



# FORMULATION AND EVALUATION OF ANTIFUNGAL AND ANTIBACTERIAL CREAM

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## ABSTRACT

The goal of the study was to formulate a cream with a composition for treating fungal skin infections and which enhances skin properties. This formulation belongs to a medicinal cream that has two antifungal active components. It reveals a formula for treating fungal skin infections, as well as other components that can help improve skin issues. For skin infections, the topical approach is the best option. 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. Garlic oil and clove oil are the active pharmaceutical ingredients (API) used to treat fungal skin infections. 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. Several experiments 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. The medicated cream was 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, in improved version. Most topical preparations are meant to be applied to the skin and therefore basic knowledge of skin and its physiological function and biochemistry is very important for designing topical formulations. Sweat and fatty acids secreted from sebum influence the pH of the skin surface. It is advocated that the acidity of the skin helps in limiting or preventing the growth of pathogens and other organisms. One promising avenue is the use of herbal gels, which harness the power of natural compounds derived from plants to combat microbial infections. The aim of the study is to formulate and evaluate antibacterial gel containing *Pongamia pinnata*.

**KEYWORDS:** Fungal Skin Infections, Garlic Oil, Clove Oil, Peppermint Oil, Antifungal Cream

## INTRODUCTION

The last few decades have witnessed an increase in fungal infection. Fungal infections are evolving diseases in sanatorium institutions. 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. *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis* are part of the normal flora of humans and can be isolated from oral cavity, vaginal and other parts of body sites from normal healthy people.[4] 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 Clove oil is reported to have very strong antifungal activity against a lot of fungal species The essential ingredient liable for its antifungal activity is eugenol 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* 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. For the reason that delivery of mankind, there has been a relationship between life, ailment and plant life. There are no facts that humans in prehistoric instances used artificial medicines for his or her ailments however they tried to make use of the things they might without difficulty procure. Microbes that enters the frame thru ingestion, inhalation, direct contact, cutaneous contamination and ascending infection after which stumble upon epithelial cells, macrophages, lymphocytes of limitations formed with the aid of mucous, sub cutaneous junctions or layers of pores.

## MATERIAL AND METHOD

### Materials

Propylene glycol, Beeswax, Stearyl alcohol, Cetyl alcohol, Triethanolamine, Propyl paraben, Methyl paraben, Liquid Paraffin, Stearic acid, Peppermint oil were purchased from Royal Drug and Pharmaceuticals Mumbai. Garlic Oil and Clove oil are extracted from Garlic and Clove respectively by steam distillation in the laboratory.

#### 1. Garlic

**Synonyms:** Allium; lissan (Hindi).

**rce:** Garlic is obtained from ripe bulb of All sativum Li

**Family:** *Liliaceae*.

**Chemical Constituents:** Allicin, Alliin, volatileand fatty oils, mucilage and albumin

#### 2. Clove

**Synonyms:** Caryophylli; Clove buds and Cloveflower, Lavang (Hindi).

**Biological Source:** Clove is obtained from dried flower and buds of Eugenia caryophyllus tree.

**Family-** Myrtaceae

#### Chemical Constituents

Clove contains Volatile oil (16-21%):- Phenol group mainly in the form of Eugenol (80-88%), acetyl eugenol (10-15%);  $\alpha$  and  $\beta$  - Caryophyllene. Also present Pyrogallol tannins, methyl furfural and dimethyl furfural Collection of sample Garlic and clove crude drugs are purchased from the market.

#### Extraction of Oil

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.

