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Formulation and Evaluation of Anti-Fungal Gel from Datura Leaves (Datura Stramonium)

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ABSTRACT

In order to fight Candida albicans, a common fungal pathogen that causes a variety of illnesses, including oral and vaginal candidiasis, this study focusses on creating and testing an antifungal herbal gel with *Datura stramonium* extracts as the active ingredient. Alkaloids with antibacterial and antifungal qualities, including atropine, scopolamine, and hyoscyamine, are found in the plant *Datura stramonium*, which is well-known for its therapeutic uses. These bioactive substances will be extracted from the plant's leaves and added to a gel formulation as part of the experiment. By using techniques like Minimum Inhibitory Concentration (MIC) determination and antifungal activity assays, the gel's efficacy will be assessed based on its capacity to stop *Candida albicans* growth in vitro. Utilising *Datura stramonium's* inherent qualities for its possible medicinal use, the gel aims to offer a topical, simple-to-apply substitute for treating fungal infections. In order to guarantee the gel's safety and effectiveness as a topical antifungal treatment, the study also evaluates the gel's stability, pH, and risk for skin irritation. The project's overall goal is to provide a plant-based, sustainable treatment alternative with fewer adverse effects than traditional antifungal medications.

Introduction:

Gel: A gel is a semi-rigid mass of lyophilic sol in which the sol particles have completely absorbed the dispersion medium. A network of cross-linked polymers that has swelled in a liquid medium is called a gel. The way these two elements interact has a significant impact on its characteristics. [1]

History of Gels: Thomas Graham, a Scottish chemist from the 19th century, borrowed the word "gel" from gelatin. Gelation is the process by which a gel is formed.

Applying a chemical to the skin to treat or cure skin disorders is known as topical medicine delivery. These topical medication delivery techniques are frequently used when other routes of administration are ineffective, such as in cases of localized fungal infections of the skin. Its deeper skin penetration improves absorption. In no way is topical application better than conventional dosage forms. They are often regarded as more efficient and less hazardous than conventional formulations due to the bilayer composition and structure. Drug carriers that allow for proper localization or penetration of the treatment within or through the skin have been used in an attempt to enhance the local effects of topical dose forms

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and lessen their systemic effects. It increases the medication's bioavailability by preventing the liver from metabolizing it and inhibiting GI discomfort. Wherever topical therapies are applied, they start working right away. Topical drugs, such as ophthalmic, vaginal, and rectal pharmaceuticals, are delivered locally through the skin. [2]

CLASSIFICATION OF GELS:

1. **Based on Composition -**

- **Hydrogels**: Water-based gels with a high water content, such as polyacrylamide and alginate, that are frequently utilized in cosmetic and medicinal applications.
- **Organogels**: Usually organic solvents (like oleo gels) are utilized instead of water.
- **Biogels**: Biogels are made from biological components, such as agarose and gelatin.

2. **Based on Structure -**

- **Polymeric Gels**: composed of network-structured polymers (e.g., silicone gels).
- **Non-Polymeric Gels**: made up of surfactants or tiny molecules. (e.g., certain food gels).

3. **Based on the Nature of the Gelation Process -**

- **Physical Gels**: created by non-covalent processes such as van der Waals forces or hydrogen bonds. (e.g., gelatin).
- **Chemical Gels**: created by covalent bonding, frequently with the help of cross-linking chemicals. (e.g.,polyurethane gels).

4. **Based on Thermal Response -**

- **Thermoreversible Gels**: Melts and gels again when heated and cooled. (e.g., gelatin).

5. **Based on Swelling Behavior -**

- **Swelling Gels**: able to expand in bulk and absorb a large amount of solvent. (e.g., superabsorbent polymers).
- **Non-Swelling Gels** Regardless of the surroundings, keep the volume comparatively consistent.

6. **Based on Gel Stability -**

- **Stable Gels**: Maintain their structure and properties over time (e.g., silicone gels).
- **Unstable Gels**: May undergo phase separation or degradation (e.g., certain biological gels).

7. **Based on Functionality -**

- **Active Gels**: Contain substances that provide additional functionality, such as antimicrobial properties (e.g., some wound dressings).

Passive Gels: mostly act as stabilizers or transporters without any active ingredients. These divisions aid in comprehending the various uses and characteristics of gels in disciplines such as materials science, food science, medicine, and cosmetics.[3]

APPLICATIONS OF GEL:

Gels are materials that can be used in a variety of industries since they are adaptable. Here are a few noteworthy uses:

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1. **Pharmaceuticals:** Drug delivery systems employ gels to provide regulated drug release. They can be prepared for injection, oral, or topical administration.
2. **Cosmetics and Personal Care:** Because of their smooth texture and hydrating properties, gels are frequently found in skincare products (like moisturizers and sunscreens) and hair products (like styling gels).
3. **Food Industry:** Gels like gelatin are used to thicken, stabilize, or gel food products, like sauces, jellies, and sweets.
4. **Biotechnology:** Gels are used for DNA separation and analysis in molecular biology procedures like agarose gel electrophoresis.
5. **Environmental Applications:** Because of their high surface area and porosity, gels are utilized as pollutant absorbents and in water purification.
6. **Medical Devices:** Gels are utilized in hydrogels for tissue engineering and regenerative medicine as well as wound dressings.
7. **Construction:** Gels can be added to paints and sealants to increase their resilience to environmental influences and durability.
8. **Textiles:** Gel compositions can be applied to fibers and textiles to enhance their antibacterial and moisture-wicking qualities.
9. **Adhesives:** Because of their flexibility and potent bonding properties, several gel formulations are utilized in adhesives. These uses demonstrate how crucial gels are for improving functionality in a range of sectors [4]

 Ideal properties of a gel include:

- **Gel strength**: A gel's strength is based on the amino acid content and the ratio of its α chain to β component.
- **Water-holding capability:** One key metric for evaluating a gel's quality is its capacity to retain water.
- **pH:** A gel's pH has a significant impact on its properties. While a gel's melting temperature drops with increasing pH, its strength improves as pH falls.
- **Mechanical properties:** A gel's mechanical strength can be raised by the ionic interaction of chitosan and polyelectrolytes.
- **Colour:**A gel's color is a crucial aesthetic characteristic that is influenced by the extraction process and the raw components employed.
- **Spreadability:** The degree to which a gel spreads when applied is known as its spreadability. The spreading value of a gel determines its therapeutic effectiveness

A gel should have following desirable qualities:

- Being clear and homogeneous
- Being easily broken when shaken
- Being inert.
- Not being sticky
- Not interacting with other formulation components
- Being stable
- Not irritating the skin.
- Hydration without greasiness: Gels are lightweight and provide hydration without the heaviness of creams.
- Cooling effect: The gel texture can soothe and cool inflamed or irritated skin. [5]

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Composition of Skin:

- In terms of weight and surface area, the skin is the biggest organ in the body. Its surface area is roughly 16,000 cm. Skin makes up 8% of an adult's body weight. It is the live body's outermost layer or tissue. The skin has a defense system against the outside world. When exposed to sunshine, the skin can create a beneficial molecule called vitamin D. The skin serves as a sensory organ and aids in controlling body temperature. Different biological components, such as melanocytes, erythrocytes, keratinocytes, etc., are found in skin.[6]

It has multi-layer structures because of different components like cells and fibers.

