of drug administration, the development of topical drug delivery systems with systemic effects appears to be advantageous for a variety of medications.

Aim: The goal of the study was to formulation and evaluation of herbal antifungal cream of garlic oil and clove oil.

Method: To achieve this aim extraction process used. Extraction of herbs followed by steam distillation process and formulation were prepared through emulsification method.

Materials: This formulation belongs to a medicinal cream that has two antifungal active components i.e. Garlic Oil and Clove Oil. It also includes two types of primary and secondary emulsifiers, waxy materials, co-solvents, two preservatives, a buffering agent, a humectants, and water in the cream base. When the active components are combined, they provide a potent antifungal effect.

Assessment: Evaluation were done to assess the physicochemical characteristics of formulated cream, such as visual inspection, pH measurement, extrudability, expandability, skin irritant test, etc. The cream was further analyzed with the use of nutritional agar for antifungal activity.

Outcome: The formulated cream was found good in consistency and color; however the smell of the garlic was quite unpleasant, so the odor of the garlic was masked with peppermint oil, which also acting as an additional antifungal agent.

KEYWORDS: Fungal skin infections, Garlic oil, Clove oil, Peppermint oil, candidacies

INTRODUCTION

Fungal infections are evolving diseases in sanatorium institutions. Herbal medicines are also called as botanical medicine or phytomedicines used to alleviate human illness and for the maintenance of general health. These will be use for treating fungal skin infections, as well as other components that can help improve skin issues. Garlic oil and clove oil are the main ingredients used to treat fungal skin infections.

Herbal antifungal creams have gained prominence today due to several compelling reasons:

1. **Natural Antifungal Properties**: Many plants possess inherent antifungal compounds. For instance, essential oils and herbal extracts contain phenolic groups that serve as primary antimicrobial agents, effectively combating various fungal pathogens.
2. **Reduced Side Effects**: Herbal treatments are often associated with fewer adverse effects compared to synthetic antifungal agents. This makes them a preferred choice for individuals seeking treatments with minimal toxicity.
3. **Immunomodulatory Benefits**: Traditional medicinal systems, such as Traditional Chinese Medicine (TCM), utilize herbal preparations that not only exhibit antifungal activity but also modulate the immune system. This dual action can enhance the body's natural defenses against fungal infections.
4. **Addressing Antifungal Resistance**: The overuse of synthetic antifungal agents has led to the emergence of resistant fungal strains. Herbal

antifungal creams offer alternative mechanisms of action, which can be effective against resistant pathogens.

5. **Holistic Approach:** Herbal antifungal creams often incorporate multiple plant extracts, providing a broad spectrum of activity and addressing various aspects of skin health, including hydration and repair.

Mechanis of action:

Garlic (Allium sativum) exhibits antifungal properties primarily due to its bioactive compounds, notably allicin and ajoene. These compounds disrupt fungal growth through several mechanisms:

1. Allicin:

Membrane Disruption: Allicin interacts with the lipid components of fungal cell membranes, increasing permeability and leading to cell leakage. This disruption compromises the integrity of the fungal cell, resulting in cell death.

Enzyme Inhibition: Allicin inhibits key enzymes involved in fungal metabolism by reacting with thiol groups (-SH) in proteins. This inhibition impairs essential cellular processes, hindering fungal growth and replication.

2. Ajoene:

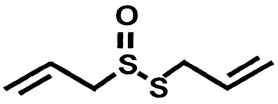
Growth Inhibition: Ajoene, a sulfur-containing compound derived from allicin, has been shown to inhibit the growth of fungi such as Candida albicans. While the precise mechanism is not fully understood, ajoene's antifungal activity contributes to the overall efficacy of garlic-based treatments.

In summary, the antifungal efficacy of garlic-based creams is attributed to the synergistic actions of allicin and ajoene, which compromise fungal cell integrity and inhibit vital metabolic processes, leading to the elimination of fungal pathogens.