#### 3.Karanja oil

carbopol934p, triethanolamine, propyl paraben, methyl paraben, propylene glycol, water. Biological source

**TABLE 1: FORMULATION 1(F1) TABLE OF TWO PHASE**

Part A- Oily Phase			Part B-Aqueous Phase		
Ingredients	Quantity	Activity	Ingredients	Quantity	Activity
Clove Oil	5%	Anti- fungal	Propylene glycol	5%	Humect ants
Garlic Oil	5%	Anti- fungal	Triethanolami ne	2%	Stabilizer
Stearyl alcohol	5%	Emollient	Methyl paraben	0.01%	Preservative
Cetyl alcohol	6.5%	Binding agent	Propyl paraben	0.04%	Preservative
Mineraloil (Liquid paraffin)	5%	Moisturizer	Distilled Water	Up to 100%	Solvent base
Stearic acid	2.5%	Emulsifying agent			
White Beeswax	1.5%	Thickening agent			

**Table 2: Formulation 2 (F2)**

Part A- Oily Phase			Part B-Aqueous Phase		
Ingredients	Quantity	Activity	Ingredient s	Quantity	Activity
Peppermint oil	5%	Antifungal& Flavoring agent	Propylene glycol	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	Up to 100%	Solvent base
Mineraloil (Liquid paraffin)	5%	Moisturizer			
Stearic acid	2.5%	Emulsifying agent			
White Beeswax	1.5%	Thickening agent			



### Preparation of oil phase

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

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

Total Oil phase was then slowly pour into the aqueous phase at 65-70°C and mixed for 10 to 15 Minutes. When the temperature 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.

#### 1. Physical examination (Organoleptic 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

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 from 0.25 s<sup>-1</sup> to 1.0 s<sup>-1</sup>. Viscosity determinations were performed at our room temperature.

#### 3. Spreadability

Spreadability 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 interval. Aim and Objective

#### Aim

Formulation and evaluation of antibacterial and antifungal cream containing clove oil and karanj oil

#### Objective

1. To determine effect of herbal constituent against *S. aureus* and *E. coli*.
2. To determine the preparation and formulation of anti- microbial gel.
3. To demonstrate anti-microbial activity of herbal gel.

#### Biological source

Biological source of karanj is *Pongamia pinnata* Linn Pierre belonging to the family (Fabaceae) Leguminosae.

#### 2. Clove

**Synonyms:** Caryophylli; Clove buds and Cloveflower, Lavang (Hindi).

**Biological Source:** Clove is obtained from dried flower and buds of *Eugenia caryophyllus* tree.

**Family-** Myrtaceae

#### Chemical Constituents

Clove contains Volatile oil (16-21%):- Phenol group mainly in the form of Eugenol (80-88%), acetyl eugenol (10-15%);  $\alpha$  and  $\beta$  - Caryophyllene. Also present Pyrogallol tannins, methyl furfural and dimethyl furfural Collection of sample Garlic and clove crude drugs are purchased from the market.

### HERBAL FINDINGS AND USE

Concentrated fruits or seeds extract can be found in various herbal preparations are widely available in market today. *Pongamia pinnata* preparation oil is widely available and employed by practitioner of natural health for treatment of rheumatism.

*Pongamia pinnata* is rightly called as Biodiesel plant, being considered as excellent source of Biodiesel. This plant is a multipurpose



tree with immense medicinal and economic value. The present work reports oral bioavailability of karanjin in rat plasma and a validated RP-HPLC-UV method for determination of karanjin.

The plant shows the properties for the agriculture like insect pest management, as a biofuel, as a good source of crop macro and micronutrients, as a soil binder etc and medical industry as an anti-microbial, anti-ulcer, anti-diarrhoeal, anti-plasmodial, anti-inflammatory, anti-oxidant antiviral, properties. Pongamia oil, leaf and cake was found to be the good nutritional value as a soil fertility management, pesticide, acaricide and nematicide in agriculture.

Karanjin is initially recognized as an antioxidant, several kinds of research have proven that it is truly multifaceted in other industries as well. This precious molecule requires further understanding to fully capture its mechanism in many terms and benefits. Hence, this review has provided a comprehensive view concerning the initial ideas of the medicinal and agricultural purposes of karanjin.

