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Formulation and Evaluation of Eucalyptus Oil Loaded In Herbal Gel for Its Antibacterial Activity

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ABSTRACT:
Originally, the majority of antibacterial compounds was derived from plants. The term "herbal medicine" describes the use of any seed or leaf oil for therapeutic purposes. In addition to conventional dosage forms, herbal medications are also made in gel form. A gel is a semisolid substance that has a gel-like consistency and is applied topically to various body parts. Formulating and evaluating an antibacterial herbal gel using local medicinal plants was the aim of the project. For the purpose to formulate gel, the oil from the chosen plants was extracted in various ratios at random.The topical formulation was created and evaluated for a uniformity drug content, physical parameters, and Spreadability (SP). As shown by the results, the MIC values for coriander and eucalyptus herbal gel against Bacillus subtilis and Staphylococcus aureus were 50% v/v and 50% v/v, respectively. The second formulation (ECG50) displayed the highest drug content (65%), as well as the formulation's maximum stability and zone of effect.
A human pathogen that can lead to infections of the skin or mucous membranes is the herpes simplex virus. Antibacterial, antimicrobial, antiviral, anti-inflammatory, and local analgesic properties are possessed by geranium, licorice, rosemary, sumac, and mellifluous plants. These herbs also have the effect of controlling the synthesis of viral proteins. The design, formulation, and assessment of the gel comprising these eucalyptus oils are the objectives of this study.
Keywords: - Antibacterial, Herbal Gel, Eucalyptus oil, Formulation, Evaluation.

I. INTRODUCTIONS:

The gels as semisolid systems consisting of either suspensions made up of small inorganic particles, or large organic molecules interpenetrated by a liquid. Where the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system. Single phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner in that no apparent boundaries between the dispersed macromolecules and the liquid. Single phase gels and jellies can be described as three-dimensional networks formed by adding macromolecules such as proteins, polysaccharides, and synthetic macro molecules to appropriate liquids. Many polymer gels exhibit reversibility between the gel state and sol, which is the fluid phase containing the dispersed or dissolved macromolecules. However, formation of some polymer gels is irreversible because their chains are covalently bonded.

The three-dimensional networks formed in two phase gels is and jellies is formed by several inorganic colloidal clays. Formation of these inorganic gels is reversible. This review focuses mainly on water-based gels and jellies. Gel structure, the basis for understanding the physical properties associated with gels, is examined forts, followed by the rheology of gels. The physical properties of gels and jellies can be classified based on two groups.

A) Transitional properties and rheological properties, yield point and rupture.
B) Spectrophotometric and thermal techniques are used to identify gel microstructures (physical junction zones) and their related transitional properties.

For example, nuclear magnetic resonance (NMR) spectroscopy measures the structural and dynamic characteristics of the polymer just prior to aggregation and gel formation and circular dichroism (CD) spectroscopy measures the conformational changes of the polymer during network formation.

Mechanical techniques are used to determine rheological properties of gels. These techniques employ either small deformation measurements that yield viscoelastic parameters or large deformation measurements that generate complete stress strain profile, which include failure parameters.

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The majority of gels are formed by the aggregation of colloidal sol particles, the solid or semisolid system so formed being interpenetrated by a liquid.

Gelation of lyophilic sols Gels formed by lyophobic sols can be divided into two group depending on the Nature of the bond between the chain of the network of gel.

Type1 are irreversible system with a three-dimensional network formed by covalent bond between the micro molecules.

Type 2 gels are held together by much weaker intermolecular bonds such as Hydrogen bond. These gels are heat reversible, a transition from the sol to gel occurring on either heating or cooling. Poly solution, for example, gel on cooling below a certain temperature referred to as the gel point. Because of their gelling properties poly are used as jellies for the application of drugs to the skin. On the application gel dried rapidly leaving a plastic film with the drug in intimate contact with the skin.

