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Formulation and evaluation of polyherbal antibacterial cream by using Tulsi, betel leaf and liquorice extract

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Abstract
The skin, the body's largest organ, serves as a protective barrier against environmental stressors. Its condition reflects overall health and aging. Herbal medicine, involving plant parts such as seeds, leaves, stems, bark, and roots, is gaining popularity for its cost-effectiveness and safety. Many pharmaceuticals are derived from traditional herbs known for their bioactive compounds like carbohydrates, alkaloids, glycosides, tannins, flavonoids, and phenolic compounds, which offer therapeutic effects including antimicrobial and anti-inflammatory properties. This study aims to develop an antifungal polyherbal cream using Neem, Guduchi, Mint, Tulsi, Piper betel, and *Glycyrrhiza glabra*. Tulsi and Piper betel exhibit broad-spectrum antimicrobial, anti-inflammatory, antioxidant, and anti-cancer activities, while *Glycyrrhiza glabra* is known for its anti-inflammatory, antiviral, antimicrobial, and hepatoprotective effects. The formulation involves extracting plant compounds using ethanol and assessing their phytochemical properties, aiming to create an effective, standardized, and internationally accepted herbal product for skin health, highlighting traditional medicine's role in modern healthcare.

Keywords: Anti-bacterial activity, polyherbal cream, Tulsi, liquorice, betel leaf

Introduction
The largest organ of the body is skin and it forms an ultimate shielding barricade against environmental stress enhancers such as transmittable pathogens, ultra-violet radiations, dust and chemical agents which causes ageing and other infections. The condition of general inner health and aging can be judged by skin [1]. Herbal medicine sometimes referred to as botanical medicine or herbalism it involves the use of plants or parts of plants to treat injuries or illnesses [2]. The parts of plants like seeds, leaves, stems, bark, roots, flowers etc and their extracts used in herbal medicine as teas and tinctures, topical applicators, pills, capsules and other formulation. Some of the pharmaceutical medications on the market are extracts of some of these traditional herbs. The lower cost, and often safer use, has attracted many medical professionals [3]. Plant material is an important source to use against some serious diseases in the world. Traditional medicinal methods, especially the use of medicinal plants in the form of powder, paste, etc. Medicinal herbs play an essential role in fulfilling the basic health needs in developing countries. The medicinal value of these herbs lies in some chemically active compounds present in plants and secondary metabolites that can give a definite physiological action to the human body. The maximum crucial of those bioactive materials of vegetation are carbohydrates, alkaloids, glycosides, tannins, flavonoids, phenolic compounds, etc. In recent years, infections have greatly increased and spread, and antibiotics resistance effects have become an ever-increasing therapeutic problem. Natural products and secondary metabolites of higher plants may show a new source of antimicrobial activity with possibly novel mechanisms of action. Infectious illness therapy using natural items is successful while also minimising many of the negative side effects frequently linked with synthetic antimicrobials. Therefore, it is important to carry out screening and investigate plant metabolites in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their chemical constituents [4]. Polyherbal compositions are combinations of two or more plants. In Ayurveda, the pharmacological formulation is based on two principles and is used as a single drug. Polyherbal formulation involves the use of many drugs. The "Sarangdhar Samhita" of Ayurveda discusses the idea of mutualism that underlies polyherbal formulations.

Despite the fact that single-plant formulations do include active phytoconstituents, they frequently contain insufficient amounts to have the intended therapeutic effects. According to scientific studies, combining plants with different levels of potency produces better outcomes than doing so alone. Synergism is created by beneficial herb-herb interactions. Pharmacokinetic or pharmacodynamic synergisms are also possible [5]. 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. Two phase of cream i.e. oil-in-water and water-in-oil [6]. The Cosmetic product are the best choice to reduce skin disorders. The use of cosmetic is not only developing an attractive external appearance, but towards achieving good health by reducing skin disorders. Natural herbal skin creams are moisturize, hydrate and nourish the skin. The present work is develop herbal cream which can produce Anti-fungal effect. Present polyherbal cream comprising drugs like Neem, Guduchi, Mint [7].

