A REVIEW:- STANDARDISATION AND EVALUATION OF ROSEMARY GEL AS A ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY

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ABSTRACT:

Rosemary (Rosmarinus officinalis L.), a fragrant evergreen herb from the mint family (Lamiaceae), is well known for its powerful health benefits. Its leaves are packed with natural compounds like rosmarinic acid, carnosic acid, and essential oils that have strong antioxidant, anti-inflammatory, and antimicrobial properties. Recent studies suggest that rosemary may help in treating infections, metabolic problems, and brain-related conditions.This research focuses on how rosemary and its key compounds can protect and heal the skin. It explores the latest findings on how these compounds work at the molecular level to fight inflammation, speed up wound healing, prevent infections, and even show promise in cancer prevention. The study also highlights the rich phytochemicals in rosemary and its potential use in skincare, especially for its antimicrobial and antifungal powers.

Keywords: Rosemary, Gel formulation, Antimicrobial activity, Antifungal activity, Standardization.

INTRODUCTION:

Microbial infections are a serious global health concern, causing illness and even death in many parts of the world. These infections are caused by harmful microorganisms like bacteria and fungi, some of which have become resistant to commonly used drugs. Bacterial infections from organisms such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa can cause anything from mild skin problems to severe conditions like pneumonia or sepsis. Fungal infections, such as those caused by Candida albicans or Aspergillus species, can be especially dangerous for people with weakened immune systems.Because of the growing resistance to traditional antibiotics and antifungal treatments, there is an urgent need for new and effective alternatives. One promising solution is the use of plant- based natural products with healing and antimicrobial properties.This study focuses on Rosemary (Rosmarinus officinalis), a medicinal plant known for its strong antioxidant and antimicrobial effects. The aim was to develop a topical (oil-in-water) formulation using rosemary extract to fight against harmful bacteria and fungi. Four different extraction methods were tested, and the best-performing extract was used in the final formulation. The product was then studied for its effectiveness, stability, and potential use in treating skin infections.

Things that can cause skin infections:

- Small cuts, wounds, or scrapes
- Not keeping the skin clean or too much moisture
- A weak immune system
- Sharing personal items that aren't clean (like towels or razors)
- Touching infected people or dirty surfaces

Rosemary isn't just a flavorful herb—it's packed with amazing health benefits! One superstar compound, rosmarinic acid, does so much more than fight infections and reduce inflammation. Recent research shows it can even help fight cancer by targeting different parts of cancer cells.In the U.S. and Europe, rosemary is popular not only as a tasty spice but also as a natural antioxidant used to keep food fresh and support health. Its extracts have been studied for protecting the liver, helping with Alzheimer's disease, and stopping the growth of new blood vessels that feed tumors.Some special chemicals in rosemary, like 1,8-cineole and alpha-pinene, can actually break through bacterial walls, causing harmful bacteria to leak and die. Others, like carnosic acid and rosmarinic acid, stop bacteria from growing by blocking their enzymes and protein-making processes.Rosemary works against a wide range of bacteria, including tough, drug-resistant strains like E. coli and the fungus Candida albicans. This power comes from its rich mix of natural compounds, including carnosic acid, rosmarinic acid, chlorogenic acid, camphor, and more.All these amazing chemicals make rosemary a natural powerhouse for fighting infections, protecting your body, and even supporting overall health.

Rosemary Leaf and Its Plant Family

Rosemary is part of the Lamiaceae family, which is one of the biggest and most important groups of flowering plants. This family has about 236 groups (called genera) and between 6,900 and 7,200 species all over the world.
It used to be called Labiatae because

their flowers have petals shaped like two lips — a top and a bottom lip — but most scientists now use the name Lamiaceae.This family is famous for plants that produce strong, natural oils. Many well-known herbs belong to this family, like basil, lavender, mint, rosemary, sage, and thyme. These herbs are used both for cooking and for their health benefits.Many plants in this family contain important natural chemicals like terpenes, iridoids, flavonoids, and phenolic compounds.