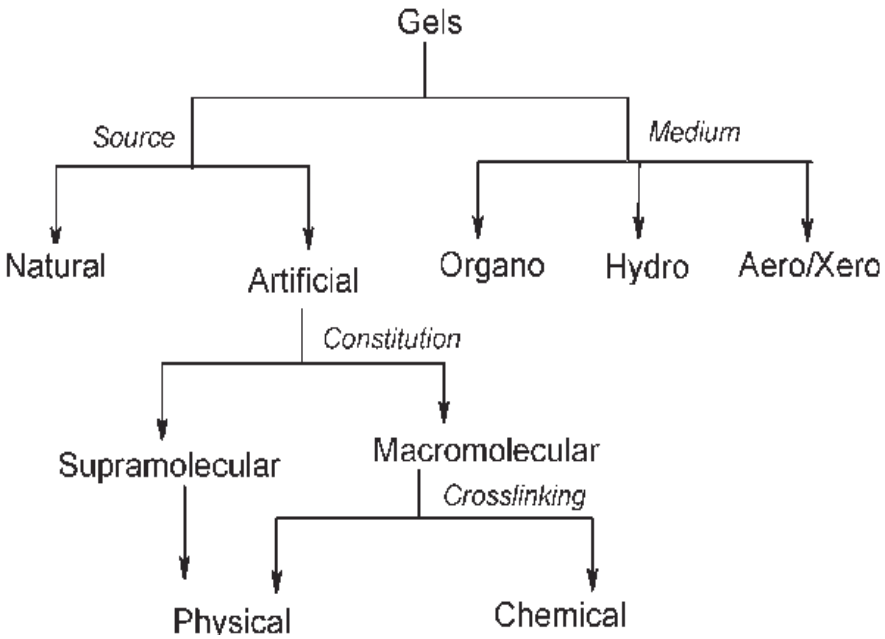

Fig.No.01: Classification of Gel

Topical Drug Delivery System:

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. 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 dominates 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:

1. Avoidance of first pass metabolism.
2. Convenient and easy to apply.
3. Reduction of doses as compared to oral dosage forms.
4. Improve patient compliance.
5. Provide suitability of self-medication.
6. Ability to easily terminate the medications, when needed
7. Ability to deliver drug more selectively to a specific site

Disadvantages of Topical Drug Delivery System:

1. Skin irritation of contact dermatitis may occur due to the drug and/or excipients.
2. Drug molecule with poor skin permeability cannot be given through topical drug delivery system.
3. Allergenic reactions may develop at the application site.

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4. Drug molecule with of larger particle size is difficult to absorb through the skin.

Anatomy of skin:

The skin is the largest organ in the body and has a surface area of about 1.5-2 square meters in adults. In many parts often body, it contains accessory structures: glands, hair and nails. It varies in thickness, being thickest on the palms of the hands and soles of the feet. There are two main layers: the superficial layer is called the epidermis, and the layer below is the dermis. Between the dermis and underlying structures is subcutaneous layer composed of areolar tissue and adipose (fat) tissue.

Epidermis:

This is composed of stratified keratinized squamous epithelium. There are no blood vessels on nerve endings in the epidermis, but its deeper layers are bathed in interstitial fluid from the dermis, which provides oxygen and nutrients, and drains away as lymph.

There are several layers (strata) of cells in the epidermis, which extend from the deepest basal layer to the most superficial stratum corneum (a thick horny layer).

The cells of the epidermis originate in the basal layer, which is made up of cubical nucleated, highly active epithelial cells that are constantly dividing. As new cells are formed,