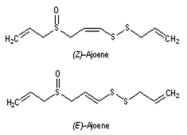
- A majority of the world's population still relies on herbal medicines to meet its health needs and are often used to provide first-line and basic health services.1, 2 The demand for plant based medicine is increasing in both developing and developed countries, due to their nontoxicity, less side effects and easy availability at affordable prizes.3, 4
- Creams are biphasic topical preparations usually applied to the skin and mucous membranes such as the rectum or vagina. They are semi solid emulsions containing oil-inwater (O/W) or water-in-oil (W/O) type preparations. Oilin-water (O/W) emulsions are used mostly because they contain water washable bases, whereas water-in oil (W/O) emulsions are emollient and cleansing agents.
- The use of cosmetics not only used for developing an attractive external appearance, but also achieving longevity of good health by reducing skin disorders. The herbs used in cosmetic preparation also have properties like antioxidant, anti-inflammatory, antiseptic, emollient, anti kerolytic activity and antibacterial activity. Cosmetic products are used to protect the skin from exogenous and endogenous harmful agents and enhance the beauty and attractiveness of skin.5
- Increase in 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]
- Fungal infection caused by Candida has become more prevalent than Escherichia coli and Pseudomonas.sp., Aspergillus sp. and other sp[2].
- 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)[3]

- Candida albicans is the most common species in the genus which has been implicated in Candidiasis. The infections range from superficial skin to systemic diseases.
- Treatment with herbs is an ancient method for curing diseases. Since the vedic time humans have used medicinal plant material to cure any disease or to give a satisfactory treatment against that disease. Plants are also known for treating the infectious and non infectious skin disorders. The antimicrobial effect of some plants is attributed to the number of phytoconstituents like flavonoid, tannins, triterpenes etc.[5]

The purpose of the current study is also based on the medicinal property of a plant i.e. Garlic (Allium sativum) and clove oil (Eugenia caryophyllus) Garlic oil shows a wide range antimicrobial activity. Alliin is the main chemical constituent in garlic oil which shows antimicrobial activity. This oil consists of sulfur containing six compounds such as i. allicin, ii. alliin, iii. ajoene, iv. diallyl disulfide, v. dithiin and vi. Sallylcysteine. These large amounts of sulfur compounds give the smell and taste to the garlic. Diallyl disulfide is an important component in garlic and being a powerful antibiotic and antifungal compound [6]. Clove oil is reported to have very strong antifungal activity against a lot of fungal species [7].



Allicin



- The essential ingredient liable for its antifungal activity is ugenol from the clove. Eugenol is the major volatile compound of extracted oil from clove buds (S. aromaticum L). It is reported that clove oil possesses tough antifungal activity against C. albicans, C. neoformans [8].

Aim of this present work is to prepare various cream formulations with herbal antifungal extract of garlic oil, clove oil and peppermint oil by using emulsification method. And evaluate the cream properties like various organoleptic property, viscosity, spreadability, tube extrudability, and microbiological study, to check the antimicrobial property.

MATERIAL AND METHOD

Table 1: Material & their Collection

Material Name	Material Collected from
Propylene glycol	Royal Drug and Pharmaceuticals Mumbai
Beeswax	
Stearyl alcohol	
Cetyl alcohol	
Triethanolamine	
Propyl paraben	
Methyl paraben	
Paraffin	
Stearic acid	
Peppermint oil	
Garlic Oil	Extracted from Garlic by steam distillation in the laboratory
Clove oil	Extracted from Clove by steam distillation in the laboratory

Table2: Plant Profile

Ingredient Name	Garlic	Clove
Synonym	Allium; lassan (Hindi)	Caryophylli; Lavang (Hindi)
Biological Source	bulb of Allium sativum Linn	dried flower and buds of Eugenia caryophyllus tree
Family	Liliaceae	Myrtaceae
Chemical Constituents	Allicin, Alliin, volatile and fatty oils, mucilage and	Volatile oil (16-21%):- Phenol group mainly in the form of Eugenol (80-88%), acetyl eugenol (10- 15%); α and β -

	albumin	Caryophyllene. Also present Pyrogallol tannins, methyl furfural and dimethyl furfural
Plant Image		

Collection & Authentification of herbs:

Garlic and clove crude drugs are purchased from the market of Balaghat, (M.P.) and authenticated by Botanist of Govt. JST College, Balaghat(M.P.). Other chemical mentioned in table1 purchased form Royal Drug and Pharmaceuticals Mumbai.