Pongamia pinnata plant is used for anti-inflammatory, cardioprotective, anti-plasmodial, anti-nociceptive, anti-diarrhoeal, anti-ulcer, anti-hyperglycaemic, antihyperammonemic and antioxidant, antibacterial, antiviral, anticonvulsant activity.

### Phytochemical constituents

There are various chemical constituents isolated from the plant *P. pinnata*. Flavonoid and its derivatives are the most common constituents for isolation. The derivatives of flavonoids are flavones, flavans and chalcones. Sesquiterpene, diterpene, triterpenes, steroids, amino acids, disaccharides, fatty acids and ester compounds are also detected in this plant. Flavones are the most common constituents extracted from *P. pinnata*. The flavone class of compounds is distributed in all parts of this plant. Karanjin is considered the first compound to be extracted from this plant. Karanjin is a major chemical constituent of *P. pinnata*. In the current studies, explored the anti-ulcerative property of karanjin, a furano-flavoid isolated from the seeds of karanj. Karanjin is known as the main active principle in Karanj, which is effective against large number of insects.

### Traditional uses

1. The leafy foods are utilized in people solutions for stomach growths in India, the seeds for keloid cancers in Sri Lanka and a powder got from the plant for growths in Vietnam 23. In Sanskrit India, seeds were utilized for skin diseases. Today, the oil is utilized as a liniment for stiffness.
2. Leaves are dynamic against *Micrococcus*; their juice is utilized for cold, hacks, the runs, dyspepsia, tooting, gonorrhea and uncleanliness.
3. Pulls are utilized for cleaning gums, teeth and ulcers.
4. Bark is utilized inside for draining heaps. They are likewise utilized for beriberi.
5. Juices from the plant as well as the oil are clean. It is supposed to be a superb solution for tingle, herpes and pityriasis versicolor.1
6. Powdered seeds are esteemed as a febrifuge, tonic and in bronchitis and beating hack.
7. Blossoms are utilized for diabetes.
8. Juice of the root is utilized for purging foul ulcers and shutting fistulous injuries.
9. Youthful shoots have been suggested for ailment.
1. Ayurvedic medication depicted the root and bark as alexipharmic, anthelmintic and helpful in stomach amplification, ascites, biliousness, sicknesses of the eye, skin and vagina, tingle, heaps, splenomegaly, growths, ulcers and wounds; the fledglings, considered alexiteric, anthelmintic, aperitif and stomachic, for irritation, heaps and skin sicknesses; the leaves, anthelmintic, stomach related and diuretic, for irritations, heaps and wounds; the blossoms for biliousness and diabetes; the leafy foods for keratitis, heaps, urinary releases and infections of the cerebrum, eye, head and skin, the oil for biliousness, eye illnesses, tingle, leukoderma, ailment, skin sicknesses, worms and wounds.
2. Unani framework utilizes the debris to reinforce the teeth, the seed, carminative and depurative, for chest objections, ongoing fevers, ear infection, hydrocele and lumbago; the oil is utilized as fuel for cooking and lamps.

### Pharmacological activity

1. Anti-Plasmodial activity
2. Anti-Inflammatory activity
3. Anti-diarrhoeal Activity
4. Antioxidant and Anti-hyperammonemic Activity
5. Anti-ulcer Activity
6. Anti-hyperglycaemic and Anti-lipidperoxidative Activity
7. Anti-lice Activity
8. Neuroprotective Activity
9. Anti-viral activity

### Anti- microbial and antifungal testing of herbal

1. The antimicrobial exercises of various not entirely set in stone by altered agar well dissemination strategy. Culture of *Staphylococcus aureus* and *E.coli* at 37 °C in 10 ml Mueller Hinton stock was utilized. The way of life were acclimated to



