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FORMULATION AND EVALUATION OF TROPICAL GEL OF TINOSPORA CORDIFOLIA AS ANTIFUNGAL AGENT

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INTRODUCTION

COSMETICS

"Any article intended to rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying. Promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetics"

A good cosmetic should have the following properties.

1) All the raw materials used should be of highest quality and they should bestandardized for their qualities.
2) All water used in cosmetics should be distilled or purified water.
3) The color used should be the permitted color.
4) The perfumes used should be compatible with other ingredients of thepreparation.
5) They should be tolerated all conditions particularly extreme of temperature and humidity condition to which they are likely to encountered in the market.
6) They should be economic.

COSMOCEUTICALS

Cosmeceuticals refers to the combination of cosmetics and pharmaceuticals. Cosmeceuticals are cosmetic products with biologically active ingredients purporting to have medical or drug-like benefits.

Cosmeceuticals are topically applied, but they contain ingredients that influencethe biological function of the skin. Cosmeceuticals improve appearance, but they do so by delivering

nutrients necessary for healthy skin. Cosmeceuticals typically claim to improve skin tone, texture, and radiance, while reducing wrinkling. Cosmeceuticals are the fastest-growing segment of the natural personal care industry.

Characterization of Cosmeceuticals

1) The product has pharmaceutical activity and can be used on normal or near-normal skin.
2) The product should have a defined benefit for minor skin disorders (cosmeticindication).
3) As the skin disorder is mild the product should have a very low-risk profile.

SKIN

The skin is the largest organ of the body, covering about 1.7 m and comprising approximately 10% of the total body mass of an average person. The primary function of the skin is to provide a barrier between the body and the external environment.

This barrier protects against the permeation of ultraviolet (UV) radiation, chemicals, allergens and microorganisms, and the loss of moisture and body nutrients. In addition, the skin has a role in homeostasis, regulating body temperature and blood pressure. The skin also functions as an important sensory organ in touch with the environment, sensing stimulation in theform of temperature, pressure, and pain.

Human skin is composed of four main regions: the stratum corneum, the viable epidermis, dermis and subcutaneous tissues A number of appendages are associated with the skin: hair follicles andeccrine and apocrine sweat glands.

From a skin permeation viewpoint, the stratum corneum provides the main barrier and therefore the structure of this layer will be discussed in most detail. The other layers and appendages contribute important functions and are important target sites for drug delivery.

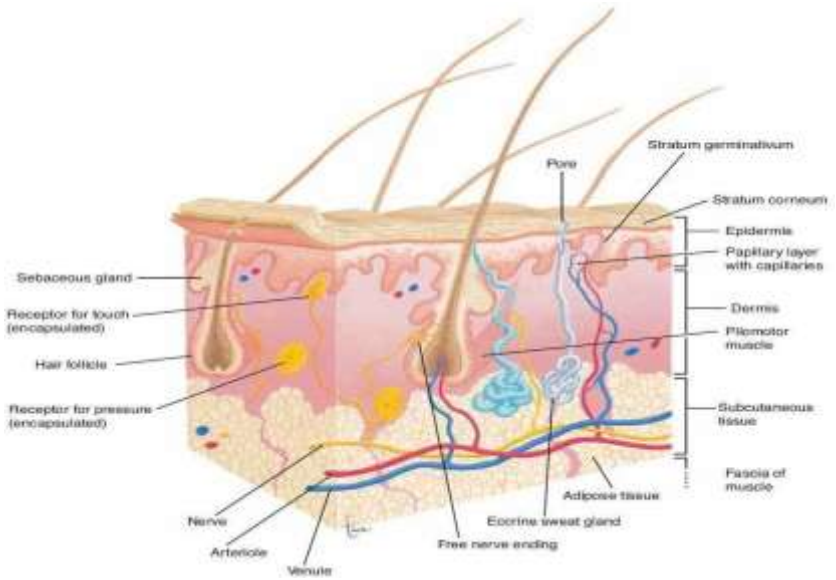


Fig 1: structure of skin.

1. Epidermis

The epidermis is the most superficial layer of the skin and provides the first barrier of protectionfrom the invasion of substances into the body.