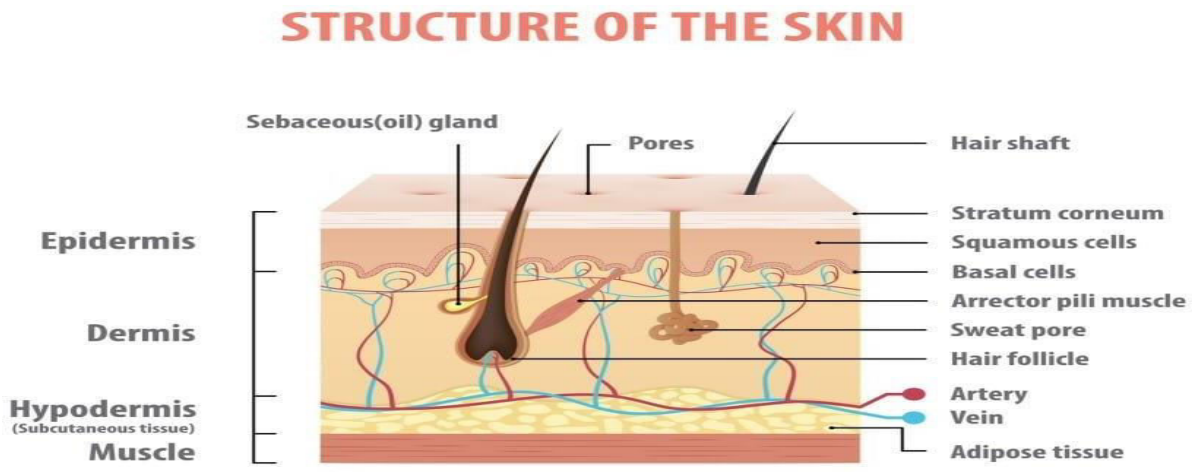

Fig. no.01: Structure of skin

The skin consists of skin layers:

A. <u>The Epidermis:</u>

The epidermis is the skin's outermost layer, and it is roughly 0.2 mm thick. This stratum is devoid of capillaries and veins. The location of the body affects the thickness of the epidermis. Keratinocytes and dendritic cells are the two primary cell types that make up the epidermis. It also includes a variety of other cells, such as Langerhans cells and melanocytes.

The outermost layer is classified into five sub layers and these are

1) Stratum corneum
2) Stratum lucidum
3) Stratum granulosum
4) Stratum spinosum
5) Stratum Basal

1) Stratum corneum: The stratum corneum is the outermost layer of the epidermis. It is also known as the horny cell layer, and it is between 8 and 15 μm thick. The layer, which has a hexagonal form, helps

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shield the skin from profound dehydration. Its primary ingredient, "ceramide," plays a significant part in water retention.

2) **Stratum lucidum:** This thin, transparent layer of dead skin cells makes up the stratum lucidum. It only appears on the palms of the hands and soles of the feet where there is thick skin.

3) **Stratum granulosum:** This 3µm-thick layer is also known as a granular cell layer. It has two to four granular cell layers. Because the keratin fibers are filling the cells more and more, the cells have a flatter structure.

4) **Stratum spinosum:** Also known as the prickle cell layer, it is between 50 and 150 µm thick. It is made up of many cells that might vary in structure and form.

5) **Stratum basal:** The deepest sublayer of the epidermis, the stratum basal is made up of a single layer. Keratinocytes are generated in the stratum basal and exhibit upward migration to the outer surface. Turnover is the term used to describe the movement of keratinocytes. It takes days for this procedure to complete one cycle, and keratinocytes alter as well.

B. The Dermis:

1. The dermis, or at least the skin, is where the majority of the magic occurs. Collagen, elastin, and fibroblasts make up the majority of the dermis. This layer serves a number of purposes.
2. Blood and lymphatic vessels, which remove waste materials and poisons from the skin, are found in the dermis.
3. The dermis contains sweat glands. Through your pores, they produce perspiration, which cools your body and eliminates contaminants.

C. The Subcutaneous layer:

1. The layer of fat connecting your bones to your muscles and bones is the deepest layer of skin. It reaches a depth that your skincare products' active compounds can never.
2. The layer beneath the skin functions similarly to a thermostat. In an emergency, it can be used as a source of energy in addition to protecting the body
3. Additionally, fat serves as a filter, preventing harm to your organs, muscles, and bones.
4. Lastly, the deepest oil-producing sebaceous glands, hair follicular roots, nerve endings, and extra blood arteries are found in the subcutaneous layer. [6]

Candida Albicans:

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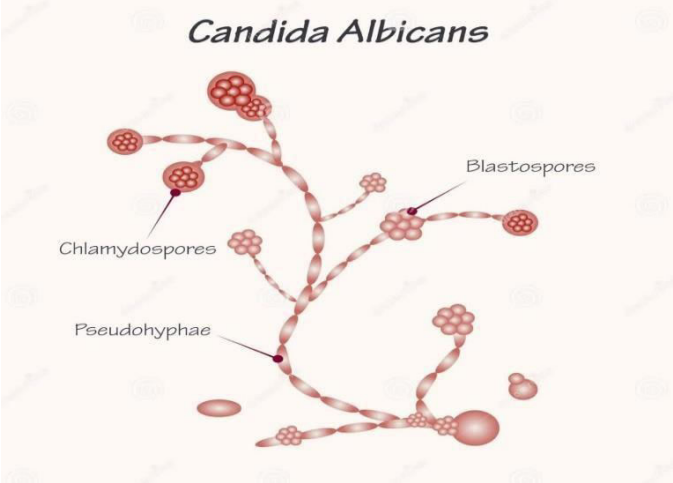

Fig. no. 02: Candida Albicans

Small levels of the yeast species Candida albicans are typically found in the human microbiome, especially in the mouth, gut, and vagina. Although generally benign, it can turn opportunistic and lead to infections, especially in people with weakened immune systems or disturbed microbiomes. Oral thrush, vaginal yeast infections, and systemic candidiasis are just a few of the many possible manifestations of Candida infections. Itching, redness, and pain are indications of overgrowth that can be exacerbated by conditions like diabetes, antibiotic use, and hormonal changes. Antifungal medications and addressing underlying risk factors are frequently necessary for effective management.