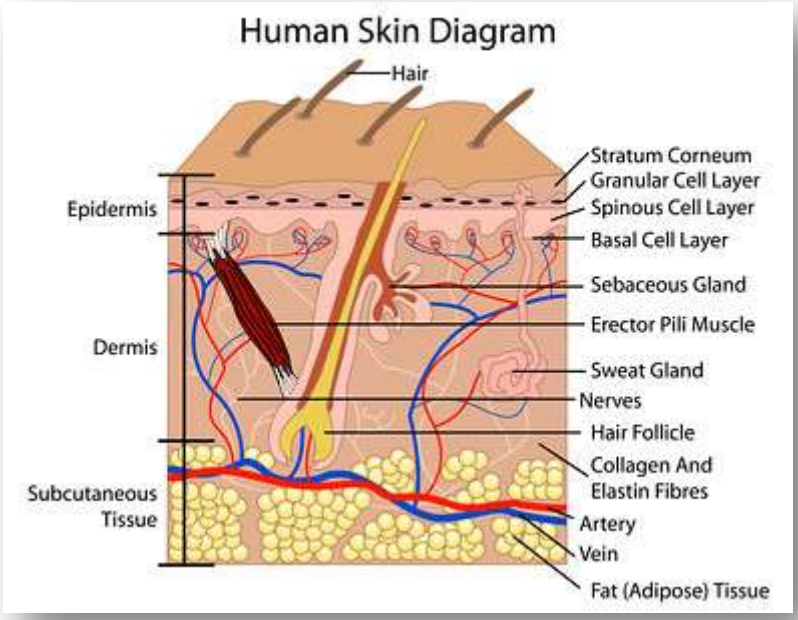

Fig.No.02 Anatomy of Skin

They are pushed upwards, away from the basal layer and further from their blood and nutrient supply. As they progress towards the skin surface, their shape and structure gradually change. By the time they reach the skin surface, they are flat, thin, non-nucleated, dead cells, or Squamosin which the cytoplasm has been replaced by the fibrous protein keratin. The surface cells are constantly rubbed off and replaced by those beneath. Complete replacement of the epidermis takes about a month. Hairs, secretions from sebaceous glands and ducts of Sweat glands pass through the epidermis to reach the skin surface. Upward projections of the dermal layer, the dermal papillae, anchor the dermis securely to the epidermis and allow passage and exchange of nutrients and wastes to the lower part of the epidermis. This arrangement stabilizes the two layers, preventing damage due to shearing forces.

Skin color is affected by various factors:

- Melanin, a dark pigment derived from the amino acid tyrosine and secreted by melanocytes in the deep germinative layer, is absorbed by surrounding epithelial cells. The amount is genetically determined and varies

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between different parts of the body, between people of the same ethnic origin and between ethnic groups. The number of melanocytes is fairly constant, so the differences in color depend on the amount of melanin secreted. It protects the skin from the harmful effects of ultraviolet rays in sunlight Exposure to sunlight promotes synthesis of melanin.

- Normal saturation of hemoglobin and the amount of blood circulating in the dermis give white skin its pink color. When oxygen saturation is very low, the skin in white people may appear bluish (cyanosis).

Dermis:

The dermis is tough and elastic. It is formed from connective tissue, and the matrix contains collagen fibers interlaced with elastic fibers. Rupture of elastic. Fibers Occult's when the skin is overstretched. Resulting in permanent stripe, or strictly marks, which are typically found in pregnancy and obesity, Collagen Fibers.

Bind water and give the skin its tensile strength, but as this ability declines with age, wrinkles develop. Fibroblasts, monophases and mast cells are the main cells found in the dermis. The subcutaneous layer, containing areolar tissue and varying amounts of adipose (fat) tissue, lies under the dermis. Structures found in the dermis are:

- Small blood and lymph vessels
- Sensory nerve endings
- Sweat glands and their ducts
- Hairs, arrestor pill. Muscles and sebaceous glands.

Subcutaneous

The subcutis is the innermost layer of the skin, and consists of a network of fat and collagen cells. The subcutis is also known as the hypodermis or subcutaneous layer, and functions as both an insulator, conserving the body's heat, and as a shock-absorber, protecting the inner organs. It also stores fat as an energy reserve for the body. The blood vessels, nerves, lymph vessels, and hair follicles also cross through this layer. The thickness of the subcutis layer varies throughout the body and from person to person.

SOL-GEL TRANSITION (GEL POINT):

Sol-Gel transition may be dependent on polymer concentration or temperature. Spectrophometric (SP) methods, as mentioned previously are used to probe Sol-Gel transitions that depend on the critical gelling concentration. Thermal methods, including differential scanning calorimetric are used to measure Sol-Gel transitions, or melting temperature, of thermo reversible gels. A relationship for estimating the heat of gelation was derived by Aldridge and Ferry in which the dependence of the Sol-Gel transition temperature on polymer concentration was considered.

The critical gelling concentration is the concentration below which no macroscopic gel is formed under the prevailing experimental condition; rather, a sol is formed by the polymer and solvent. This concentration depends on polymer-polymer and polymer –solvent interactions, the hydrophilic – lipophilic character of the polymer, and the molecular weight and flexibility for the chain. Furthermore, polymers that require ions to form gels have critical gelling concentration that depend on the concentration these additives and many other variables. There are two thermal gel points associated with thermo reversible gels. Shifts in temp may cause gel formation at the setting point or gel liquefaction at the melting point. In addition, temp hysteresis may occur in some gels in which the gel setting point is lower than the melting point. The hysteresis behavior indicates that junction zones constitute a family of association that than set of identical cross linkages. Agarose gels show temp hysteresis; the gel sets at about 40◦C and melts at about 90◦C

GEL-FORMING SUBSTANCES AND THEIR PHARMACEUTICAL USES:

Forming hydrophilic polymers are typically used to prepare lipid free semisolid dosage forms, including dental, dermatological, nasal, ophthalmic, rectal, and vaginal gels and jellies. Gel vehicles containing therapeutic agents are especially useful for application to mucous membranes and ulcerated or burned tissues because their high-water content reduces irritancy. Furthermore, these hydrophilic gels are easily removed by gentle rinsing or natural flushing with body fluids, reducing the propensity for mechanical abrasion. The superior optical clarity of synthetic polymer gels, such as those composed of poloxamer and carbomer, has led to the current interest in developing therapeutic.