They also have large amounts of phenolic acids such as rosmarinic acid, which help fight bacteria and viruses, reduce inflammation, and act as antioxidants to protect the body.

Scientific classification of Rosmarinus officinalis L :

Kingdom: Plantae

Sub kingdom:Tracheobionta Super division:Spermatophyta Division: Magnoliophyta Class:Magnoliopsida

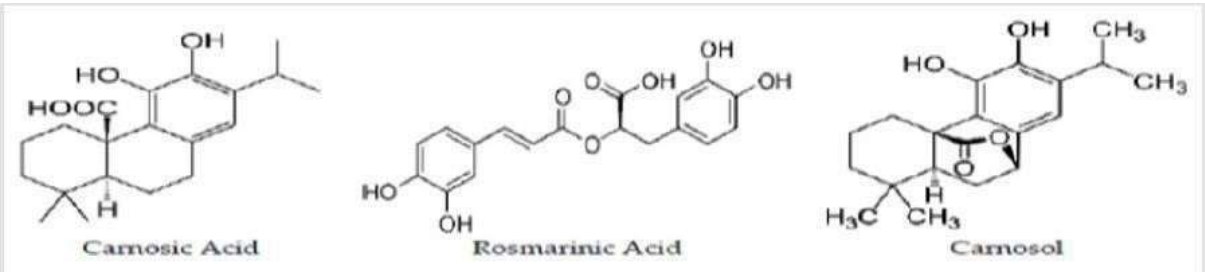
Sub class: Asteridae

rder:Lamiales Family: Lamiaceae Genus:Rosmarinus L.

Species: officinalis
Binomial nomenclature : Rosmarinus officinalis L.

Rosemary is a dense, bushy plant that stays green all year and grows up to about 1 meter tall. It has small, narrow leaves that are 1–4 cm long and 2–4 mm wide. The leaves are leathery, dark green on top, and lighter with a fuzzy texture underneath. They have curved edges, a strong central vein, and a very distinctive smell.
In traditional medicine, rosemary leaves are used because they can fight bacteria .

Natural Compounds in Rosemary



Rosemary is much more than just a fragrant herb used in cooking—it's a treasure chest full of powerful natural compounds that bring amazing health benefits. Packed with special plant chemicals called phytochemicals, rosemary contains important groups like phenolic

acids, flavonoids, diterpenes**,** and essential oils that work together to support your body.One superstar compound is rosmarinic acid, known for its strong ability to fight inflammation and protect cells from damage with its antioxidant power. Alongside it, carnosic acid and carnosol shine as defenders against harmful microbes and protectors of brain health.

Rosemary's chemical makeup is quite complex, with other helpful players like rosmanol, rosmadial, and flavonoids adding to its wide range of benefits. The essential oils in rosemary—such as 1,8-cineole, camphor, α- pinene, and borneol—also contribute to its impressive antioxidant and antimicrobial actions.

Antimicrobial Activity:

The antibacterial activity of rosemary has been determined in various assay types based on either MIC or MBC. In this regard, Sienkiewicz et al.rosemary (Rosmarinus officinalis, L.) These authors reported the inhibition of microbial growth by both essential oils, presented as MIC values. Antibiotic susceptibility was carried out using disc diffusion. The inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol. They interact with the cell membrane, causing changes in genetic material and nutrients, altering the transport of electrons, leakage of cellular components and production changes in fatty acid. In addition, it also produced an interaction with the membrane of proteins that produced the loss of membrane functionality and its structure Portugal.

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The antimicrobial activities of EOs of rosemary and sage against 13 bacterial strains and 6 fungi by the microdilution technique. Compared with bifonazole, rosemary EO showed better antifungal activities especially against C. albicans, Trichophyton tonsurans (T. tonsurans), and Trichophyton rubrum at lower MICs (15.0–30.2 μL).