Identification of Allicin by IR:

Key Peaks in Allicin's IR Spectrum:

- C=O (Carbonyl Stretching) - ~1690-1750 cm^{-1}

Allicin contains a thiosulfinate (R-S(O)-S-R) functional group, where the S=O bond contributes a strong peak in this region.

- S=O (Sulfoxide Stretching) - ~1030-1070 cm^{-1}

The sulfoxide (S=O) functional group in allicin produces a strong absorption in this region.

- C-H (Aliphatic) - ~2800-3000 cm^{-1}

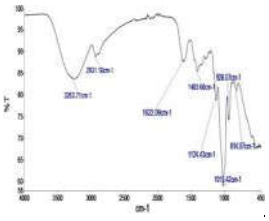
Allicin has aliphatic carbon-hydrogen (C-H) bonds, resulting in medium to strong peaks in this range.

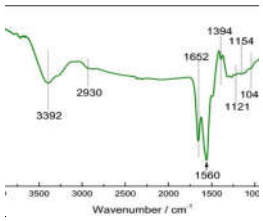
- C-S (Carbon-Sulfur Stretch) - ~600-700 cm^{-1}

The C-S bond in allicin contributes weak to medium absorption bands in this region.

Interpretation of IR the Spectrum:

- Look for the strong peak around 1030-1070 cm^{-1} (S=O stretch), which is a defining feature of allicin.
- Identify the C=O stretch (~1690-1750 cm^{-1}) if present.
- Verify the C-H stretching (~2800-3000 cm^{-1}) to confirm the presence of aliphatic groups.
- Examine the low-frequency region (600-700 cm^{-1}) for C-S stretching





(Standard Peak) **(Sample Peak)**

Sample were meet standard IR peak of Allicin.

Extraction of Oil from herbs:

Oil of garlic and clove was extracted by the steam distillation method by using the Clevenger apparatus in the laboratory. Garlic and clove cleaned properly and separately filled in the RBF with solvent arranged the assembly properly by attaching Clevenger apparatus and condenser and heat. The obtained oils are separated and fill in an airtight container.

Formulation of Antifungal Cream [9]

Table 3: Formulation 1(F1) Table of two phases

Part A- Oily Phase			Part B- Aqueous Phase		
Ingredients	Qty.	Activity	Ingredients	Quantity	Activity
Clove Oil	5%	Anti-fungal	Propylene glycol	5%	Humectants
Garlic Oil	5%	Anti-fungal	Triethanolamine	2%	Stabilizer
Stearyl alcohol	5%	Emollient	Methyl paraben	0.01%	Preservative
Cetyl alcohol	6. 5%	Binding agent	Propyl paraben	0.04%	Preservative
Mineral oil Liq.paraffin)	5%	Moisturizer	Distilled Water	Upto 100%	Solvent base
Stearic acid	2. 5%	Emulsifying agent			

White Beeswax	1. 5%	Thickening agent			

Table 4: Formulation 2 (F2) Table of two phases

Part A- Oily Phase			Part B- Aqueous Phase		
Ingredients	Quantity	Activity	Ingredients	Quantity	Activity
Peppermint oil	5%	Flavoring agent	Propyleneglycol	5%	Humectant
Clove Oil	5%	Anti-fungal	Triethanolamine	2%	Stabilizer
Garlic Oil	5%	Anti-fungal	Methyl paraben	0.01%	Preservative
Stearyl alcohol	5%	Emollient	Propyl paraben	0.04%	Preservative
Cetyl alcohol	6. 5%	Binding agent	Distilled Water	Upto100%	Solvent base
Mineral oil (Liq. paraffin)	5%	Moisturizer			
Stearic acid	2. 5%	Emulsifying agent			
White Beeswax	1. 5%	Thickening agent			