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PHYSICAL AGING:

The gel physically ages as it moves toward equilibrium, making the history of a gel sample an important consideration when measuring physical properties. Aging reflects changes in gel microstructure, where non covalent crosslink is breaking and reforming. Furthermore, instabilities caused by the non-equilibrium state arise in some polymer gels; two examples are retrogradation and syneresis. Retro gradation is the spontaneous reversion of a polymer solution to a gel on standing. Polyvinyl alcohol dissolved in water undergoes retro gradation, whereby the stereo regular chains form microcrystalline aggregates as the solution ages. Polyvinyl alcohol gels retro grade, forming crystalline domains. Amylase, which is the linear polysaccharide fraction of starch, underground retro gradation, reducing the physical stability for solution and gels over time.

Syneresis is the process whereby liquid is liberated spontaneously from the gel matrix. This instability arises from the non-equilibrium state of the gel established as it was or because of a change in external conditions. An equilibrium, elastic contraction forces of polymer chains are usually balance by solvent swelling forces, resulting from an osmotic pressure differential. With changes in temperature, for example, the osmotic pressure shifts, causing an elastic contraction of polymer chains. The contractive response squeezes excess liquid out of the matrix. Agar and carrageen are example of gels the exhibit syneresis.

RHEOLOGICAL PROPERTIES:

Like the transitional physical properties, the rheological properties of gels are not easily characterized because they depend strongly on the attributes of the polymer, history of the gel sample, and experimental conditions. Most often, the apparent viscosity or gel strength increases with an increase in the effective crosslink density of the gel or in the concentration and average molecular weight of the polymer. However, a rise in temperature may increase or decrease the apparent viscosity, depending on the molecular interactions between the polymer and solvent. In addition, the direction of change in apparent viscosity may not be readily predictable when additives such as ions, nonelectrolytes, solvents or non-solvents, and other compatible polymers are mixed with a gel.

RIGIDITY:

The modules of rigidity, or shear modulus, are defined as the ratio of shear stress to strain. It is a measure of a gels ability to resist deformation. The minimum rigidity for a strong gel to resist deformation under its own weight is equal to about gpl which is the product of the acceleration due to gravity(g), density(p), and a linear dimension of the sample. Therefore, the minimum rigidity is about 100 Pa (103 Dyn/cm) for a gel sample 1 cm long.

RUPTURE STRENGTH:

Rupture strength is equal to the stress at which a strong gel ruptures or fails rather than undergoing further strain. The rupture strength is determined by large-deformation measurements on instruments such as the Instron tester (Motor In coder), where a tensile stress is applied to the sample. However, strong gel samples can only be tested in tension if they can support their own weight, and most physically crosslinked gels are relatively weak, making this type of test difficult.

OBJECTIVES OF RESEARCH

1. To formulate the herbal gel of eucalyptus oil for topical drug delivery system.
2. To evaluate the herbal gel for topical drug delivery system.
3. To study antibacterial activity for eucalyptus oil loaded herbal gel by disc diffusion assay.
4. The objective of this research work was to formulate the Herbal gel which does not cause any side effects or adverse reactions.
5. To study use of genus eucalyptus & to formulate safe and stable anti-bacterial gel.
6. To develop topical drug delivery system and to prevent first pass metabolism.
7. To overcome the limitation of conventional oral and parenteral route of drug administration.
8. Externally, the antiseptic, slightly anesthetic, anti-bacterial, and warming properties of Eucalyptus make it a valuable resource treatment of burns, sores, ulcers, scrapes, boils, and wounds. Applied topically as, it also helps relieve the pain of rhe

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II. MATERIALS AND METHODS:

EUCALYPTUS OIL:

Fig.No.03 Eucalyptus oil

- **Biological Source-** Oil obtained from distillation of fresh leaves of Eucalyptus globulus.
- **Family**- Myrtaceae.
- **Chemical Constituents-**

✔ 80% Cineole/ Eucalyptol Pinene
Camphene
✔ Phellandrene
✔ Citronellal
✔ Geranyl acetate

DESCRIPTION

1. **Color** - colorless or pale yellow
2. **Oduor**- aromatic &camphourous
3. **Taste**- pungent &Camphourous
4. **Solubility**-Soluble in 90% Alcohol

- **Synonyms -** Eucalyptus, Dinkum oil, Nilgiri.