The opportunistic pathogenic yeast Candida albicans is a common part of the human gut flora. It can survive without a human body as well. 40–60% of healthy people have it in their gastrointestinal and oral tracts. Although it is normally a commensal organism, it can become harmful in immunocompromised individuals in a variety of situations. Among the few species of the genus Candida, it is the cause of the human infection known as candidiasis, which is caused by an excess of the fungus.
Jimson weed, or Datura stramonium, is a plant that contains a number of bioactive substances, especially alkaloids like scopolamine and atropine. Although there isn't much study precisely on how Datura stramonium affects Candida albicans, certain studies indicate that extracts from the plant may have antifungal qualities.
Datura stramonium may impact Candida albicans by disrupting the integrity of the cell membrane, which would increase permeability and ultimately cause cell death. The plant's alkaloids have the ability to disrupt the fungal cells' regular physiological functions, which may prevent them from growing and reproducing. Furthermore, these substances may change the expression of genes related to Candida's virulence and biofilm production, which would lessen the pathogen's pathogenicity. Furthermore, some research suggests that Datura stramonium extracts have a synergistic impact by increasing the efficacy of traditional antifungal drugs. This combination may increase the effectiveness of treatment for Candida infections, especially when resistance to common antifungal medications is a problem. To clarify the exact mechanisms and assess Datura stramonium's efficacy and safety in clinical settings, more investigation is needed. [7]

OBJECTIVES

Aim: -The aim is Formulation and evaluation of Anti-fungal gel from Datura leaves *(Datura stramonium).*

❖ RESEARCH OBJECTIVES

➢ **Pre-formulation Studies**

A. Plant Collection.

B. Drying.

C. Grinding

D. Phytochemical screening

E. Evaluation (Anti-fungal activity) MIC

➢ **Formulation parameters**

➢ **Evaluation parameters**

1. Physical evaluation.
2. Homogeneity
3. Measurement of pH.
4. Viscosity.
5. Spreadability
6. Skin irritation test
7. Stability study as per ICH guidelines

REVIEW OF LITERATURE:

Drug profile: Datura Stramonium

1. **Kingdom**: Plantae
2. **Division**: Magnoliophyta
3. **Class**: Magnoliopsida
4. **Order**: Solanales
5. **Family**: *Solanaceae*
6. **Genus**: *Datura*
7. **Species**: *Datura Stramonium*
8. **Common names**: thorn apple, Common thorn apple, Datura,Devil's trumpet, jimsonweed, Moonflower
9. **Morphological characters-**

Leaves: alternating on stems on long petioles (up to 10 cm) that smell terrible when crushed; dark green (occasionally purple), hairless, egg-shaped to broadly triangular (5–25 cm long and 4–25 cm wide), with conspicuous veins and coarsely and irregularly serrated or lobed margins. Flowers: big (up to 10 cm long), funnel-shaped, white, mauve, or purplish at each stem fork Fruits/Seeds: Egg-shaped capsules that are green at first but turn brown as they mature. They are 3–7 cm long and 2–3.5 cm wide, coated in thin spines that can reach a length of 10 mm, and they are held upright on the plant. Many flat, kidney-shaped, dark brown to black seeds. [8]

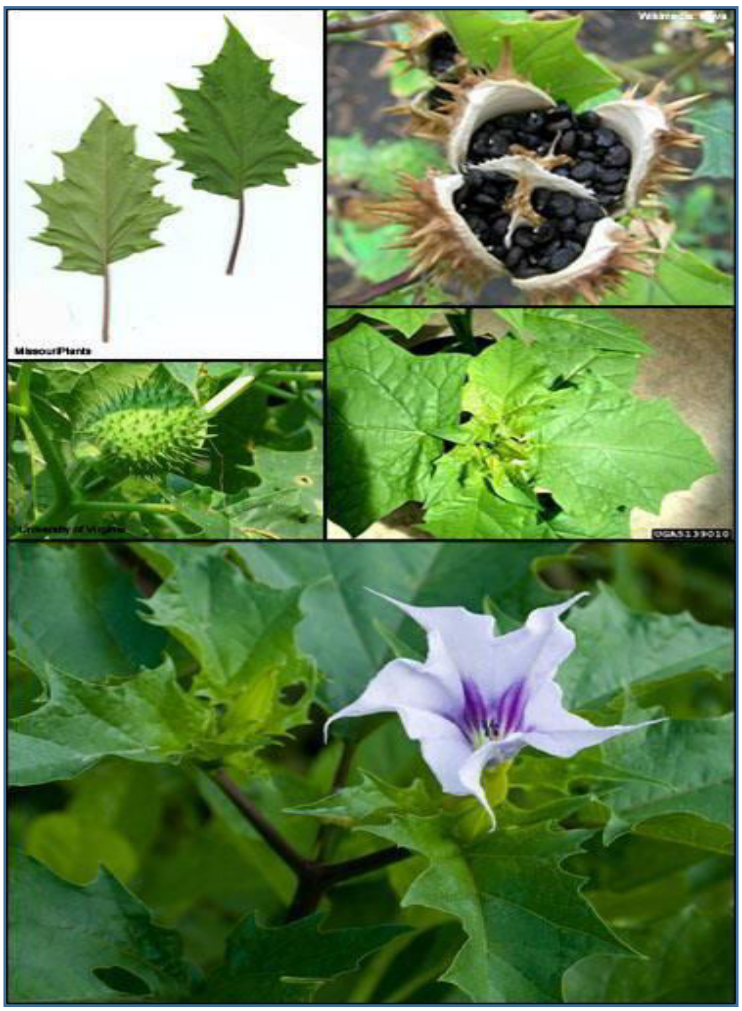

Fig no. 03 : Parts of Datura

10.Biological Source Datura: The dried leaves and flowering tops of Datura metel linn and Datura metel war-fastuosa, which are members of the Solanaceae family, make up this herb.