- roughly 105 CFU/ml with clean saline arrangement.
- In this technique, supplement agar plates were cultivated with 0.2 ml for 24 hr stock culture of *E. coli* and *S.aureus*. The agar plates were permitted to harden. A clean 8 mm drill was utilized to cut wells of equidistance in each plate 0.5 ml of detailing hatched at 37 degree Celsius for 24 hr in a hatchery.
  - Agar dispersion procedure was applied to concentrate on the antibacterial impact of the recently referenced separates. Last convergence of 5%, 10%, 15%, 20%, 30%, 40%, half of the sound leaf gel in Mueller agar were acquired for each concentrate independently. Mueller Hinton agar plates were cleaned with a suspension of *Staphylococcus aureus* arranged as referenced before, utilizing sterile q-tip. Plugs were eliminated from every agar plate creating openings.
  - To each opening 1gm of gel weakened in 10L refined water from various centralization of each concentrate was added and permitted to diffuse at room.

### 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

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

### Herbal Antifungal Agents

Medicinal plants are of great importance to the health of individuals and communities, and their importance lies in the chemical substances that produce a definite physiological action on the human body. Many of the pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis, and quinine while their purification and quantification makes them more predictable and chemical processing can sometimes modify their effects in desirable ways.

Herbal remedies tend to have a more complex and subtle mix of chemicals and can sometimes offer access to drugs or combinations of drugs that the pharmaceutical industry has not yet exploited. These natural compounds formed the basis of discovering modern drugs. Some of the antifungal drugs most recently introduced in clinical practice are echinocandins and sordarines derived from natural products. Therefore, there is a need to develop new antifungal agents providing new mechanisms of action, with a broad spectrum of antifungal activity, fewer dose-limiting side effects, and economic. Some of the plants having wide fungal activity are listed in Table 1. Which will be proved beneficial for the pharmaceutical industry when formulated. Herbal formulations always have attracted considerable attention due to their good activity and comparatively lesser side effects when compared to synthetic drugs. Karanja commonly known as Indian beech is one of the many plants with diverse medicinal properties, where all its parts have been used as traditional medicine in the treatment and prevention of several kinds of ailments in many countries such as for treatment of piles, skin diseases, and wounds. Ayurveda as well modern medical science is using its benefits of useful phytochemical source and potency in healing a number of ailments including skin allergies. This plant is having proven immunomodulation, anti-viral and anti-allergic actions. Dr. Gupta's IAFA has conducted several Ayurveda research programs to establish the abundant medical uses of this herb. IAFA have included this herb in most of the skin allergy related products. Single herb extract therapy with Karanja, is one among the unique method performed by Dr. Gupta in healing various skin diseases with difficult prognosis. Allergic conditions are showing high success rates in the cure with the help of Indian beech plant or Karanja.

Several different classes of flavonoid derivatives, such as flavans, flavones and chalcones and several types of compounds including terpenes, steroid and fatty acids have been isolated from different parts of this plant. The pharmacological and Ayurveda studies revealed that various types of preparations, extracts and single compounds of this species exhibited a broad spectrum of biological activities such as antioxidant, antiallergic, antimicrobial, anti-inflammatory, and anti-diabetic activities.

## PHARMACOGNOSTIC CHARACTERS

### 1.KARANJ

**Kingdom** Plantae

**Family** Fabaceae

**Synonym** *Milletia pinnate*

**Common Name** Karanj, Indian Beech Tree

### Biological Source

Root and flowers *Pongamia pinnata* (i.e., Indian beech nut tree, Karanja), a tree found along the coast or rivers in India, contain a bioflavonoid that possesses many differing properties, including anti-inflammatory and antisolar effects.



### Geographical Source

*Pongamia pinnata* (Indian beech) Flowers. Kannur, Kerala, India.