I. Preparation of oil phase [6]

All the ingredients like white beeswax, stearic acid, stearyl alcohol, cetyl alcohol were melted in a stainless steel container. To this mixture liquid paraffin was added and allowed to melt. The temperature was then kept between 65 to 70°C.

II. Preparation of Aqueous phase [6]

Water was heated to 65 to 70°C. To this aqueous medium pre weighed all the reagent like propylene glycol, triethanolamine, propyl paraben and methyl paraben were added; Then the temperature of the aqueous phase was maintained at 65 to 70°C

III. Development of Cream formulation [6]

Total Oil phase was then slowly pour into the aqueous phase at 65-70°C and mixed for 10 to 15 Minutes. When the temperture of both the medium were at the same temperature, the aqueous phase was slowly added to the oil phase with moderate agitation and was kept stirred until the temperature dropped to 40°C. Garlic oil and clove oil was added to it. The o/w emulsion was then cool down to room temperature to changed a thick cream base. In case of Formulation 2(F2), extra reagent peppermint oil was added at the final stage, and immediately transfers in to a container, and closed tightly.

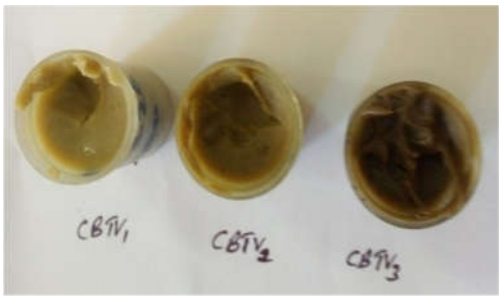


Figure 1: Antifungal cream formulation

RESULTS AND DISCUSSION:

1. Physical examination (Organolectic properties)

The prepared herbal antifungal creams were inspected visually for their colour, appearance, odor, and consistency. The pH was measured in each herbal antifungal cream, using a pH meter, which was precalibrated with standard buffer solutions at pH 4, 7, 9. The pH meters electrode was inserted in to the cream 10 min before the reading at room temperature. The standard pH of a topical preparation should be within the pH range matching to the pH of the skin, namely, 4.5- 6.5.

2. Viscosity [12, 13, 14]

The viscosity of formulated creams was measured by Brook field ViscometerNDJ-8S using spindle S 94 at varying speed and shear

rates. The measurements were done over the range of speed setting from 0.15, 0.25, 0.35, 0.45 and 0.55 rpm in 60 s between two successive speeds as equilibration with shear rate ranging from0.25 s-1 to 1.0 s-1. Viscosity determinations were performed at our room temperature.

3. Spredability [12, 13, 14, 15]

Spredability property of a formulation was calculated by an apparatus designed by Muttimer et al.; it made of a wooden block, which was connected by a pulley at a one end. A rectangular shaped ground glass was set on this block. An excess amount of cream (about 3-4 gm) under study was placed on this ground plate. The herbal antifungal cream was then kept in between this plate and a glass plate having the same dimension of fixed ground plate and attached with the hook. A fixed 1 Kg load was placed on the upper of the plates for about 4-5 minutes to expel all the entrapped air and to provide a uniform film of the cream between the plates. Excess of the cream was scrapped off from the boundaries. The top plate was then subjected to drag of 80 Gms. With the help out of string attached to the hook and the time (in seconds) required by top plate to cover a distance of 10 cm be noted. A less intervalindicates better Spredability. Spredability measured in unit gm.cm/sec Spredability of the cream may be determined by the following equation,