11.Chemical constituents: Higher concentrations of major alkaloids, such as scopolamine and hyoscyamine, as well as lesser alkaloids, are found in thorn apples. Every part of the plant has the ability to be both deadly and healing; the bioactive compound in the leaves, seed, fruit, bark, stem, root, and seed coat showed pharmacological effects. The phytoconstituents of the plant demonstrated strong nauseating and therapeutic efficacy.[8]

Table no. 01: Phytoconstituents of Datura

Sr. No.	Phytochemicals	Parts
1	Carbohydrates, fat, protein, ash, fiber	Seed coat
2	Phytate, tannin, oxalate	Seed
3	Calcium, tannin and oxalate, iron, potassium, phosphorous	Seed coat
4	Glycosides, saponins, flavonoids, alkaloids, pheno phlobatanins	Leaves
5	Scopolamine, atropine, fastunine, daturaolone	Seed
6	Hyoscine, norhyoscine, hyoscimine, tropine	Root
7	Daturanolone and daturadiol	Fruits
8	Hyoscine and hyoscyamine	Whole plant
9	Scopolamine and fastusine	Pericarp

12 .Uses of Datura stramonium:

- Antibacterial
- Anti-fungal
- Antimicrobial
- Anesthetic
- Anti-stress
- Antioxidant
- Anti-cancer [9]

Excipient profile:

➢ **Ethanol:**

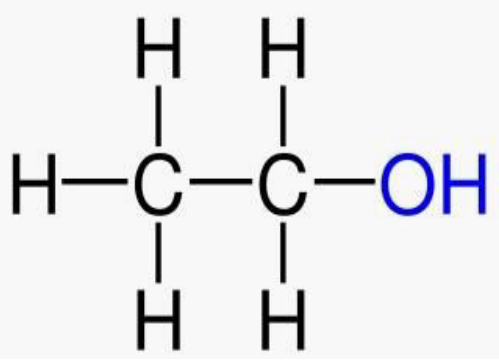

Fig. no.04: chemical structure of Ethanol

TABLE no. 02: Properties of ethanol

Molecular formula	C2H6O
Molecular weight	46.07 gm/mol.
IUPAC Name	Ethanol
Melting point	-114.1°C
Boiling point	78.37°C
pH	7.33
Odour	Mild, rather pleasant like wine
Solubility	highly soluble in water
Source	Sugarcane, Grape, Corn

Uses:

- Ethanol is frequently used in beverages, as a fuel additive, as a solvent in businesses and labs, and in the creation of personal care products.
- Ethanol is used in the production of medications, plastics, lacquers, polishes, plasticizers, and cosmetics.
- It is also a common disinfectant found in hand sanitizers, antiseptic wipes, and medical wipes to eradicate bacteria and viruses.
- In medicine, ethanol is used as an antidote for ethylene glycol and as a topical anti-infective.[10]

➢ **Carbopol:**

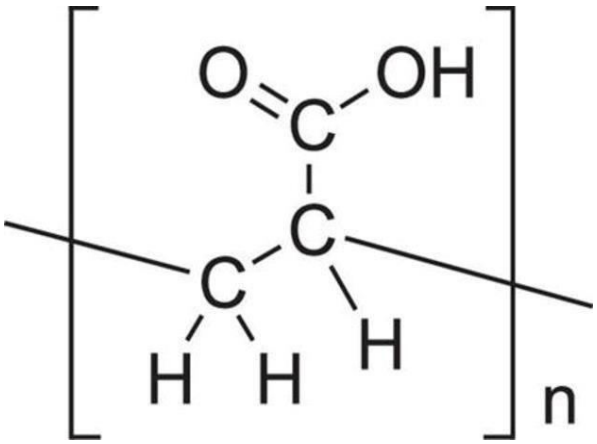

Fig. no. 05: chemical structure of Carbopol

TABLE no. 03: Properties of Carbopol

Molecular formula	$C_5H_{10}O_2$
Molecular weight	102.13 gm/mol
IUPAC Name	Poly (Acrylic acid)
Melting point	12.5^0 C
Boiling point	116^0 C
pH	5.5 – 6.5
Odour	Slightly acetic odour
Solubility	water-soluble
Source	polymerization of acrylic acid

Uses:

- Applied to creams, lotions, and gels to improve texture and stability; used as a thickening agent in topical formulations, gels, and suspensions.
- Applied as a lubricant in specific medical devices and in formulations of controlled-release drugs.
- Applied to paints, coatings, and adhesives to improve performance and consistency.[11]

➢ **Triethanolamine:**

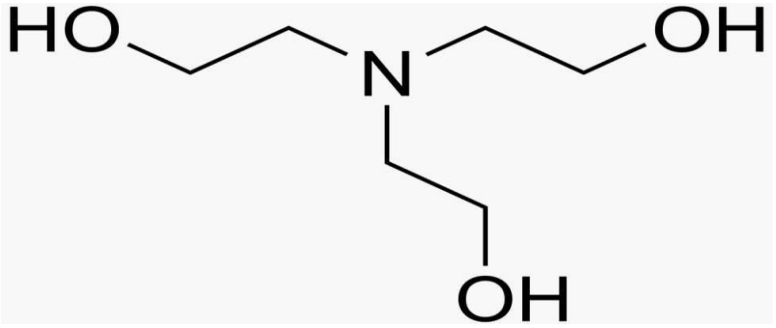

Fig. no.06: chemical structure of Triethanolamine

TABLE no. 04: Properties of Triethanolamine

Molecular formula	$C_6H_{15}NO_3$
Molecular weight	149.19 g/mol
IUPAC Name	2-[bis(2-hydroxyethyl) amino] ethanol
Melting point	21.5 °C
Boiling point	350 °C
pH	10.5
Odour	Slight ammonical
Solubility	Soluble in chloroform
Source	Reaction of ethylene oxide with aqueous ammonia

Uses:

- pH Adjuster: It can adjust the pH of products to ensure they are gentle on the skin.
- Pharmaceutical Formulations: It is used as a stabilizer and pH adjuster in pharmaceutical formulations, particularly in topical products.
- Active Ingredient Solubilizer: TEA can help dissolve certain active ingredients in ointments and creams.[12]

➢ **Propylene glycol:**

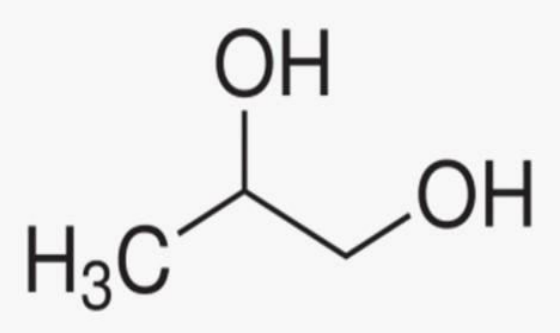

Fig. no.07: chemical structure of Propylene

TABLE no.05: Properties of Propylene glycol

Molecular formula	C3H8O2
Molecular weight	76.09 g/mol
IUPAC Name	propane-1,2-diol
Melting point	-59 °C
Boiling point	188.2 °C
pH	9.3 to 10.5
Odour	Odourless
Solubility	Soluble in water
Source	Propylene glycol can be produced from petroleum or from renewable sources like vegetable oil

Uses:

- Food, cosmetics, and pharmaceuticals
- Paint and plastics
- Fire-fighting training and theatrical productions
- Liquid detergents
- Plastics [13]