Tulsi is an important symbol of the Hindu religious tradition. Although the word 'Tulsi' gives the connotation of the incomparable one, its other name, Vishnupriya means the one that pleases Lord Vishnu. Found in most of the Indian homes and worshipped, its legend has permeated Indian ethos down the ages. Known in English as Holy Basil and botanically called Ocimum sanctum, Tulsi belongs to plant family Lamiaceae. It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties. Tulsi has been described as of two types- vanya (wild) and gramya (grown in homes). Although having identical usage, the former has darker leaves. Tulsi is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarrhal fever, otalgia, lumbago, hiccough, ophthalmia, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders [8-9].

Piper betel Linn. A member of the piperaceae family is an edible plant with leaves that have been traditionally used in India, China, and Thailand. The betel plant is an evergreen and perennial, creeper, with glossy heart shaped and white catkin. The genus piper (Piperaceae) is largly distributed in tropical and subtropical region of the world [10]. Betel leaves possess activity like antidiabetic, antiulcer, antiplatelet aggregation, antifertility, cardiotonic; antitumour, antimutagenic, respiratory depressant and antihelmenthetic wound healing property. Piper betel is used to treat alcoholism, bronchitis, asthma, leprosy and dyspepsia, antihistaminic, antioxidant property antimicrobial activity anti-inflammatory radioprotective and immune-modulatory property [11].

Glycyrrhiza glabra L. (Fabaceae) generally known as mulaithi, sweet wood or liquorice is a small perennial herb native to the mediterranean region, central and southwest asia. This herb is cultivated in various parts of the world including Italy, Russia, France, UK, USA, Germany, Spain, China and Northern India. It has provided us some of important bioactive constituents for life saving drugs, used in the armamentarium of modern medicine. It is widely used in ayurvedic formulations [12].

- **Topical Drug Delivery**

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentrations. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. The skin comprises multiple layers, with the outermost layer termed the epidermis. The epidermis consists of several layers including the Stratum corneum, Stratum lucidum, Stratum granulosum, Stratum spinosum, and Stratum germinativum. Directly beneath the epidermis lies the dermis, followed by subcutaneous fatty tissues [13].

Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solutions, as well as medicated adhesive systems are also in use.

- **Advantages of Topical Drug Delivery System**
  - Avoidance of first pass metabolism.
  - Convenient and easy to apply.
  - Abilityto easily terminate the medications, when needed.
  - A relatively large area of application in comparison with buccal or nasal cavity
  - Ability to deliver drug more selectively to a specific site.
  - Providing utilization of drugs with short biological half-life,
  - Improving physiological and pharmacological response.
  - Improve patient compliance.
  - Provide suitability for self-medication.

Disadvantages of Topical Drug Delivery System

Skin irritation of contact dermatitis may occur due to the drug and/or excipients.

Poor permeability of some drugs through the skin.

Possibility of allergenic reactions [14].

Plant profile

- **Tulsi**

Ocimum tenuiflorum, commonly known as holy basil or Tulsi, is an aromatic perennial plant in the family Lamiaceae. It is native to tropical and subtropical regions of Australia, Malesia, Asia, and the western Pacific. It is widely cultivated throughout the Southeast Asian tropics.