### Cultivation And Collection

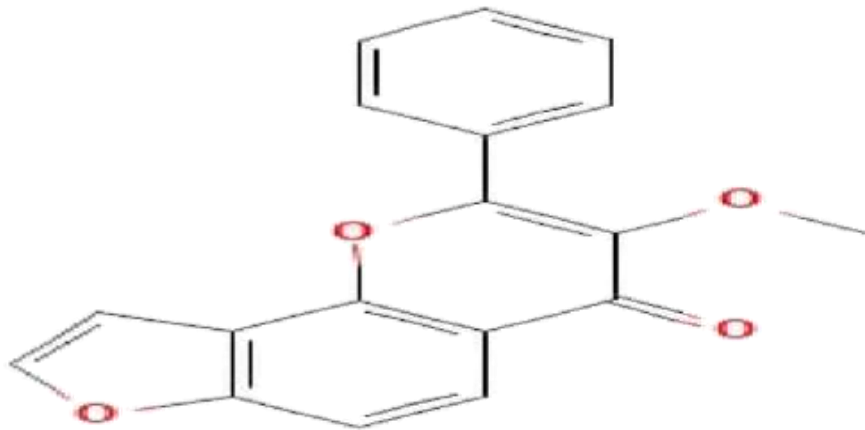
*Pongamia pinnata* grows in a fairly moist situation on porous and well drained soil. *Pongamia pinnata* grows almost anywhere, even on gravelly, sandy and saline soil. It can grow even in the crevices of rocks.

### Morphological Character

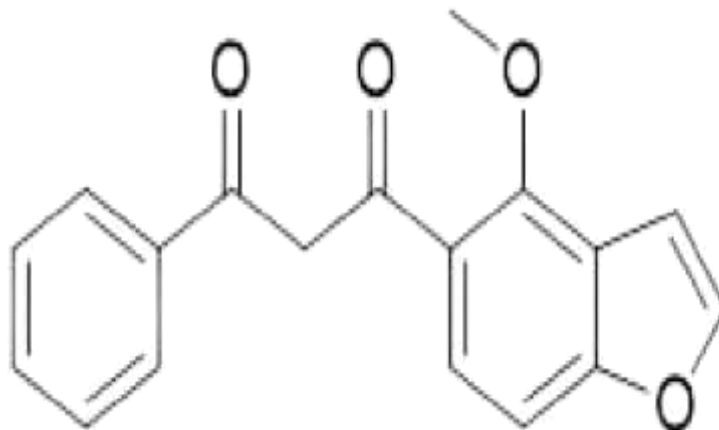
Leaves - 8-10 inch long, pale green, leaflets 5-7 oblong or ovate, 2-4 inch long subcoriaceous, midrib and natural nerves. Flowers – . The flowers are lavender, pink white in colour, finely pubescent, and 15-18 mm long. Seed- Seeds are bean-like, 1.5-2.5 cm long x 1.2-2.0 cm wide, dark brown in colour. They are oily, containing about 30-40% oil.

### Chemical Constituents

It consists of chemical constituents including karanjin, pongamol, tannin and karanjachromene. karanjin



Pongamol



## 2 CLOVE OIL

**Synonyms** Clove buds, Clove flowers.

### Biological Source

Clove consists of the dried flower buds of *Eugenia caryophyllus* Thunb., belonging to the family Myrtaceae.

### Geographical Source

Clove tree is a native of Indonesia. It is cultivated mainly in the islands of Zanzibar, Pemba, Brazil, Amboiana, and Sumatra. It is also found in Madagascar, Penang, Mauritius, West Indies, India, and Ceylon.