S= M x L/T

Where,

L= length moved by glass slide T= Time in seconds M=Weight in pan & S= Spredability

4. Tube extrudability

In this present work, the method adopted for evaluating cream formulation for extrudability was based upon the quantity in percentage cream extruded from tube on application of finger pressure 7kg. More quantity extruded improved was extrudability. The both the formulation F1 and F2 was filled in a clean, lacquered aluminium collapsible tube containing about 5 gm of cream which contains in a nasal tip of 5 mm hole and applied the pressure on the tube by the help of finger tip. The tube extrudability property was determined by, quantity of cream formulations were extruded from the tube tip as when the pressure was applied on the tube body.

5. Microbiological studies

All types of broad, non-resistance microorganism like staphylococci, streptococci, dermatophytes or yeast or molds can be protected by tropical formulations with anti microbial agent have enormous use in dermatology preparation were infections are often mixed. Since herbal anti fungal cream containing antimicrobial extracts asactive constitutent, it is expected to protect from microbial growth. To determination of an anti microbial activity of herbal antifungal cream Disk diffusion method was followed. For this study standard media was prepared with 65 g Sabouraud Dextrose Agar, and 28 g Nutrient Broth. Both the sample cream formulation was compared with standard Fluconzole. Finally the zone of inhibition diameters was measured with the help of zone reader.

Antifungal Evaluation:

- Materials: Herbal antifungal cream, fungi.
- Media: Sabouraud Dextrose Agar (65 g), and Nutrient Broth (28 g).
- Sample: Herbal antifungal cream, candida albicans.
- Standard: Fluconazole

Candida albicans Showing antifungal activity

Figure 2: Antifungal activity of Herbal antifungal cream(F2) on organism

Antifungal activity of Herbal antifungal cream on organism

Table 5. Effect of antifungal activity

Organism	Extract	Test	Standard
Candida Albicans	Ethanol	Susceptible	Susceptible

Table 4. Showing Diameters of Inhibition Zones

Organism	Plant Extract	Zone of inhibition [mm]	
		Test Sample (avgas diameter)	
Candida Albicans	Ethanol	F1 - 9	F2 - 11

As Herbal antifungal cream shows antifungal activity against Candida albicans it can be formulated as antifungal formulation (cream).

6. Skin irritancy test

Skin irritancy is determined with that herbal antifungal cream formulations do not affect the human skin cells or tissues. Irritancy may result in swelling, redness and inflammation on the surface of skin when some particular creams are applied without testing. Hence skin irritancy test was carried out by marking an area on the left hand dorsal surface. The cream was applied with a spatula to that marked specified area and time was noted. Irritancy, erythema, edema was checked for regular intervals upto 24 hours. There was no prominent irritation because of the applied herbal antifungal cream hence it was safe to use.

7. Phase separation

The formulated creams were kept intact in a closed container at 25 – 30° C not exposed to light. Phase separation was observed carefully every 24 hrs. for 30 days. Any change in phase separation was checked.

8. **Stability Studies**

The developed formulation was subjected to accelerated stability testing for about 6 weeks. Temperatures were maintained as per (ICH guidelines). At the end of the studies, samples are to be analysed for the physical properties.

Observation

Table 6. Evaluations results

Sr. No.	Evaluation Parameter	Results	
		F1	F2
1	Colour	Buff yellow to creamish	Buff yellow
2	Appearance	Smooth	Smooth
3	Odor	Pungent (strong garlic oil)	Pleasant peppermint
4	Consistency	No phase separation	No phase separation
5	Viscosity	66430 cps.	65720 cps.
6	Spread ability((gm.cm/sec)	13.43	17.00
7	pH	7.5	7.4
8	Extrudability	96.11 %	89.52%
9	Skin irritancy test	No Irritancy, erythema, edema	No Irritancy, erythema, edema
10	Phase separation	No	No
11	Microbiological studies [zone of inhibition]	9mm	11mm

The prepared both formulations showed good spread ability, no evidence of phase separation and good consistency during the study period. Though stability parameters like visual

appearance, is same but the F2 shows better fragrance compare to the formulation F1. And both the formulations showed that there was no significant variation during the study period.