Kingdom: Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Lamiales

Family : Lamiaceae
Genus : Ocimum
Species : *O. Tenuiflorum*

- **Chemical constituents**

1. **Essential Oils:** Eugenol, Methyl eugenol, Linalool, β-Caryophyllene, α-Terpineol.
2. **Flavonoids:** Apigenin, Luteolin, Rosmarinic acid, Caffeic acid.
3. **Ursolic acid**: Has anti-inflammatory, antimicrobial, and anti-cancer properties.
4. **Oleanolic acid**: Known for its hepatoprotective, anti-inflammatory, and antioxidant effects.
5. **Carvacrol**: Exhibits antimicrobial properties and is found in the essential oil.
6. **Estragole**: Contributes to the aroma and has antimicrobial properties.
7. **Vitamins**: Tulsi contains vitamins A and C, which are essential for immune function, skin health, and antioxidant protection.
8. **Minerals**: Includes calcium, iron, and zinc, which are important for various bodily functions, including bone health, blood production, and immune response.
9. **Alkaloids**: These compounds have various pharmacological effects, including pain relief and antimicrobial properties.
10. **Tannins**: Contribute to the astringent properties of Tulsi and have antioxidant effects.
11. **Amino Acids:** Tulsi contains essential amino acids that are important for protein synthesis and overall health.
12. **Glycosides:** These compounds contribute to the overall medicinal effects of Tulsi, including its role in supporting heart health and regulating blood sugar levels.

- **Pharmacological activity**

1. Antimicrobial Activity

Tulsi exhibits broad-spectrum antimicrobial activity against bacteria, viruses, and fungi. This is
primarily due to essential oils like eugenol, thymol, and carvacrol, which make it effective in treating infections and boosting immunity.

2. Anti-inflammatory Activity

Compounds such as eugenol, ursolic acid, and rosmarinic acid contribute to Tulsi's anti-inflammatory properties. These help in reducing inflammation and associated conditions like arthritis and inflammatory bowel disease.

3. Anti-cancer Activity

Research indicates that Tulsi has potential anti-cancer properties. It can inhibit the growth of cancer cells and induce apoptosis (programmed cell death) in various types of cancers.

4. Analgesic Activity

The herb has pain-relieving properties, which can help in managing pain associated with various conditions, including headaches, muscle pain, and arthritis.

5. Adaptogenic Activity

Tulsi is well-known for its adaptogenic properties, helping the body adapt to stress and restore physiological balance. It enhances the body's resilience to physical, chemical, and biological stressors.

6. Antioxidant Activity

Tulsi contains numerous antioxidants, including phenolic compounds and flavonoids, which help neutralize free radicals, thereby protecting cells from oxidative damage and reducing the risk of chronic diseases.

7. Respiratory Benefits

Tulsi is traditionally used to treat respiratory conditions such as asthma, bronchitis, and colds. Its anti-inflammatory, antimicrobial, and immunomodulatory properties help alleviate respiratory symptoms and enhance lung function [15].

- **Betel Leaf**

The betel, Piper betel, is a species of flowering plant in the pepper family Piperaceae, native to Southeast Asia. It is an evergreen, dioecious vine, with glossy heart-shaped leaves and white catkins. Betel plants are cultivated for their leaves which are most commonly used as flavoring in chewing areca nut.

Kingdom: Plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Magnoliids
Order : Piperales
Family : Piperaceae
Genus : Piper
Species: *P. betle*

- **Chemical constituent**

1. **Essential Oils:** Chavicol, Eugenol Estragole, Safrole, Carvacrol Caryophyllene
2. **Phenolic Compounds:** Hydroxychavicol, Chavibetol, Eugenol, Allylpyrocatechol
3. **Alkaloids:** Arecoline, Guvacine, Guvacoline
4. **Flavonoids:** Flavones, Flavanones Flavonols
5. **Terpenes:** β-Caryophyllene, α-Pinene, β-Pinene, Limonene, Camphene
6. **Other Constituents:** Vitamins, Mineral, Amino acids

- **Pharmacological activity**

1. Antimicrobial Activity

Betel leaf exhibits significant antimicrobial properties against a variety of pathogens, including bacteria, fungi, and viruses. The essential oils and phenolic compounds such as chavicol, eugenol, and hydroxychavicol are primarily responsible for these effects.

- **Bacterial Infections:** Effective against pathogens like *Streptococcus mutans* (dental caries)

and *Staphylococcus aureus* (skin infections).