➢ **Methyl paraben:**

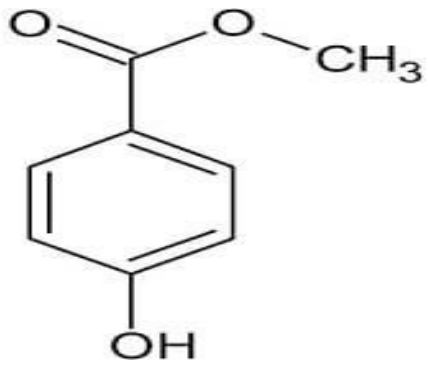

Fig. no.08: chemical structure of Methyl

TABLE no.06: Properties of Methyl paraben

Molecular formula	C8H8O3
Molecular weight	152.15 g/mol
IUPAC Name	methyl 4-hydroxybenzoate
Melting point	124–127°C
Boiling point	270–280°C
pH	4–8
Odour	characteristic
Solubility	freely soluble in most oils, waxes, and fatty alcohols
Source	found naturally in some fruits, like blueberries

Uses:

- Cosmetics
- Preservatives
- Food
- Pharmaceuticals
- Personal hygiene products [14]

➢ **Glycerin:**

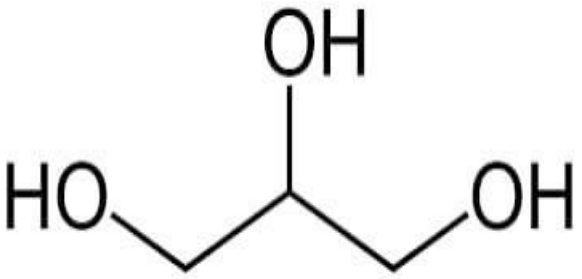

Fig. no.09: chemical structure of Glycerin

TABLE no.07: Properties of Glycerin

Molecular formula	C3H8O3
Molecular weight	92.09382 g/mol
IUPAC Name	Glycerol.1,2-Ethanediol. Propane-1,2,3-triol
Melting point	18.1–18.2 °C
Boiling point	290 ° C
pH	6.0
Odour	does not have any characteristic odour
Solubility	Highly soluble in water and alcohol, but insoluble in chloroform, ether, and volatile oils
Source	variety of sources, including plants, animals, and petroleum

Uses:

- Skincare
- Moisturize
- Reduce wrinkles
- Protect skin
- Prevent acne [15]

In 2024, S. D. Bahekar et al. conducted a study titled **"Formulation and evaluation of antifungal gel using aloe vera and betel leaves."** They created four different formulations because betel leaves and aloe vera are medicinal plants that contain a variety of phytoconstituents. In the global market, herbal formulations are growing in popularity. For the treatment of candidiasis, a topical gel comprising aloe vera and betel leaf extract, together with 3 grams of Carbopol 940 as a gelling agent, was successfully created and tested. Regular 1% clotrimazole gel (25 mm) and commercial herbal gel (20 mm) worked better against Candida albicans in the antifungal study than the herbal gel formulation's ethanolic extract (23 mm). Phytoconstituents and phytochemicals in aloe vera and betel leaf extract are responsible for their antifungal properties. Thus, it may be said that a topical gel containing 0.8% aloe vera and betel leaf extract could be used in place of manufactured herbal gel.

The study **"Formulation and evaluation of antifungal activity of gel of crude methanolic extract of leaves of Ipomoea carnea Jacq" by Kusum Kaushik et al. (2020)** includes eight formulations. The goal of the current investigations was to create a herbal antifungal gel of Ipomoea carnea. As a result, formulation F8 (6% Ipomoea carnea extract, 1% carbopol, and 1% lavender oil) was effectively created and provided a better zone of inhibition than the antifungal allopathic gels (Itraconazole; Itromed 1% and herbal marketed Himalaya V gel) that were now available on the market. In addition to increasing the antifungal action, herbal formulations offer a substitute for some fungi that have demonstrated resistance. Preclinical investigations have demonstrated that the gel is safe and non-irritating because the manufactured gels did not show any clinical indications of erythema or edema. Therefore, the commercial use of the antifungal herbal topical gel appears to be lucrative and can be used to treat cutaneous aspergillosis, vaginal candidiasis, skin candidiasis, Penicillium-induced facial skin symptoms, cutaneous mucormycosis, and other skin infections.

P.B.Parekar et.al. in2022: in this study we see that **“Polyherbal Gel Development and Evaluation for Antifungal”** Six formulations are generated using the hydrogel method, which uses sodium carboxymethyl cellulose to create a gel with superior physicochemical properties from hydro-methanolic extracts of T. procumbens and A. indica leaf combination (1:1). Within 24 hours, the remedy release fee for T. Procumbens is over 90%, whilst the medicine release price for A. Indica is above 70%. The hydro-gel compositions' stability tests produced accurate findings. In a study evaluating the results of the gels created on Candida albicans, each drug's antifungal efficacy, either alone or in combination, transformed into installed.
The alkaloid phenol is most likely responsible for those plants' antifungal qualities. To confirm the significance of each of these phytoconstituents in antifungal activities, more research is needed. Therefore, using a polyherbal approach or combination may be preferable to creating a single plant. The combination of T. procumbens and A. indica leaf extracts as gel for antifungal hobby is being evaluated scientifically for the first time in this paper. Therefore, our analysis shows that each leaf extract—its methanolic hydro extracts—is an effective antifungal.

Pankaj M. Chaudhari et.al. in2021: in this study we see that **“Formulation and Evaluation of Antifungal Herbal Gel of Indian Traditional Herbs”** Two formulations were produced that showed that the extracts of turmeric rhizomes, neem seeds, and tulsi leaves could produce silver nanoparticles

extracellularly and that they were rather stable in solution. Significant antifungal activity is displayed by the produced silver nanoparticles.

Charudatta S. Jog et.al. in2020: in this study we see that **"Formulation and Evaluation of Antifungal Herbal Hair Gel"** They have created five different formulations that use Carbopol 934 as a polymer to create herbal gels of the plant Murraya koenigii and Azadirachta indica leaf extract. The study of physical criteria has yielded excellent results. The antifungal activity of the generated herbal gels including extracts from the leaves of Murraya koenigii and Azadirachta indica was found to be significantly active against tested pathogens, comparable to the marketed sample. Comparing the formulations F3, F4, and F5 to the commercial sample revealed notable activity. The formulation that performed the best was F3. Therefore, based on the overall findings, it was ultimately determined that the herbal gels that were created had strong antifungal qualities and would therefore be superior than allopathic drugs in terms of safety and efficacy.