Antifungal Activity:

The antifungal activity was evaluÀated by testing the plant as a powder in the culture medium, testing the EOs by fumigation and incorporation into the culture medium, and testing the MEs using the well-diffusion method. The radial growth of each tested fungus was measured daily and compared to that of the control. The inhibition percentage was calculated on the 7th day for all applied techniques. In general, all the concentrations applied by the different methods have significantly reduced or completely inhibited the growth of the tested mold. Anti- fungal chemotherapy is used in the treatment of both superficial and deep fungal infection. Figure 1 show fungal infections commonly seen in the different layers of skin. The antifungal activity of rosemary essential oil was tested against Candida albicans, Candida dubliniensis, Candida parapsilosis, and Candida krusei. Such dermatophytes are the most common agents causing topical mycoses . It was found that an oil concentration of 8% was capable of inhibiting the growth of Candida sp. A similar study evaluated the effect of R. officinalis hydroalcoholic extract against two dermatophytes, Microsporum gypseum and Trichophyton rubrum, and showed that a concentration of 10% R. officinalis extract was responsible for 86% inhibition of fungal growth.

Skin Diseases & Conditions Treated by Rosemary Gel :

1. **Fungal Infections** Ringworm (Tinea corporis) Athlete's foot (Tinea pedis)

Candidiasis (skin yeast infection.

2. **Bacterial Skin Infections**

Acne (caused by Propionibacterium acnes) Impetigo (mild cases)

Minor wound infections.

3. **Inflammatory Skin Conditions**

Eczema (Atopic dermatitis) – helps reduce redness and itching Psoriasis – soothes scaling and inflammation.

4. **Other Skin Issues**

Burns and minor cuts (due to wound-healing and antiseptic effects) Insect bites and stings (anti-inflammatory action)

Hyperpigmentation and premature aging (antioxidant activity protecting skin cells)

Ringworm fungal infection:

Ringworm of the body (tinea corporis) is a rash caused by a fungal infection. It's usually an itchy, circular rash with clearer skin in the middle. Ringworm gets its name because of its appearance. No worm is involved.Tinea corporis is most commonly caused by dermatophytes belonging to one of the three genera, namely, Trichophyton (which causes infections on skin, hair, and nails), Microsporum (which causes infections on skin and hair), and Epidermophyton (which causes infections on skin and nails).While tinea corporis occurs worldwide, it is most commonly observed in tropical regions.42 The lifetime risk of acquiring tinea corporis is estimated to be 10– 20%.6 Tinea corporis occurs most frequently in post-pubertal children and young adults.

Ringworm on an arm:

Ringworm often causes a ring-shaped rash that is itchy, scaly and slightly raised. The rings usually start small and then expand outward. Ringworm of the body is related to athlete's foot (tinea pedis), jock itch (tinea cruris) and ringworm of the scalp (tinea capitis). Ringworm often spreads by direct skin-to-skin contact with an infected person or animal. Mild ringworm often responds to antifungal medications applied to the skin. For more-severe infections, you may need to take antifungal pills for several weeks.

Symptoms : Signs and symptoms of ringworm may include:

A scaly ring-shaped area, typically on the buttocks, trunk, arms and legs Itchiness
A clear or scaly area inside the ring, perhaps with a scattering of bumps whose color ranges from red on white skin to reddish, purplish, brown or gray on black and brown skin
Slightly raised, expanding rings A round, flat patch of itchy skin Overlapping rings.

Causes :
Ringworm is a contagious fungal infection caused by common mold-like parasites that live on the cells in the outer layer of your skin. It can be spread in the following ways:

Animal to human : You can contract ringworm by touching an animal with ringworm. Ringworm can spread while petting or grooming dogs or cats. It's also fairly common in cows.

Object to human : It's possible for ringworm to spread by contact with objects or surfaces that an infected person or animal has recently touched or rubbed against, such as clothing, towels, bedding and linens, combs, and brushes.

Soil to human : In rare cases, ringworm can be spread to humans by contact with infected soil. Infection would most likely occur only from prolonged contact with highly infected soil.