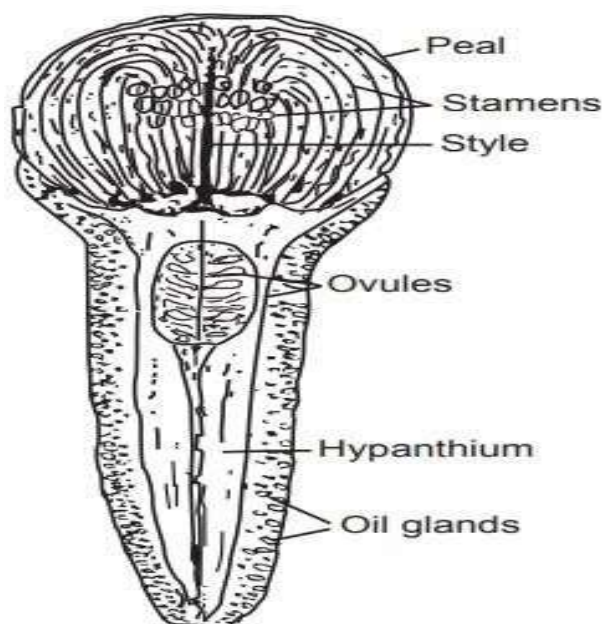
### Cultivation and Collection

Clove tree is evergreen and 10 to 20 m in height. The plant requires moist, warm and equable climate with well-distributed rainfall. It is propagated by means of seeds. The seeds are sown in well-drained suitable soil at a distance of about 25 cm. The plants should be protected against pests and plant diseases. Initially it has to be protected from sunlight by growing inside a green house or by constructing frames about 1 m high and covering them with banana leaves. As the banana leaves decay gradually more and more sunlight falls on the young seedlings and the seeds are able to bear full sunlight when they are about 9 months old. The seedlings when become 1 m high, they are transplanted into open spaces at a distance of 6 m just before the rainy season. The young clove trees are protected from sun even for a longer period by planting banana trees in between. The drug can be collected every year starting from 6 years old till they are 70 years old.

Clove buds change the colour as they mature. At the start of the rainy season long greenish buds appear which change to a lovely rosy peach colour and as the corolla fades the calyx turns yellow and then red. The buds are collected during dry weather in the month of August to December. The collection is done either by climbing on the tree or by using some ladders or with the help of mobile platforms. In some places the trees are even beaten using bamboo sticks for the collection of the bud. The drugs which are collected are then separated from the stalks and then placed on coconut mats for drying under sun. The buds lose about 70% of its weight, whereas drying and change their colour to dark reddish-brown. The dried clove is graded and packed.

### Characteristics

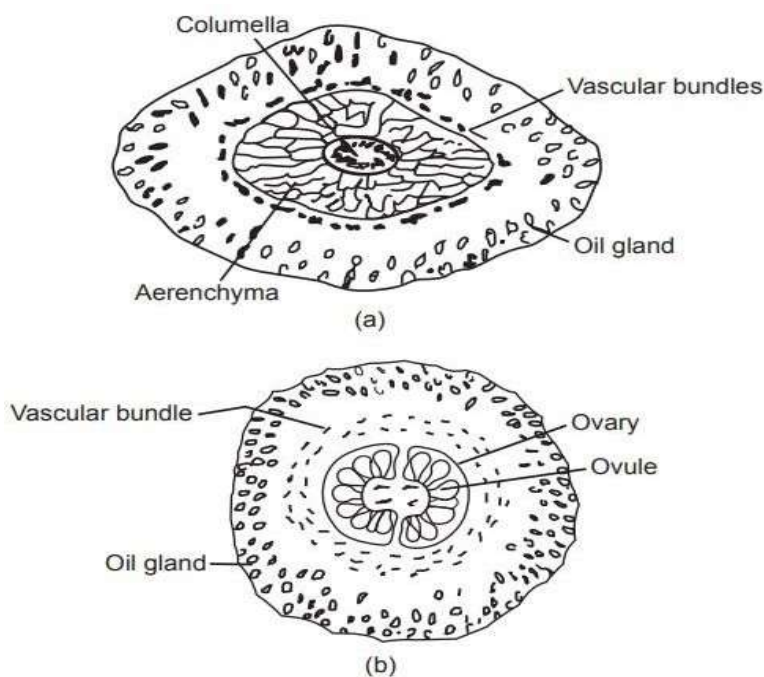
Clove is reddish-brown in colour, with an upper crown and a hypanthium. The hypanthium is sub-cylindrical and tapering at the end. The hypanthium is 10 to 13 mm long, 4 mm wide, and 2 mm thick and has schizolysigenous oil glands and an ovary which is bilocular. The Crown region consists of the calyx, corolla, style and stamens. Calyx has four thick sepals. Corolla is also known as head, crown or cap; it is doinshaped and has four pale yellow coloured petals which are imbricate, immature, and membranous. The ovary consists of abundant ovules. Clove has strong spicy, aromatic odour, and pungent and aromatic taste.