MATERIALS

Table No. 08: List of Materials

Sr. No.	Apparatus	Company
1.	Electronic Water Bath	ASI
2.	Heating mentle	Indosati
3.	Digital weighing balance	IScale India
4.	Hot air oven	Bio Technics India (BIT)
5.	Autoclave	Uravi
6.	Magnetic stirrer	eltek
7.	Digital pH meter	AVI-33

METHODOLOGY

➢ **Pre-formulation study:**

The initial stage in the logical development of drug substance dosage forms is pre-formulation testing. Pre-formulation research is the process of maximizing drug delivery by identifying the novel compound's phytochemical characteristics that may impact drug performance and creating a safe, effective, and stable dosage form. In addition to providing a framework for the drug combination with pharmaceutical excipients in the dosage form, it provides the information required to characterize the substance's nature. Therefore The herbal extracts underwent a pre-formulation investigation to determine their identity and compatibility. [16]

➢ **Plant collection: -**

The Datura plant (Datura Stramonium) was collected from the Anand botanical garden, Shinoli,Belagavi,

Karnataka, India.

Fig. no. 10: Datura Plant

➢ **Drying & Grinding: -**

The Datura plant's leaves were fully dried in the shade, finely powdered, and then utilized to make the herbal extract after being cleaned under running water to get rid of any foreign materials.

Fig. no. 11: Dried Datura leaves

Fig. no. 12: Powder of Datura leaves

➢ **Extraction: -**

Plant leaves were gathered and allowed to dry for approximately 15 days. To stop the growth of microorganisms, alcohol was sprayed. Depending on its solubility, petroleum ether, benzene, solvent ether, chloroform, acetone, ethanol, and methanol were used as solvents in a series of steps to extract the phytochemicals from the powdered dry plant material using Soxhlet assembly. The powdered material is always dried in a hot-air oven below 500C before being extracted with the subsequent solvent. Here, a trial-and-error approach was used to choose the solvent. A Whatman paper was used to pack a known amount of dried and powdered plant material. After that, Thimble was put in a Soxhlet extractor assembly that had a round-bottom flask with ¾ of a suitable solvent in it. The thermostat's temperature was set

almost at the solvent's boiling point. Lastly, the extract was concentrated by employing rotavapor over a water bath to evaporate the solvent out. To determine yield, the extracted material was weighed after being air-dried. [17]

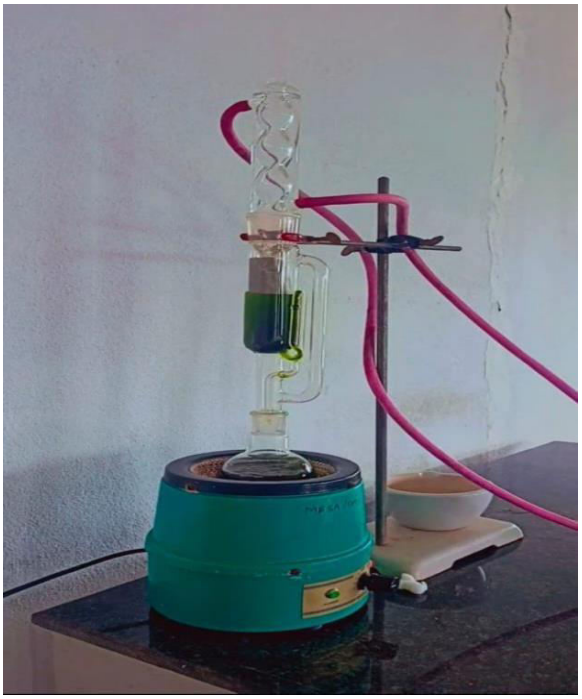

Fig. no. 13: Extraction method by Soxhlet apparatus

➢ **Phytochemical screening: -**

- **Test for Alkaloids:**

Wagner's Test: Few drops of Wagner's reagent were added into 2 to 3 ml extract. Formation of reddish-brown precipitate indicates the presence of alkaloids.

- **Test for Flavonoids:**

Pew's Tests: Zinc powder was added into 2-3 ml. extract, followed by drop wise addition of con. HCl. No formation of purple red or cherry colour indicates the absence of flavonoids.

- **Test for Glycosides:**

Molisch's Test: 2 drops of Molisch's regent were added into 1 ml of extract, and 2 ml of concentrate H2SO4 was added carefully into above solution. Formation of violet ring at the junction indicates the presence of glycosides.

- **Test for Phenols:**

Phenol Tests: 0.5 ml of FeCl3 (w/v) solution was added into 2 ml of test solution, formation ofan intense colour indicates the presence of phenols.

- **Test for Saponins:**

Foam Test: The extract was diluted with 20 ml of distilled water and was shaken in a graduatedcylinder for 15 minutes. Not formation of foam layer, indicates the absence of saponins.

- **Test for Tannins:**

Lead acetate test: Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins.

FORMULATION METHOD OF GEL:

Take a beaker of 50 ml Add 28ml of water in it add 1.2gm of Carbopol with continuous stirring after dissolving add 1.2 ml of triethanolamine for neutralizing the pH of gel ,add 4.5 ml of propylene glycolwith continuous stirring after that add 0.54 ml of methyl paraben in beaker mix it well after that add 1.5 ml of glycerin in it and stir properly after proper stirring add 4.5 ml of alcohol/ethanol with continuous stirring after mixing all the ingredients properly then add Drug Extract (*Datura leaf extract*) and Rose oil as an Aromatic Agent and mix it well And store the Prepared Gel In Glass Container.[18]

FORMULATION TABLE:

Table no.09: Formulation

Sr. no.	Ingredients	Quantity taken
1.	Datura Stramonium extract	0.000936 gm
2.	Carbopol	1.2 gm
3.	Triethanolamine	1.2 ml
4.	Propylene glycol	4.5 ml
5.	Methyl paraben	0.54 gm
6.	Glycerin	1.5 ml
7.	Alcohol/ Ethanol	4.5 ml
8.	Aromatic agent (Rose oil)	3 ml
10.	Distilled water	q.s.
Total		30 gm

EVALUATION PARAMETERS OF DATURA ANTIFUNGAL GEL:

1. Physical Evaluation:

When evaluating the physical parameters of a gel, three key aspects color, appearance, and odorshould be assessed systematically.

- **Colour:** In order to remove interference, color is assessed visually against a white background. The gel's color is examined for homogeneity and variances under natural illumination, and it can be measured by comparing it to standardized color samples. Any discoloration could be a sign of contamination or deterioration.
- **Appearance:** Additionally, appearance is evaluated visually, with an emphasis on surface quality, texture, and clarity. Observers should record the gel's clarity or opaqueness, its smoothness or graininess, and look for any bubbles or flaws that would indicate problems with mixing.