Treatment :

The standard treatment of tinea corporis is with topical antifungals and there is evidence of the superiority of topical antifungals over the use of placebo.84,98 Localized or superficial tinea corporis usually responds to topical antifungal therapy applied to the lesion and at least 2 cm beyond the lesion once or twice daily for 2–4 weeks.24 Commonly used topical antifungal agents include azoles (e.g. econazole, ketoconazole, miconazole, clotrimazole, miconazole, oxiconazole, sulconazole, sertaconazole, eberconazole, and luliconazole), allylamines (e.g. naftifine, terbinafine), benzylamine (butenafine), ciclopirox, and tolnaftate.18,24,99–110 In this regard, nystatin, which is an effective treatment for Candida infections, is not effective for tinea corporis.24 In a 2013 meta-analysis of 65 trials (trials with a common comparator and head-to-head trials) involving 14 topical antifungals, there was no significant difference among the antifungals regarding the outcome of mycologic cure at the end of the treatment.Some authors suggest the addition of a topical corticosteroid to the topical antifungal agent, especially in individuals with inflammatory dermatomycosis.

MATERIALS AND METHOD:

Plant material: Fresh rosemary leaves were collected, authenticated, and shade dried.

Chemicals: Ethenol**,** carbopol 934, triethanolamine, PEG 400, and distilled water.

Microbial strains: E. coli, S. aureus, C. albicans, A. niger.

Authentication of Plant Material:

Dried rosemary (Rosmarinus officinalis L.) leaves were purchased from a certified online herbal store. The plant material was authenticated based on its morphological characteristics such as color, odor, and texture, in comparison with standard descriptions available in the Indian Pharmacopoeia. The identification was confirmed by a qualified botanist, and the specimen was preserved for future reference.

Extraction of Rosemary Esenssial Oil By Hydrodistilation Methods : Materials :

- **Fresh rosemary leaves** – 500 g
- **Distilled water** – 1.5 liters (approx.)
- **Anhydrous sodium sulfate** – a few grams (optional, for drying oil)
- **Filter paper** – as needed

Apparatus :

- Clevenger-type hydrodistillation apparatus
- Round-bottom flask (1–2 L capacity)
- Liebig condenser
- Heating mantle or hot plate
- Measuring cylinder
- Pipette or syringe
- Amber glass vials (10 mL)
- Beakers, funnel
- Digital weighing balance
- Thermometer (optional)

Methodology (Step-by-Step Procedure) :

1. To begin the experiment, 500 grams of fresh rosemary leaves are collected and thoroughly washed to remove dirt and impurities. The leaves are air-dried at room temperature for about 30–60 minutes to remove surface moisture. They are then chopped into smaller pieces using a clean knife to increase the surface area, which facilitates better extraction.
2. A Clevenger-type hydrodistillation apparatus is set up, including a 1-liter round-bottom flask, a Liebig condenser, and an oil collection arm. The chopped rosemary leaves (500 g) are placed into the round-bottom flask, and approximately 1.5 liters of distilled water are added, enough to completely submerge the plant material. The setup is securely connected and checked for airtightness.
3. The flask is heated using a heating mantle, and the mixture is brought to a gentle boil. As the water boils, steam passes through the rosemary leaves, carrying volatile essential oil vapors into the condenser. These vapors condense into liquid form, collecting in the oil separator arm of the apparatus. The essential oil floats on top of the water layer due to its lower density.
4. The distillation continues for approximately 3 hours, or until no more oil is observed forming in the collection tube. After cooling, the essential oil (6 mL) is carefully collected using a pipette and dried with anhydrous sodium sulfate to remove residual water. The purified oil is transferred into a clean, labeled amber glass vial for storage.

HYDRODISTILLATION METHOD :

Principle :

Hydrodistillation is a technique for isolating essential oils from plants by applying heat to water and plant material together. When heated, the water produces steam that carries the plant's fragrant and volatile compounds. This steam mixture is then cooled down to turn back into liquid, allowing the essential oil to separate from the water due to their different densities.