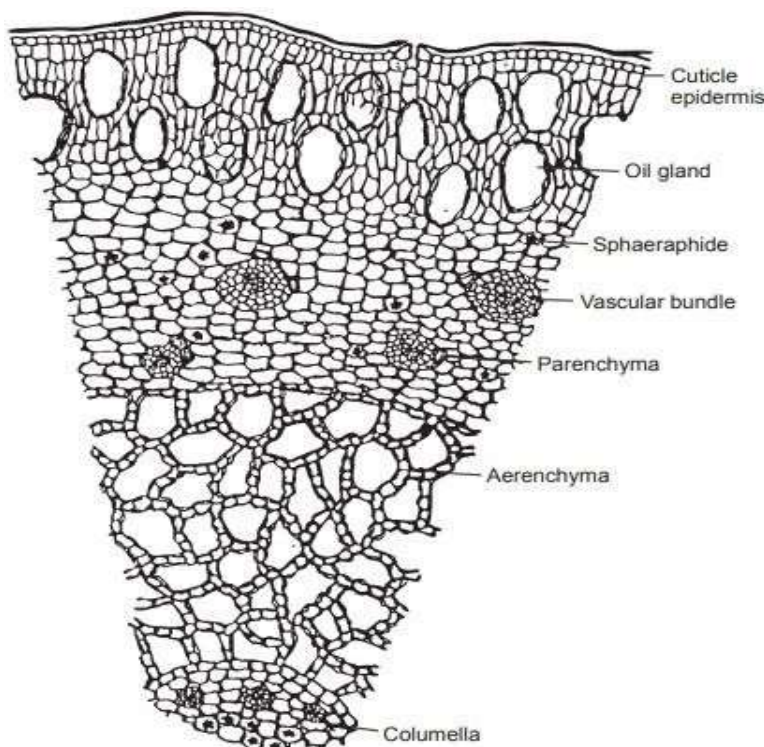
CLOVE BUD

### Microscopy

The transverse section should be taken through the short upper portion which has the bilocular ovary and also through the hypanthium region. The transverse section through the hypanthium shows the following characters. It has a single layer of epidermis covered with thick cuticle. The epidermis has ranunculaceous stomata. The cortex has three distinct region: the peripheral region with two to three layers of schizolysigenous oil glands, embedded in parenchymatous cells. The middle layer has few layers of bicollateral vascular bundle. In the inner portion it has loosely arranged aerenchyma cells. The central cylinder contains thick-walled parenchyma with a ring of bicollateral vascular bundles and abundant sphaeraphides. The T.S. through ovary region shows the presence of an ovary with numerous ovules in it.



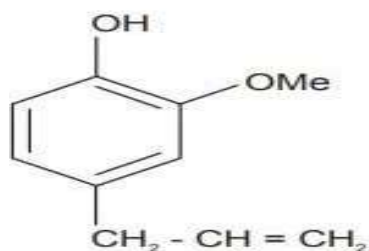
(a) T.S. passing through hypanthium. (b) T.S. passing through ovary



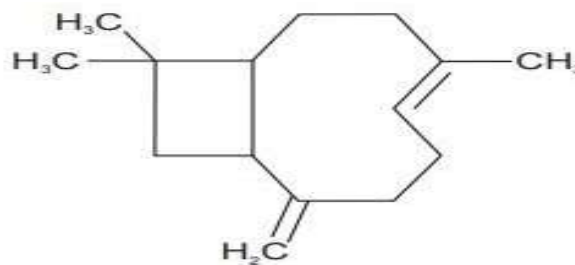
### Transverse section of clove flower bud