- **Fungal Infections:** Demonstrates antifungal activity against Candida albicans and other fungal species.
- **Viral Infections:** Shows potential antiviral activity against viruses such as influenza and herpes simplex.

2. Anti-inflammatory Activity

Betel leaf has potent anti-inflammatory properties, which are beneficial in reducing inflammation and pain. Compounds like hydroxychavicol and eugenol help inhibit the release of pro- inflammatory mediators.

Arthritis: Reduces inflammation in joint-related disorders.

Wound Healing: Applied topically to reduce inflammation and promote healing of wounds and ulcers.

3. Antioxidant Activity

The phenolic compounds and flavonoids in betel leaf provide strong antioxidant effects, protecting cells from oxidative stress and damage caused by free radicals.

Cell Protection: Helps in preventing cellular damage and aging.

Chronic Disease Prevention: May reduce the risk of diseases associated with oxidative stress, such as cancer and cardiovascular diseases.

4. Anti-cancer Activity

Betel leaf has shown potential anti-cancer properties, with studies indicating its ability to inhibit the growth of cancer cells and induce apoptosis (programmed cell death).

Inhibition of Carcinogens: Some compounds in betel leaf can neutralize carcinogens and prevent their interaction with DNA.

Cancer Cell Suppression: Research indicates efficacy against various cancer cell lines, including those of oral, breast, and lung cancers.

5. Anti-diabetic Activity

Betel leaf can help in regulating blood glucose levels and improving insulin sensitivity, making it beneficial for managing diabetes. Blood Sugar Regulation: Compounds in betel leaf help lower blood glucose levels and improve glucose metabolism.

6. Gastroprotective Activity

Betel leaf is known for its protective effects on the gastrointestinal tract, aiding in digestion and preventing ulcers.

Anti-ulcer Activity: Reduces gastric ulcers and promotes the healing of the stomach lining.

Digestive Aid: Enhances digestive function and alleviates symptoms of indigestion and constipation.

7. Respiratory Benefits

Betel leaf is used in traditional medicine to treat respiratory conditions such as cough, asthma, and bronchitis.

Expectorant Properties: Helps in expelling mucus from the respiratory tract. Bronchodilator Effect: Eases breathing by dilating the bronchi and bronchioles.

8. Analgesic Activity

The analgesic (pain-relieving) properties of betel leaf make it useful in managing pain from various conditions.

Pain Relief: Applied topically or consumed to relieve pain from headaches, arthritis, and injuries.

9. Immunomodulatory Activity

Betel leaf has immunomodulatory effects, helping to enhance the body's immune response.

Immune Enhancement: Stimulates the immune system, increasing the production of antibodies and other immune cells [16].

- **Liquorice**

Liquorice or licorice is the common name of *Glycyrrhiza glabra*, a flowering plant of the bean family Fabaceae, from the root of which a sweet, aromatic flavouring is extracted. The liquorice plant is an herbaceous perennial legume native to Western Asia, North Africa, and Southern Europe.

Kingdom: Plantae
Clade : Tracheophytes
Clade : Angiosperm
Clade : Eudicots
Clade : Rosids
Order : Fabales
Family : Fabaceae
Subfamily: Faboideae
Clade : Inverted repeat-lacking clade
Genus : Glycyrrhiza
Species : *G. glabra*

- **Chemical constituent**

1. **Triterpenoid Saponins:** Glycyrrhizin, Glycyrrhetinic Acid
2. **Flavonoids:** Liquiritin, Isoliquiritin, Glabridin, Licochalcone A
3. **Coumarins:** Herniarin, Umbelliferone
4. **Polysaccharides:** These compounds contribute to the demulcent (soothing) and expectorant properties of liquorice, making it effective in treating coughs and sore throats.
5. **Sterols:** β-Sitosterol, Stigmasterol
6. **Amino Acids:** Liquorice contains essential amino acids that contribute to its nutritional and therapeutic properties.
7. **Essential Oils:** Anethole, Eugenol

- **Pharmacological activity**

1. **Anti-inflammatory Activity**

Liquorice has significant anti-inflammatory properties. Glycyrrhizin and its metabolite, glycyrrhetinic acid, inhibit the enzyme 11β-hydroxysteroid dehydrogenase, which leads to increased local concentrations of cortisol and hence an anti-inflammatory effect.