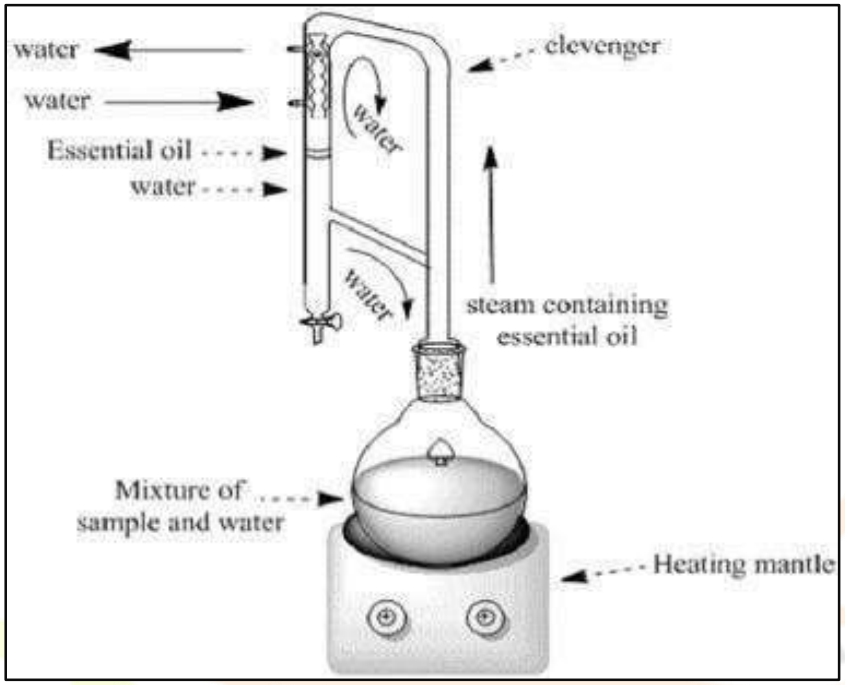

Fig. Hydrodistilation Setup Method

USES:

- **Extraction of Essential Oils**

It is widely used to extract essential oils from aromatic plants such as lavender, peppermint, eucalyptus, and rose. The essential oils have applications in perfumery, aromatherapy, cosmetics, and flavoring.

- **Isolation of Volatile Compounds**

Hydrodistillation helps isolate volatile compounds from plant tissues for chemical analysis and research.

- **Food Industry**

Essential oils obtained via hydrodistillation are used as natural flavoring agents and preservatives in food products.

- **Quality Control and Research**

Researchers use hydrodistillation to study the chemical composition of plant materials and monitor quality in commercial products.

EVALUATION OF ROSEMARY OIL :

1. Physical Evaluation

Parameter	**Method/Instrument Expected**	**Observation/Result**
Colour	Visual examination	Pale yellow to light green

Odour	Organoleptic	Organoleptic
Appearance	Visual Clear	transparent liquid
Solubility	Solvent test	Soluble in alcohol and oils, insoluble in water

2. Chemical Evaluation

Parameter	**Test/Principle**	**Observation/Inference**
Acid Value	Titration with KOH	Determines free fatty acids (purity indicator)
Saponification Value	Reflux with alcoholic KOH	Indicates molecular weight of esters present
Peroxide Value	Iodometric titration	Measures oxidation/rancidity level

3. Phytochemical Tests

These tests show what active compounds are present in rosemary oil.

Compound	Test Result
Phenols	Present
Flavonoids	Present
Alkaloids	Present
Tannins	Present
Saponins	Present
Terpenoids	Present

Main active chemicals : cineole, camphor, α-pinene, borneol, and rosmarinic acid. These give the oil its medicinal and aromatic properties.

4. Instrumental Tests

Used to check chemical compounds more deeply.