The epidermis is subdivided into five layers or strata.

- stratumbasale
- stratum spinosum
- stratum granulosum
- stratum lucidum
- stratum corneum

2. Dermis and Subcutaneous Fat

The dermis is a tough but elastic support structure that houses nerves, blood vessels, lymphatics, and cutaneous appendages (pilosebaceous units, eccrine and apocrine sweat glands).

It is thicker (averages 1 to 4 mm) than the epidermis which is about as thin as piece of paper. The dermis varies in thickness. It is very thick on the back (almost 1 cm); it is very thin on the eyelid.

The dermis has two main zones, the papillary dermis and the reticular dermis. The subcutaneous fat is an important layer and plays a role in shock absorption, energy storage,

andmaintenance of bodyheat.

3. **Other cellular components of the epidermis**

Melanocytes are dendritic, pigment-producing cells located in the basal layer. The pigment theymanufacture is called melanin.

Langerhans cells are dendritic cells derived from the bone marrow in the stratum spinosum that have animmunologic function. They are identical to tissue macrophages and present antigens tolymphocytes.

Permeation through skin

A penetrant applied to the skin surface has three potential pathways across the epidermis: through sweat ducts, via hair follicles and associated sebaceous glands, or across the continuous stratum corneum These pathways are not mutually exclusive, with most compounds possibly permeating the skin by a combination of pathways and the relative contribution of each being related to the physicochemical properties of the permeating molecule.

Function of skin

The skin is the largest organ of the human body and serves several important functions. Here are somekey function of the skin.

1. **Protection**: The skin acts as a barrier that protects the body from external factors such as pathogens, UV radiation, chemicals, and physical injuries.
2. **Regulation:** The skin helps regulate body temperature through processes like sweating andshivering. It also plays a role in maintaining fluid balance.
3. **Sensation**: The skin contains sensory receptors that allow us to feel touch, pressure, temperature, andpain.
4. **Excretion**: The skin helps eliminate waste products from the body through sweating.
5. **Vitamin D synthesis**: The skin plays a crucial role in the production of vitamin D when exposed tosunlight.
6. **Immunity:** The skin is part of the body's immune system, acting as a defense against pathogens andinfections.

Overall, the skin is essential for maintaining the body's overall health and well-being. The skin serves various functions such as protection, regulation, sensation, excretion, vitamin D

synthesis, andimmunity**.**

TOPICAL GEL

Gels are semisolid systems in which a liquid phase is constrained within a three dimensional polymeric matrix of natural or synthetic gums in which a high degree of physical or chemical crosslinking has been established.

Most topical gels are prepared with organic polymers, such as carbomers, that impart an aesthetically pleasing, clear, sparkling appearance to the products and are easily washed off from the skin with water. The type of base used in formulating a topical dermatological product greatly influences its effectiveness. Bases containing large amounts of oleaginous substances provide an emollient effect to dry irritated skin. More importantly, bases made up of non-volatile oleaginous substances (e.g. hydrocarbon bases) can form an occlusive barrier on the skin that prevents escapeof moisture from the skin into the environment.

As a result, moisture accumulates between the skin and the ointment layer that cause hydration ofthe stratum corneum. Hydration of stratum corneum all 'opening up' of intra and inter-cellular channels and pathway for easier passage of drug molecules. Additionally, the moisture layer provides a medium for dissolution of the drug that is otherwise dispersed as fine particles in the ointment base. Since only the dissolved drug presented to the skin, as an individual molecular entity is able to enter the stratum corneum, skin occlusion generally results in enhanced percutaneous drug absorption**.**

Fig 2a gel

Fig 2b-gel

FUNGAL SKIN INFECTION

Fungal skin infections, including those caused by Candida albicans, are common skin conditionsthat occur due to fungal overgrowth on the skin. These infections can manifest as red, itchy rashes, scaling, or blisters on the skin. Fungal skin infections are typically treated with antifungal medications, such as antifungal gels, creams, or oral medications, depending

on the severity of theinfection.