2. Antiviral Activity
Liquorice has demonstrated antiviral effects against various viruses, including hepatitis C, herpes simplex, and HIV. Glycyrrhizin interferes with viral replication and boosts the immune response against viral infections.

3. Antimicrobial Activity
Liquorice exhibits antimicrobial properties against a range of bacteria and fungi. It has been shown to be effective against *Streptococcus mutans*, the bacteria responsible for dental cavities, and Helicobacter pylori, which causes stomach ulcers.

4. Hepatoprotective Effects
Liquorice is known for its liver-protective properties. Glycyrrhizin helps to reduce liver damage and fibrosis, making it useful in managing chronic liver diseases such as hepatitis.

5. Anti-ulcer Activity
Liquorice helps in the treatment of peptic ulcers. Deglycyrrhizinated liquorice (DGL) is often used to soothe and heal ulcers by promoting mucus production and cell regeneration in the stomach lining.

6. Antioxidant Properties
Liquorice contains flavonoids and other compounds that exhibit strong antioxidant properties, protecting cells from oxidative damage and reducing the risk of chronic diseases such as cancer and cardiovascular diseases.

7. Immunomodulatory Effects
Liquorice modulates the immune system, enhancing the body's defense mechanisms. It has been shown to stimulate the production of interferon, a key component of the immune response to viral infections.

8. Endocrine Effects
Liquorice affects the endocrine system by mimicking the action of certain hormones. For example, it can lead to elevated levels of aldosterone, causing sodium retention and potassium loss, which is important to consider in patients with hypertension or other cardiovascular issues.

9. Anti-cancer Activity
Some studies suggest that liquorice has anti-cancer properties, with its components inhibiting the growth of cancer cells and inducing apoptosis. The flavonoids in liquorice contribute to these effects by interfering with various signaling pathways involved in cancer progression.

10. Respiratory Benefits
Liquorice is used in the treatment of respiratory conditions like asthma, bronchitis, and cough. Its expectorant properties help to clear mucus from the airways, while its soothing action relieves throat irritation [17].

Materials and Instruments

Table 1: Instruments used for work

Sr. No.	Name Of Instrument
1.	Soxhlet Apparatus
2.	Electronic weighing balance
3.	pH meter
4.	Brookfield viscometer
5.	Heating mantle
6.	Electronic water bath

Table 2: Chemicals used for work:

Sr. No.	Chemicals
1.	Ethanol
2.	Clove oil
3.	Cinnamon oil
4.	Stearic acid
5.	Cetyl alcohol
6.	Potassium hydroxide
7.	Glycerine
8.	Propyl paraben
9.	Methyl paraben
10.	Distilled water