Instrument	Purpose
FTIR	Shows types of bonds or functional groups
UV- Visible Spectrophotometer	Measures phenols and flavonoids
Viscometer	Measures phenols and flavonoids

Phytochemical Screening in Rosemary Oil :

This is a preliminary test done to find out which bioactive compounds (like phenolics, flavonoids, alkaloids, saponins, tannins, etc.) are present in a plant or its extract.

1. Phenolics

Examples: Rosmarinic acid, Carnosic acid, Carnosol Activity: Strong antioxidants, protect cells from damage.

2. Flavonoids

Examples: Apigenin, Luteolin, Diosmin

Activity: Antioxidant, anti-inflammatory, some antibacterial effects.

3. Alkaloids

Activity: Antimicrobial, helps in pain relief.

4. Saponins

Activity: Fight bacteria and fungi, support immune system.

5. Tannins

Activity: Antibacterial, antiviral, and astringent (tighten tissues)

6. Essential Oils

Examples: 1,8-Cineole, Camphor, α-Pinene, Borneol

Activity: Antimicrobial, antifungal, anti-inflammatory, aromatic.

The phytochemical screening of rosemary (Rosmarinus officinalis) showed that the plant contains useful natural compounds such as phenolics, flavonoids, alkaloids, saponins, and tannins. These were found more in extracts made with methanol and acetone than in water extracts. The methanol extract had the highest amount of phenolic and flavonoid compounds, which are good for health. When tested against bacteria, the acetone extract showed the best antibacterial activity. This means rosemary oil has strong natural properties that can help fight germs and may be a good alternative to chemical antibiotics.

Formulation in Gel :

Sr. No.	Ingredients	Quantity	Use / Function
1.	Rosemary oil	4 ml	Acts as the active ingredient — provides antimicrobial, antioxidant, and anti- inflammatory effects.
2.	Carbopol 934	0.3 gm	Used as a gelling agent to form the gel base and provide viscosity.
3.	Ethanol	0.8 ml	Serves as a co-solvent and has antimicrobial and preservative properties.
4.	PEG 400	0.8 ml	Acts as a humectant and solvent, helps mix oil and water phases, and keeps the gel smooth.
5.	Distilled water	12 ml	Used as a vehicle or solvent to dissolve and disperse other ingredients.

Preparation of Gel :

Equipment : analytical balance, graduated pipettes/syringes, 2 beakers (50–100 ml), magnetic stirrer or glass rod, pH paper or pH meter, spatula, amber jar for storage, PPE (gloves, goggles, lab coat).

1. Weigh & measure:

Weigh –0.3 g Carbopol 934 accurately. Measure 5 ml distilled water into a clean beaker.

2. Hydrate Carbopol:

Slowly sprinkle the Carbopol into the 5 ml water while stirring (magnetic stirrer or manual). Avoid clumping. Stir until uniformly dispersed.

Cover and let hydrate — overnight is ideal. If short on time, hydrate minimum 2–4 hours until smooth.

3. Make oil phase:

In a separate small beaker combine 4.0 ml rosemary oil + 0.8 ml PEG 400 + 0.8 ml ethanol. Mix until uniform.

4. Incorporate oil into gel base:

With the Carbopol dispersion stirring, slowly add the oil mixture in a thin stream or dropwise while stirring continuously. Do this slowly to avoid phase separation. Continue stirring for 5–10 minutes to homogenize.

5. Bring to final water volume:

Add the remaining 7 ml distilled water slowly while stirring so the total water becomes 12 ml. Stir gently until uniform.

6. Adjust pH (gelation step):

Measure pH. Then add TEA dropwise (use a pipette) while stirring. After each drop wait ~30–60 s and re-check pH. Stop when pH reaches 4.5–5.5.

7. Homogenize & deaerate:

Stir gently to remove lumps and evenly distribute oil. If foam/bubbles are present, let the gel stand 10– 30 min or vacuum-degas briefly.

8. Final checks & corrections:

Check appearance (smooth, homogeneous), smell, and pH (4.5–5.5).

If too thin → next batch increase Carbopol a little (e.g., +0.05 g). If too stiff → add small amounts of distilled water (0.2–0.5 ml) and mix.