It's essential to keep the affected area clean and dry, avoid sharing personal items like towels orclothing, and follow the prescribed treatment regimen to effectively clear the infection.

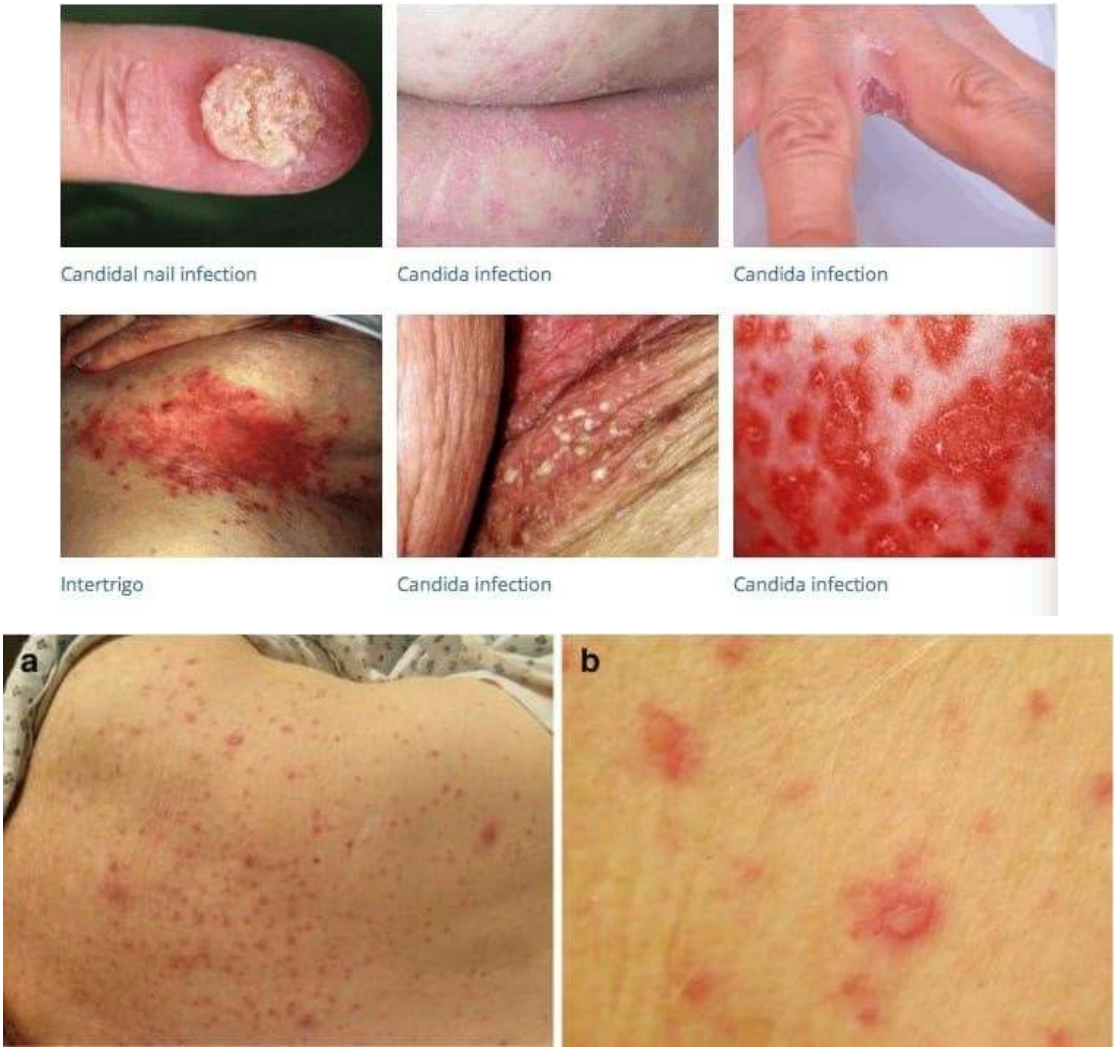


Fig 3-candida infection on various part of body.