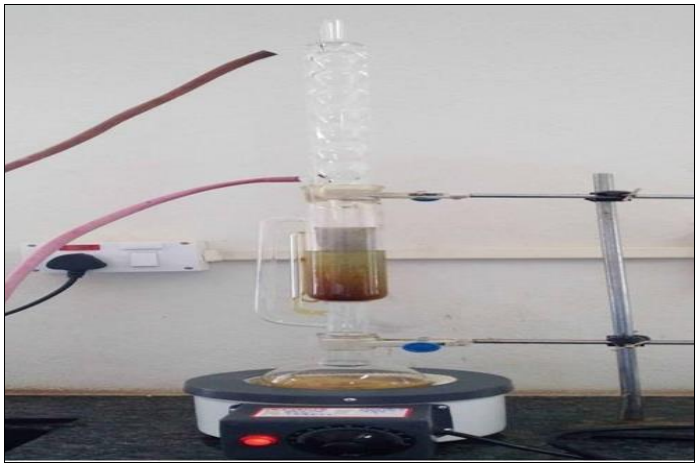

Fig 1: Soxhelet Apparatus

Experimental Methods

- **Extraction**
- **Preparation of Ethanolic Extract of polyherbs**

- The leaves of polyherbswere carefully selected washed to remove impurities and shade dried.
- The dried material was reduced to fine powder in the mechanical grinder.
- The fine powder was passed through sieve no.43 and stored in an airtight container for further use.
- About 100 gm of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus.
- The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in the test tube during the completion of the cycle and chemical test of the solvent was performed.
- After each extraction, the extract was evaporated to dryness in rotary vacuum evaporator.

Moreover, some part of the extract was preserved for preliminary Phytochemical screening for the detection of various plant constituents and rest extract was used for formulation of batch [18].

Preliminary phytochemical investigation

The ethanolic extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, saponins and glycosides. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. The following procedures were adopted to test for the presence of various chemical constituents in extract.

Test for saponins

Foam test

A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

Test for alkaloids

a) Mayer's test: 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt.

b) Wagner's test: 2-3 ml of filtrate with few drops of Wagner's reagent gives Reddish brown colour.

Test for tannins

Ferric chloride test

To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of Tannins.

For steroids

Liebermann's reaction

Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H2SO4. Blue
color appears.

Test for flavanoids

Alkaline reagent test

Test solution when treated with sodium hydroxide solution shows increase in the intensity yellow colour which becomes colourless on addition of few drops of dilute acid.

Test for terpenoids

Salkowski reaction

To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H_2SO_4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Test for reducing sugar

Benedict's test

Mix equal volume of Benedict's reagent and test extract in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Test for proteins

Biuret test

a) Add 2 ml of Biuret reagent to 2 ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.
b) To 3 ml. extract add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violate or pink colour appears [19].

Preformulating study

Preformulating studies are needed to ensure the development of a stable as well as effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the physic-chemical properties of the drug substances and its interaction with various formulation components. Goals of Preformulation study:

- To determine the necessary physicochemical parameter of a new drug substance.
- To establish its incompatibility with excipients of formulation.

Experimental Design

- **Formulation of Herbal Cream**
- **Preparation of herbal Cream**

Selection of excipients

Neem, Guduchi, Mint is collected from the ayurvedic shop from Kolhapur. The raw materials and chemicals were taken from Ashokrao mane institute of pharmacy, ambap, kolhapur. All ingredients and excipients used are given in the Table.

Methods of preparation

Water in oil emulsion-based cream was formulated. The emollient steric acid melted at 75C then add Cetyl alcohol and wait until it dissolves then add clove oil, and cinnamon oil. This is the oil phase (Part A) of our emulsion. Simultaneously prepare the water phase (Part B) by dissolving the ethanolic extract of Neem, Guduchi, Mint, KOH solution, Glycerine, methyl paraben, propyl paraben in water. Apply heating at a temperature of 75C. Immediately after heating pour the oil phase into the mortar. Add the water phase in small portions into the oil phase by continuously stirring with the help of a pestle. The colling of emollients causes the development of cream formulation. Formulation ingredients of A1 A2 A3 with the quantity required are shown in the following table [20].

Formulation table

Table 3: Evaluation of Cream

Sr. No.	Ingredients	Batches			Role of ingredient
		A1	A2	A3	
1	Ethanolic extract	0.5 gm	0.5 gm	0.5 gm	Therapeutic agent
2	Clove oil	1 ml	1 ml	1 ml	Active ingredient
3	Cinnamon oil	1 ml	1 ml	1 ml	Active ingredient
4	Stearic acid	5 gm	5 gm	5 gm	Emollient
5	Cetyl alcohol	1.6 gm	1.6 gm	1.6 gm	Emollient, Co-emulsifier
6	Potassium hydroxide	0.5 gm	0.5 gm	0.5 gm	Alkali reagent, Emulsifier
7	Glycerine	15 ml	15 ml	15 ml	Moistening reagent
8	Propyl paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
9	Methyl Paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
10	Distilled water	qs	qs	qs	Vehicle

1. Physical Evaluation

Physical parameters such as color and appearance were evaluated.