COMPOSITION FORMULA:

Name of Ingredients	Quantity Taken	Role
Eucalyptus oil	2ml	Anti-bacterial
Carbopol 934	5gm	Gelling agent
Methyl paraben	0.0075gm	Antimicrobial preservative
Propyl paraben	0.0015gm	Antimicrobial preservative
Propylene glycol	5ml	Humectant, solvent, dispersing agent
Triethanolamine	1-2drop	PH &buffer adjusting agent
Distilled Water	q. s.	vehicle

Table No.01: Composition Formula & Role of Ingredients

INSTRUMENTS AND GLASSWARES:

Sr. No	Instruments	Glasswares
1	Lab stirrer (mechanical stirrer)	Beaker
2	Sonicator	stirrer
3	UV-visible spectroscopy	Volumetric flask
4	Digital pH meter	Measuring cylinder
5	Brookfield Viscometer	Funnel

Table No.02: Name of Instruments and Glassware.

METHODS OF PREPARATION OF ANTI-BACTERIAL GEL OF EUCALYPTUS OIL:

- Carbopol 934, which had been precisely weighed, was placed in a beaker and dissolved in 50 ml of distilled water.
- Kept the beaker a side to swell the Carbopol for half an hour and then stirring should be done using lab stirrer at 1200rpm for 30 min
- Take 5 ml of propylene glycol and the necessary quantity of extract, which is 2 mL of eucalyptus oil.
- Put 5 ml of propylene glycol in a different beaker, then add weighed quantity of propyl paraben and methyl paraben, add eucalyptus oil to it and stir well.
- Following the dispersion of all carbopol, 1g of extract and preservative solutions were added while being continuously stirred.
- Lastly, the volume was made upto 100 ml by adding the remaining distilled water, and

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- Triethanolamine was added drop wise to the formulations to balance the skin pH (6.8-7) and to get the gel at the desired consistency.

MEDICINAL USE OF EUCALYPTUS OIL:

- Externally, eucalyptus oil is useful resource for treating burns, sores, ulcers, boils, and wounds due to its antiseptic, slightly anesthetic, antibacterial, and warming qualities.
- When taken internally, eucalyptus seems to help reduce the symptoms of respiratory infections, pneumonia bronchitis, colds, sore throat and flu.
- An oil or cream to reduce respiratory issues, asthma, and congestion.
- When applied topically, it also helps with stiffness, neuralgia, aching, and rheumatic discomfort.
- In addition to relieving pain and headaches, eucalyptus oil can also cure burns and wounds, repel mosquitoes, and reduce stress.

CONTRAINDICATIONS:

- Eucalyptus is very safe when taken in moderation, although it seems to be a little difficult to eliminate from the kidneys. If you have liver or kidney issues or are pregnant, you should either avoid it altogether or use it very sparingly. Don't take it all at once for longer than a few days.
- In terms of skin application, pure eucalyptus oil may be hazardous. It may result in severe neurological system issues.

III. RESULT AND DISCUSSION:

The formulation was developed with eucalyptus oil using Carbopol 934 as gelling agent. The formulation was Colorless and have characteristic odor of eucalyptus. The formulation was glossy and translucent, and had good consistency. The pH of the formulation ranged from 5.7 to 6.0, which was relevant with human skin ph. Hence it may be suitable for topical application without discomfort. Spreadability test was performed for evaluation of gel. The viscosity of formulation increased with increase in the concentration of Carbopol content. The Spreadability of the formulation was found to be good. The increase in viscosity was observed with decrease in Spreadability and vice versa. The skin irritation test was performed to evaluate the skin irritation of the formulated gel on the skin of human volunteers. The results of these tests were shown that the formulated herbal Anti-Bacterial gel was safe to use.

Physical appearance:

1. Colour:

The Colour of the formulation was checked out against white background. No any different colour particle are shown.

2. Odour:

The odour of the gel was checked by mixing the gel in water and taking the smell camphor us scent that is sharp and highly pungent.

3. Consistency:

The consistency was checked by applying gel on skin. Smoothly and easily sprayed on skin.

4. Grittiness:

The formulation was evaluated microscopically under 40 x magnifications thire is no any presence of any particulate matter or aggregates.