9. Packaging & storage:

Transfer gel into a clean amber jar. Label with formulation, date, and storage instructions. Store away from light at room temperature for short-term; refrigerate if no preservative and you want longer shelf- life.

10. Safety & note:

Wear gloves and eye protection. TEA and ethanol are irritants — handle carefully. Essential oils can cause skin sensitivity; perform patch test if for topical use. Use preservative if storing >2–4 weeks or if product will be used repeatedly.

Evaluation of Rosemary Gel :

Physical Evaluation :

1. **Colour –** to ensure it looks uniform.
2. **Consistency** – to see if the gel is smooth and not too thick or watery.
3. **Homogeneity –** to confirm all ingredients are mixed evenly.
4. **Smell (Odour) –** to check the fragrance of the gel.
5. **Touch (Feel)** – to test how it feels on the skin (soft, sticky, or smooth).

2. pH Test :

The pH of the gel was measured using a pH meter to make sure it is safe for skin (usually between 5–7).

3. Viscosity :

The viscosity (thickness) of the gel was measured using a Brookfield viscometer to know how easily the gel can be applied on the skin.

4. Spreadability :

The spreadability shows how easily the gel spreads on the surface.

It is tested by placing gel between two glass slides and measuring how quickly the slides move apart under a certain weight.

Formula:

S = \frac{M \times L}{T}

M = weight on the upper slide L = length of the glass slide
T = time taken to separate the slides

A shorter time means better spreadability.

5. Stability Studies :

The gel was kept at different temperatures for three months to check. Any change in pH

Any change in appearance or consistency This ensures the gel remains stable over time.

6. Extrudability:

Gel should come out easily from the tube/container.

7. Grittiness:

Should be smooth, no gritty particles.

8. Washability:

Should wash off easily with water.

1. Antimicrobial Study :

Test Microorganisms

Gram-positive bacteria: Staphylococcus aureus Gram-negative bacteria: Escherichia coli (E. coli) Standard (Control): Povidone Iodine

Method:

The Agar well diffusion method was used:

Nutrient agar plates were inoculated with bacterial strains.

Wells were made in the agar, and the rosemary gel was placed inside. Plates were incubated at 37°C for 24 hours.
After incubation, the zone of inhibition (clear area around the well) was measured in millimeters (mm). A larger zone of inhibition indicates stronger antibacterial activity.

Controls:

Positive control: Standard antibiotic or antifungal (e.g., Gentamicin for bacteria, Fluconazole for fungi) to compare effectiveness.

Negative control: Base gel without rosemary extract to ensure any effect is due to rosemary.

2. Antifungal Activity Study :

Test Organisms Candida albicans Aspergillus niger

Standard drug: Clotrimazole

Method :

The Agar well diffusion method was also used for antifungal testing:

Fungal cultures were spread on Sabouraud Dextrose Agar (SDA) plates.

Wells were filled with rosemary gel samples and incubated at 25–28°C for 48–72 hours. The zone of inhibition was measured to determine antifungal activity.

CONCLUSION :

The study on the standardization and evaluation of rosemary (Rosmarinus officinalis) confirmed that the plant possesses significant antimicrobial and antifungal activities. Standardization parameters such as organoleptic properties, physicochemical constants, and phytochemical analysis verified the quality, purity, and consistency of the rosemary sample.The antimicrobial evaluation revealed that rosemary extract effectively inhibited the growth of both Grampositive and Gram-negative bacteria, while the antifungal studies showed notable activity against common fungal pathogens such *as* Candida albicans *and* Aspergillus niger. *T*hese findings support the traditional use of rosemary as a natural antimicrobial and antifungal agent.In conclusion, standardized rosemary extract demonstrates strong potential as a natural source of antimicrobial and antifungal compounds, making it valuable for pharmaceutical, cosmetic, and therapeutic applications. Further studies on its active constituents and mechanisms of action are recommended to enhance its practical use in modern medicine.

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