2. Homogeneity

All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container for their appearance and presence of any aggregates.

3. pH

The pH of various creams formulations were determined by using digital pH meter. 2.5 gm of cream was accurately weighed and dispersed in 25 ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried out in triplicate and the average values are represented. The pH of dispersions was measured using pH meter.

4. Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of creams. An excess of creams (about 2 g) under study was placed on this ground slide. The cream was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. Weight of 1 kg was placed on the top of the slide for 5 minutes to expel air and to provide a uniform film of the cream between the slides. Excess of the cream was scrapped off from the edges. The top plate was then subjected to pull of 50 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 6.5 cm be noted. A shorter interval indicates better spreadability.

Spreadability was calculated using the following formula:

$S = M \times L / T$

Where, S = Spreadability,
M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and
T = Time (in sec.) taken to separate the slide completely each other.

5. Viscosity

Viscosity of herbal cream was determined by using Brookfield rotational viscometer at 5, 10 20, 30 and 50 rpm using spindle no.64. Each reading was taken after equilibrium of the sample at the end of two minutes. The viscosity determination of samples was repeated three times [21-24].

Anti-bacterial Activity In Vitro Techniques

1. Preparation of Agar Medium

1. Prepare nutrient agar (2.8 gm nutrient agar dissolve in 100 ml of distilled water). Media should be prepared using distilled water or deionized water
2. Heat with frequent agitation and boil to dissolve the medium completely. Sterilize by autoclaving at 121 °C for 15 min.
3. Check the pH of each preparation after it is sterilized, which should be between 7.2 and at room temperature. This is done by macerating a small amount of medium in a little distilled water or by allowing a little amount of medium to gel around a pH meter electrode.
4. Cool the agar medium to 40-50 °C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4 mm.
5. Allow to solidify.
6. Prior to use, dry plates at 30-37 °C in an incubator, with lids partly ajar, for not more than 30 minutes or until excess surface moisture has evaporated. Media must be moist but free of water droplets on the surface. Presence of water droplets may result to swarming bacterial growth, which could give inaccurate results. They are also easily contaminated [25-31].

2. Agar Well Diffusion Method

1. First of all, we have to prepare the media, that is nutrient agar and nutrient broth.
2. Then the agar is poured into the plates to grow the tested bacteria or organisms.
3. The broth is required for growing the tested bacteria.
4. When the media are prepared, a loop of tested bacteria like *E. coli*, and *S. aureus* were taken and added in 2-3 mL of MHB and incubated at 37 degrees Celsius.
5. Now, after 24 hr the bacteria were taken out and the turbidity of the bacteria was matched with Mc Farland (Barium chloride + sulfuric acid) to maintain the approx. amount of bacteria in each plate.
6. After matching the turbidity, by the process of a cotton bud, the bacteria were swapped on nutrient agar plates with continuous rotation at a 60-degree angle.
7. Then after drying, the well was made in the plates with borer and positive, and negative control, and extracts were added to that well.
8. Finally, it was incubated for 24 hrs at 37-degree celsius and was observed.
9. After 24 hrs, the zone of inhibition was measured. That is the measurement of inhibiting the growth of bacteria by the extracts [32].

Results and Discussion

Extraction of Tulsi, Liquorice and Betel Leaf.

Table 4: Extractive values of Neem, Guduchi, Mint

Sample	Extraction method	Solvent used	Wt. of sample	Extraction value (%w/w)
Tulsi, Liquorice, Betel leaf Fine powder	Soxhlet extraction	Ethanol	30 gm	10% w/w

Physicochemical evaluation of Cream

1. Physical Appearance

All formulation batches were found to be homogeneous Green cream preparations.