CONCLUSION

The use of herbal/bioactive ingredients in cream (cosmetic) influence biological functions of skins and provide nutrients necessary for the healthy skin against antifungal infection. The prepared formulation (F2) showed good spread ability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature but it had a drawback. In first formulation (F1) the smell was unpleasant as garlic oil was used which gives a very strong unpleasant smell. So we prepared another formulation (F2) to mask this unpleasant smell. In the second formulation peppermint oil was used to enhance the preparation and mask the odor of garlic, which was also acting as a tertiary antifungal agent here.

Conflict of interest

The authors declare no conflict of interest for current work.

REFERENCE

[1]. Alemdar S, Agaoglu S. Investigation of In vitro Antimicrobial activity of Aloe vera juice. Journal of Animal and Veterinary Advances 2009: 8(1): 99-102.

[2]. Koka S , Pancholi M, Sharma V. Formulation and Evaluation of Topical Antifungal Herbal Gels Containing Hydroalcoholic Extract of Catharanthus roseus and Aloe vera International Journal of Pharmacognosy and Phytochemical Research 2019; 11(3);173-176.

[3]. Hugo WB, Russell AD: Pharmaceutical Microbiology: Blackwell Science, 6 Ed, 2003; 35-51

[4]. Khan Z K, Gyanchandani A, Candidiasis A Review: PINSA: 1998: B 64 :1-34

[5]. Baqars S R, The role of traditional medicine in the real environment, Traditional Medicine in Africa I ssaq, East African Education Publisher LTD, Nairobi, 1995 p 141-142.

[6]. Pimpale A, Formulation and Evaluation of Antibacterial Antifungal Cream of Garlic Oil, International Journal of Trend in Scientific Research and Development (IJTSRD), No: 2456 - 6470 | Volume - 3 | Issue – 1 | Nov – Dec 2018.

[7]. Santos A.L., Chierice G. O., Alexander K.S., Riga A., Matthews E. Characterization of the raw essential oil eugenol extracted from Syzgium aromaticum L. J. Therm. Anal. Calorim. 2009;96:821-825 [8]. Panizzi L., Falmini G., Cioni P.L., Morelli I. Composition and antimicrobial properties of essential oil of four Mediterranean Lamiaceae. J. Ethopharm. 1993;39:167-170.

[9]. https://www.researchgate.net/publication/333694027 Formulation and Evaluation of Antibacterial Antifungal Cream of Garlic Oil.

[10]. Pai, S.T.; Platt, M.W. Antifungal effects of Allium sativum (garlic) extract against the Aspergillus species involved in otomycosis. Lett. Appl. Microbiol. 1995, 20, 14–18.

[12]. Akanksha D, Vikas G, Neetesh KJ, Shailendra S, Neelam B, Dinesh KJ.Formulation and Evaluation of Neomycin Sulphate Ointment containing Natural Wound Healing Agent. Curcuma longa ,International Journal of Pharmaceutical Sciences and Drug Research. 2009;1(2):116-18

[13]. Panda P and Ghosh A. Formulation and Evaluation of Topical Dosage Form of Eupatorium Odoratum Linn. And Their Wound Healing Activity. International Journal of Pharma and Bio Sciences. 2010;1(2):201-203

[14]. Purushothamrao K, Khaliq K, Sagare P, Patil SK, Kharat SS, Alpana.K. Formulation and evaluation of Vanishing cream for scalp

psoriasis. International Journal of Pharma Sci Tech. 2010; 4(1):33-41.
[15]. Chakole CM, Shende MA, Khadatkar SN. Formulation and evaluation of novelcombined halobetasol propionate and fusidic acid ointment. International Journal of ChemTech Research. 2009 Jan-March;1(1):103-16.