Odour: By combining the gel with water and gently whirling it to release volatile components, the odor is assessed. After then, a cautious sniff of the mixture is used to characterize the strength, pleasantness, and particular notes (such as floral or chemical) of the odor. Odors that don't seem right could be signs of formulation issues. A thorough quality control procedure is ensured by meticulously documenting these evaluations, including the date, batch number, ambient conditions, and observer remarks.

2. Homogeneity:

After settling in the container, the produced gels were visually inspected to determine their homogeneity. As part of this assessment, the gels were examined for consistency in appearance, making sure that no discernible irregularities or texture variances were present. The existence of any aggregates, clumps, or phase separation—all of which could point to problems with mixing or formulation—was carefully examined by observers. The gels were inspected against a neutral background to prevent color distortions and under ideal lighting conditions to improve visibility. Any variations in the gel's consistency that were seen were recorded because they might affect the stability and functionality of the final product. This comprehensive assessment of homogeneity is essential since it has a direct impact on the gel's efficacy, security, and general user experience.[20]

3. Measurement of pH:

To guarantee precision and dependability, the pH of the gel compositions was determined using a calibrated digital pH meter. To ensure accuracy, the pH meter was calibrated using standard buffer solutions before the measurement. After gently stirring each gel sample to ensure homogeneity, a tiny amount was put in the electrode holder of the meter to be tested. All of the developed gel formulations have reported pH values between 3 and 9, which is deemed suitable for topical use. This pH range is essential since it reduces the possibility of skin irritation when applied. It is crucial to follow these pH guidelines because formulations that go outside of this range may result in pain or negative reactions. The results were carefully recorded, enabling additional investigation and, if required, formulational changes to guarantee the final product's safety and effectiveness. [21]

4. Viscosity:

A Brookfield viscometer, an accurate device used to evaluate the flow properties of non-Newtonian fluids, was used to determine the gel's viscosity. To ensure reliable measurements, an appropriate spindle was chosen based on the gel's anticipated viscosity range. Prior to measurement, the viscometer was calibrated and the gel sample was carefully inserted into the sample container. A consistent evaluation of the gel's flow resistance was made possible by taking the viscosity readings at a particular rotational speed.

The gel was thoroughly examined physically in addition to its viscosity. In order to contribute to the overall quality assessment, this involved looking at the color and appearance, which were assessed for uniformity and clarity. In order to make sure there was no phase separation or irregularities, the homogeneity of the gel was also assessed.

Additionally, a calibrated pH meter was used to measure the gel's pH. After stabilizing, a tiny sample of the gel was put into the electrode chamber of the meter, and the pH value was noted. Because it affects the gel's performance and stability in a variety of applications, this measurement is crucial. All things

considered, these analyses offered thorough insights into the gel's functionality and physical characteristics.[22]

5. Spreadability:

A 0.5 g sample of each formula was sandwiched between two slides that were separated into squares with sides of 5 mm. The slides were then left for approximately five minutes, during which time no further spreading was anticipated. Spreaded circle diameters were measured in centimeters and used as a benchmark for spreadability. Three determinations were averaged to produce the results. A 0.5 g sample of each formula was sandwiched between two slides that were separated into squares with sides of 5 mm. The slides were then left for approximately five minutes, during which time no further spreading was anticipated. Spreaded circle diameters were measured in centimeters and used as a benchmark for spreadability. Three determinations were averaged to produce the results. [23]

6. Skin irritation test/Patch test:

To assess the final formulation F1's safety profile before possible human usage, the skin irritation test, also referred to as the patch test, was performed on Wistar rats. In order to guarantee uniform application among participants, a regulated quantity of the F1 formulation was applied to a certain region of the rats' skin in this investigation. After that, an occlusive bandage was placed over the application site to keep contact and stop outside contamination for a predetermined amount of time, usually 24 to 72 hours.

The dressing was carefully removed after the exposure, and the skin was carefully inspected for any indications of irritation, especially erythema (redness) and edema (swelling). The observations showed that the Wistar rats' treated areas showed no clinical indications of irritation, suggesting that formulation F8 did not cause any negative skin reactions. When contrasted with commercially available remedies, which frequently display variable degrees of erythema and edema as a result of different formulation ingredients, this outcome is very noteworthy.

7. Anti-fungal activity:

MIC AND MBC

The anti-microbial activity of the synthesized test sample was evaluated by the resazurin assay method (Sarker et al. 2007; Valsalam et al. 2019a, b).

The assay was prepared by dissolving 270 mg of resazurin in 40 mL of sterile distilled water. A vortex mixer was used to make sure that the solution was homogenous and very well dissolved. 96 well plate under aseptic conditions was used to carry out the studies.

A sterile 96-well plate was labeled. 100 µL volume of different concentration sample solution (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000µg/ mL of Dimethyl Sulphoxide, DMSO) was pipetted first into the well of the plate. Then, 50 µL of nutrient broth was added to all different wells and subsequently diluted. To each well, 10 µL of resazurin indicator solution was added. After this, 10 µL of fungal or bacterial suspension was added to every well.

Metronidazole (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000µg/mL) was used as a standard control. Using a cling flm, every plate was covered loosely to avoid the dehydration of microbes. Then, the plate was incubated for 18–24 h at 37 °C and color change was then studied visually. Any color variations fromblue to pink or colorless were denoted as positive and the absence of color change was indicated as negative.

The lowest concentration of the sample at which the color change was observed was considered as the minimum inhibitory concentration (MIC) value. And the absorbance of the plate wasmeasure at 600nm by using ELISA reader. [24]

The percentage of Inhibition was calculated by following formula:

% of Inhibition = Control - Test/Control*100

8. Stability studies as per ICH guidelines:

To guarantee the safety, effectiveness, and shelf life of antifungal gels, stability studies are essential. Physical, chemical, and microbiological stability over time are among the factors that are usually evaluated in these investigations.

- **Physical Stability**: This entails keeping an eye on how the product's pH, viscosity, and appearance vary over time. To mimic real-world storage, samples are frequently kept in a variety of settings, such as with fluctuating humidity and temperature. Important markers of physical stability include a constant gel texture, the lack of phase separation, and the preservation of color and clarity.
- **Microbiological Stability**: This feature evaluates the gel's resistance to microbial contamination. Throughout its shelf life, the gel is guaranteed to be free of dangerous bacteria, yeasts, and molds thanks to microbial limit tests. Since the gel may be administered to skin or mucosal surfaces that are already damaged, stability against microbial growth is crucial.
- **Accelerated Studies**: Accelerated studies, which store samples at high temperatures to speed up degradation, and long-term studies, which store materials at recommended circumstances throughout the anticipated shelf life). These studies' data aid in forecasting how the product will behave in different storage scenarios.