Table 5: Physical appearance of cream

Sr. No.	Batch	Color	Appearance
1	A1	Cream white	white
2	A2	White	white
3	A3	Milky white	white

2. Homogeneity

All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container.

Table 6: Homogeneity of formulation

Sr. No.	Batch	Homogeneity
1	A1	Homogeneous
2	A2	Homogeneous
3	A3	Homogeneous

3. Measurement of pH

The pH values of all prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Table 7: pH of extracts formulation.

Sr. No.	Batch	pH
1	A1	6.8 /±0.03
2	A2	7.0/±0.03
3	A3	7.2/±0.03

5. Spreadability

The time in seconds require to separate the two slides was taken as measure of spreadability.

Table 8: Spreadability of extracts formulation.

Sr. No.	Batch	Spreadability (gm.sm/sec)
1	A1	16.15/±0.005
2	A2	15.50/±0.005
3	A3	15.35/±0.005

Table 9: The samples were repeated three times.

Sr. No.	rpm	Viscosity (Cps)
1	5	3465±0.31
2	10	3670±0.29
3	20	3807±0.37

Table 10: Zone of Inhibition

Sr. No.	Details	Concn. (%)	Zone of Inhibition (*E coli*)
1	Cephalosporin	100	10
2	Cream	100	20
3	Extract	10	8
4	Saline water	5	No zone

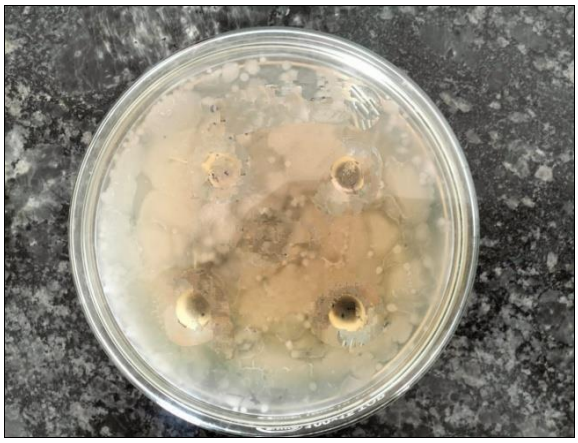

Fig 2: Zone of Inhibition

6. Viscosity

Viscosity of cream was determined by using Brookfield rotational viscometer at 5, 10, 20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes.

Conclusion

The study highlights the immense potential of medicinal plants, especially in the formulation of topical treatments like creams. The skin, the largest organ of the body, provides a crucial barrier against environmental stressors and is indicative of overall health. Herbal medicine, involving the use of plant parts such as leaves, stems, and roots, has long been utilized to treat various ailments due to its cost-effectiveness and safety. The effectiveness of these plants lies in their bioactive compounds, such as alkaloids, flavonoids, and tannins, which offer a range of therapeutic properties including antimicrobial, anti-inflammatory, and antioxidant effects.

In particular, polyherbal formulations, which combine multiple plants, have been shown to enhance therapeutic outcomes through synergistic effects. Tulsi, betel leaf, and liquorice are notable for their extensive pharmacological activities. Tulsi is revered for its antimicrobial, anti-inflammatory, and adaptogenic properties; betel leaf offers antimicrobial, anti-inflammatory, and anti-cancer benefits; and liquorice is known for its anti-inflammatory, antiviral, and hepatoprotective effects. The research aims to develop an anti-fungal polyherbal cream leveraging these plants' properties, emphasizing the need for rigorous phytochemical screening and standardization to ensure efficacy and global acceptance. The study's findings are poised to support the Ayurvedic industry in creating standardized, effective herbal formulations, highlighting the enduring value of traditional medicinal knowledge in modern therapeutic applications.

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