#### Chemical Constituents

Clove contains 14–21% of volatile oil. The other constituents present are the eugenol, acetyl eugenol, gallotannic acid, and two crystalline principles;  $\alpha$ - and  $\beta$ - caryophyllenes, methyl furfural, gum, resin, and fibre. Caryophyllin is odourless component and appears to be a phytosterol, whereas eugenol is a colourless liquid. Clove oil has 60–90% eugenol, which is the cause of its anesthetic and antiseptic properties.



Eugenol



Caryophyllene

**Chemical Tests**

1. To a thick section through hypanthium of clove add 50% potassium hydroxide solution; it produces needle-shaped crystals of potassium eugenate.
2. A drop of clove oil is dissolved in 5 ml alcohol and a drop of ferric chloride solution is added; due to the phenolic OH group of eugenol, a blue colour is seen.
3. To a drop of chloroform extract of clove add a drop of 30% aqueous solution of sodium hydroxide saturated with sodium bromide; Needle and pear shaped crystals of sodium eugenate arranged in rosette are produced immediately.

**Uses**

Clove is used as an antiseptic, stimulant, carminative, aromatic, and as a flavouring agent. It is also used as anodyne, antiemetic. Dentists use clove oil as an oral anesthetic and to disinfect the root canals. Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria and so it is used in the treatment of diarrhea, intestinal worms, and other digestive ailments. Clove oil can stop toothache. A few drops of the oil in water will stop vomiting, eating cloves is said to be aphrodisiac. Eugenol is also used as local anaesthetic in small doses. The oil stimulates peristalsis; it is a strong germicide, also a stimulating expectorant in bronchial problems. The infusion and Clove water are good vehicles for alkalies and aromatics.

**Adulterants**

The clove is generally adulterated by exhausted clove, clove fruits, blown cloves and clove stalks. The exhausted cloves are those from which volatile oil is either partially or completely removed by distillation.

Exhausted cloves are darker in colour and can be identified as they float on freshly boiled and cooled water. Clove fruits are dark brown in colour and have less volatile oil content. These can be identified by the presence of starch present in the seed of the fruit.

Blown Cloves are entirely developed clove flowers from which corolla and stamens get separated. While separation, sometimes the stalks are incompletely removed and the percentage of volatile oil in clove stalk is only 5%. As clove stalks contain prism type of calcium oxalate crystals and thick-walled stone cells which are absent in clove the clove stalk can also be detected.

**Marketed Products**

It is one of the ingredients of the preparation known as Himsagar tail (Dabur).

Sr. No.	Evaluation Parameter	Results	
		F1	F2
1	Color	Buff yellow to creamish	Buff yellow
2	Appearance	Smooth	Smooth
3	Odor	Pungent (strong garlic oil)	Pleasant peppermint
3	Consistency	No phase separation	No separation
	Viscosity	66440 cps.	65740 cps.
4	Spread ability (gm.cm/sec)	14.23	18.00
5	pH	7.5	7.4
6	Extrudability	96.15 %	89.50%
7	Skin irritancy test	No Irritancy, erythema, edema	No Irritancy, erythema, edema
8	Micro biological [zone studies of inhibition]	8mm	10mm



## RESULTS AND DISCUSSION

### 1. Physical Examination

(Organoleptic 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,

### 2. Viscosity

The viscosity of formulated creams was measured by Brook field Viscometer NDJ-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 from 0.25 s<sup>-1</sup> to 1.0 Viscosity determinations were performed at our room temperature.

### 3. Spreadability

Spreadability 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.

### 4. 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 as active constituent, 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 Fluconazole. Finally the zone of inhibition diameters was measured with the help of zone reader.

### 5. 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.

## 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.

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