5. Homogeneity:

Homogeneity was tested by visual inspection by naked eye after allowing them to set in a container they were evaluated for their appearance and presence of aggregates

6. Clarity: The clarity of various formulations was determined by visual inspection under white background there is no any particular matter.

7. Skin irritancy test:

This test was performed on 10 healthy human volunteers of either sex after obtaining consent for the same. About 0.5 gm. of gel was applied to an area of about 6cm2 on skin of hand covered with a gauze patch. The patch was held in contact with the skin for period of 1hr, the gauze was removed and residual test substance was scrapped, without altering the existing response or integrity of the epidermis. The skin was observed at 1 hr., 3hrs, 6hrs, and 12hrs.24hrs. 48hrs. and 72hrs.For any visible response on the skin. No any irritation can takes place.

8. Measurement of pH:

The pH of developed gel formulations was determined using digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each

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formulation was done in triplicate and average values are calculated.

Sr.no	Observation time	pH observed
1	After 12 hrs.	5.6
2	After 24 hrs.	6.0

Table No.04: Measurement of PH

9. Spreadability (SP):

Spreadability (SP) of formulations was determined by an apparatus suggested by Multimer 45, which was fabricated itself in laboratory and used for slide fixed on wooded block and upper slide with one end tide to glass slide and other end tied with other end tied to weight pan. An excess of gel (2-5 gm.) was placed in between two glass slides and then 1000 gm. weight was placed on slides for 5 min to compress the sample to a uniform thickness, Weight (80 gm.) was added to pan. They show good spreadability(SP).

Spreadability = m x l / t
Where,
m= weight tied to upper slide, l= length of the glass slide (6cm)
t= time in seconds

Test	Observation
Spreadability	Easily spreadable

Table No.05: Spreadability

10. Viscosity:

The viscosity of prepared gel will be measured with Brookfield viscometer at a setting of 100 RPM at 25℃. viscosity is good.

Sr no.	Formulation code	Viscosity
1.	F1	1675 dyne/cm
2.	F2	1695 dyne/cm
3.	F3	1633 dyne/cm
4.	F4	1722 dyne/cm
5.	F5	1755dyne/cm

Table No.06: Viscosity Observation

11. UV VISIBLE SPECTROSCOPY:
Sample: - Eucalyptus oil gel formulation

Stock Solution: -

1) Make a 1000micro gram/mL solution. Take 1gm of drug and dissolve in 100mL of water.
2) Stir continuously up to small particle dissolve, if any particle seen in solution filter out the solution.
3) After filtration take a solution in volumetric flask placed in sonicator bath for 10 min.

Reference Sample:
The formulation is soluble in distilled water is the reference sample

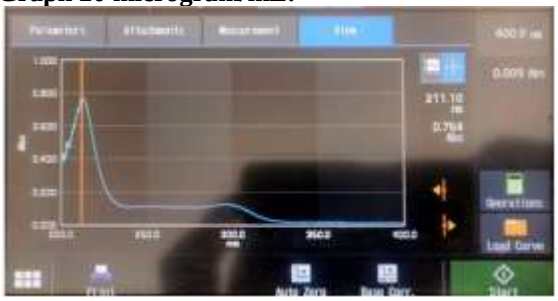
10 microgram/mL solution
Take 1mL solution into stock solution and volume make up to 10 mL
While identify UV rang of sample is
Wavelength- 210.50 nn
Absorbance – 0.445

Graph 10 microgram/mL:

Graph No.02 : 10 microgram/mL

20 microgram/mL solution
Take 2mL solution into stock solution and volume make up to 10mL
While identify UV rang of sample is
Wavelength- 211.10 nn
Absorbance – 0.76

Graph 20 microgram/mL:



Graph No.03: 20 microgram/mL

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IV. CONCLUSION:

In the present study, antibacterial gels made from eucalyptus oil were formulated and evaluated. Eucalyptus oil, a sample substance from the eucalyptus plant, was utilized in the compositions' manufacture. A green leafy plant in the Myrtaceae family, eucalyptus is also used medicinally. The main reason behind this investigation was to formulate a stable and safe Anti-Bacterial gel. To assess the gel's performance, several evaluation tests were conducted. Such as physical appearance, colour, consistency, odour, greasiness, grittiness, homogeneity, skin irritancy test, pH determination, spreadability (SP), and viscosity. From the result of performed test we conclude that gel formulation from eucalyptus oil is safe to use.

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