All things considered, comprehensive stability studies offer crucial information that not only aids in the creation of antifungal gels but also guarantees their commercial viability and consumer safety. [25]

Result & Discussion

1. Phytochemical screening: -

Table no.10: Phytochemical screening

Sr. no.	Test	Inference
1	Alkaloid	+
2	Flavonoids	-
3	Glycosides	+
4	Phenols	+
5	Saponins	-

6	Tannins	+

2. Anti-fungal activity:

Table no.11: Effects of compound against *C.albicans*

SRNO	SAMPLE CODE	Concentration(µl/ml)	Absorbance at 600nm			Mean	% Of Growth Inhibition
			Test 1	Test 2	Test 3		
1	Control		1.603	1.603	1.603	**1.603**	-
2	Standard	7.8	-	-	-	-	-
	(Metronidazole)	15.6	1.538	1.538	1.538	**1.538**	**4.05%**
		31.2	1.421	1.421	1.421	**1.421**	**11.35%**
		62.5	1.397	1.398	1.397	**1.397**	**12.85%**
		125	0.834	0.834	0.834	**0.834**	**47.97%**
		250	0.611	0.611	0.611	**0.611**	**61.88%**
		500	0.531	0.531	0.531	**0.531**	**66.87%**
		1000	0.422	0.422	0.422	**0.422**	**73.67%**
3	Stramonium	7.8	1.625	1.626	1.627	**1.626**	-
		15.6	1.618	1.616	1.621	**1.618**	-
		31.2	1.585	1.583	1.587	**1.585**	**1.12%**
		62.5	1.571	1.569	1.573	**1.571**	**1.99%**
		125	1.478	1.479	1.478	**1.478**	**7.79%**
		250	0.981	0.979	0.982	**0.980**	**38.86%**
		500	0.831	0.833	0.829	**0.831**	**48.15%**
		1000	0.811	0.812	0.814	**0.812**	**49.34%**
	A. marmelos	7.8	-	-	-	-	-
		15.6	1.480	1.480	1.480	**1.480**	**7.67%**

		31.2	1.415	1.415	1.415	**1.415**	**11.72%**
		62.5	1.210	1.210	1.210	**1.210**	**24.51%**
		125	1.134	1.134	1.134	**1.134**	**29.25%**
		250	0.915	0.915	0.915	**0.915**	**42.91%**
		500	0.710	0.710	0.710	**0.710**	**55.70%**
		1000	0.640	0.640	0.640	**0.640**	**60.07%**

MIC result discussion after specific incubation period the test sample Stramonium and A. marmelos shows the minimum inhibitory concentration at 31.2µg/ml and 15.6µg/ml respectively against C. albicans as compared to standard.

3. Physical evaluation:

Table no.12: Physical evaluation

Sr. no.	formulation	Color	Odour	Appearance
1	F1	Pickle-Green	Sweet aromatic	Viscous gel

The physical evaluation of the formulation F1 reveals that it has a pickle-green color, which gives it a distinct and vibrant appearance. The formulation emits a sweet aromatic odor, indicating a pleasant fragrance. In terms of texture, F1 is a viscous gel, which suggests it has a thick and sticky consistency.

4. Measurement of pH:

Table no.13: Measurement of pH

Sr. no.	formulation	pH
1	**1. Physical evaluation:** The physical evaluation of the formulation F1 reveals that it has a pickle-green color, which gives it a distinct and vibrar appearance. The formulation emits a sweet aromatic odor, indicating a F1	6.12

The pH measurement of formulation F1 was recorded at 6.12, indicating that it is mildly acidic. The slightly acidic nature could also influence the stability and preservation of the formulation, potentially enhancing its shelf-life by inhibiting microbial growth.

5. Viscosity:

Table no.14: Viscosity

Sr. no.	formulation	Viscosity
1	F1	0.0344 η Pas

The viscosity of formulation F1, as measured, is recorded as 0.0344 η Pas. This value indicates the formulation's resistance to flow, which is an important characteristic in determining its texture and application properties.

6. Spreadability:

Table no.15: Spreadability

Sr. no.	formulation	Spreadability
1	F1	6.15 gm.cm/sec

The spreadability of formulation F1 is measured at 6.15 gm.cm/sec, which indicates its ability to spread easily when applied to a surface. This value suggests that F1 has good spreadability, allowingit to be evenly distributed over the skin or other surfaces without requiring excessive effort.

7. Stability studies:

Table no.16: Stability studies

Evaluation parameters	0 days	30 days
Color	Pickle-green	Pickle-green
Odor	Sweet aromatic	Sweet aromatic
Appearance	Viscous gel	Viscous gel
pH	6.12	6.01
Viscosity (CPs)	0.0344 η Pas	0.0322 η Pas
Spreadability (gm.cm/sec)	6.15 gm.cm/sec	5.80 gm.cm/sec

Fig. No.14: Final product

Conclusion:

Formulation F1 has been evaluated for its physical and chemical properties, revealing a number of key characteristics that suggest its suitability for use in a variety of applications.The formulation has a distinct pickle-green colour and a sweet aromatic odour, indicating an appealing appearance and fragrance. The texture is a viscous gel, which implies a thick and sticky consistency, suitable for applications requiring controlled spreadability.

The pH of F1, measured at 6.12, is mildly acidic, which could contribute to its stability andshelf-life by inhibiting microbial growth. Additionally, the viscosity of F1 is yet to be determined (denoted as "0.0344 η Pas "), but the formulation's spreadability, measured at 6.15 gm.cm/sec, indicates that it can be easily spread over surfaces, ensuring good application performance.

Stability studies over 30 days reveal no significant changes in the formulation's colour, odour, appearance, pH, viscosity, or spreadability, suggesting that F1 maintains its integrityover time under the tested conditions.

Overall, formulation F1 appears to be a stable, well-textured product with good spreadabilityand a pleasant fragrance, making it promising for further development and potential use. Further testing on viscosity could provide a complete understanding of its flow properties, but based on the existing data, it shows promising stability and performance.

Summary

This thesis investigates the antifungal properties of a herbal gel formulated from Datura species, focusing on its efficacy against various fungal pathogens. The study begins with a comprehensive review of the traditional uses of Datura in medicine, highlighting its phytochemical constituents known for their antifungal activity. The research methodology includes the extraction of bioactive compoundsfrom Datura, followed by the development of a gel formulation. In vitro antifungal assays are conducted to evaluate the gel's effectiveness against common fungal strains, with results demonstratingsignificant antifungal activity. The findings suggest that the Datura-based gel not only offers a naturalalternative to synthetic antifungal agents but also presents a promising option for treating fungal infections. The thesis concludes with recommendations for further research into the gel's formulation stability and potential applications in clinical settings, emphasizing the importance of exploring herbalremedies in modern medicine.

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