CANDIDA ALBICANS

Candida albicans is a type of yeast that is commonly found on the skin, in the digestive tract, and in other mucous membranes of the body. While Candida albicans is a normal part of the human microbiota, overgrowth of this yeast can lead to infections, particularly in warm and moist areas of the body.

Candida albicans can cause various infections, including oral thrush, vaginal yeast infections, and skin infections. Factors such as weakened immune system, antibiotic use, diabetes, and poor hygiene can contribute to the overgrowth of Candida albicans.

Treatment for Candida albicans infections typically involves antifungal medications to helpsto eliminate the yeast and restore the balance of microorganism on the skin or mucous membrane.

Table 1: taxonomy of c.albicans.

Domain:	Eukaryota
Kingdom:	Fungi
Division:	Ascomycota
Class:	Saccharomycetes
Order:	Saccharomycetales
Family:	Saccharomycetaceae
Genus:	***Candida***

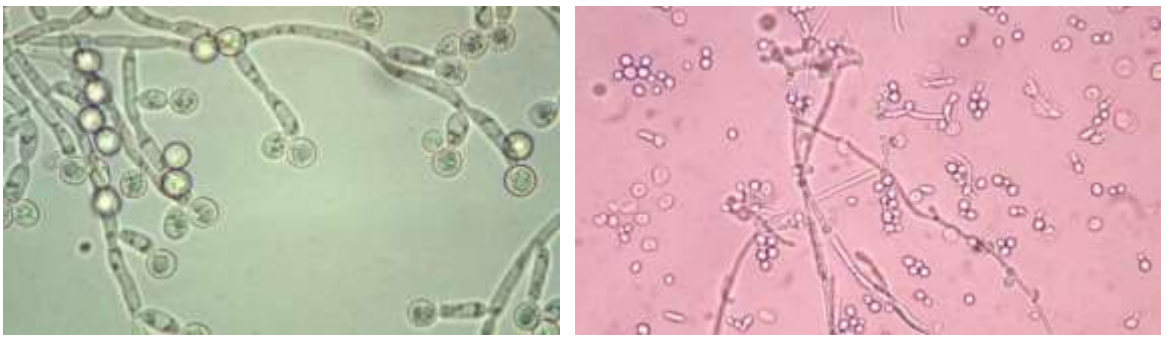


Fig 3a 3b microscopic view of c.albicans.

Distribution of Candida species responsible for candidiasis (n = 82). Candida albicans (n = 38), Candida parapsilosis (n = 16), Candida glabrata (n = 13), Candida tropicalis (n = 12), Candidadubliniensis (n = 1), Candida guilliermondii (n = 1), Candida spp. (n = 1)

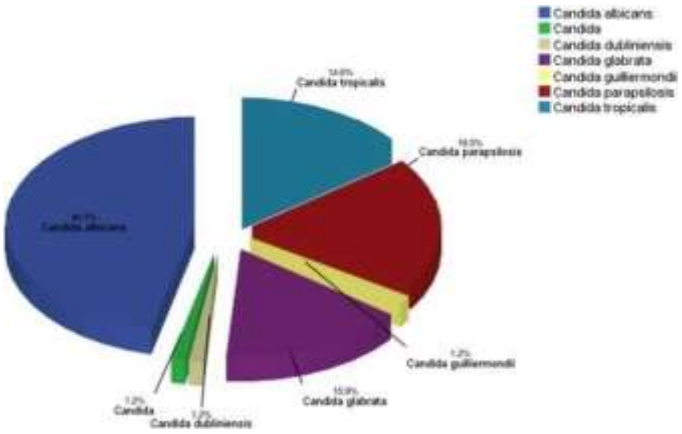


Fig 4: distribution of c.albicans.

The chart shows the distribution of Candida species responsible for candidiasis in a retrospectivestudy conducted in a teaching hospital from 2011 to 2017. The study involved 82 critically ill patients.

The most common Candida species identified was Candida albicans, accounting for 38% of cases other significant species included Candida parapsilosis (16%), Candida glabrata (13%), and Candidatropicalis (12%). Less common species were Candida dubliniensis, Candida guilliermondii, and Candida spp., each representing 1% of cases.

This data suggests that Candida albicans remains the predominant cause of candidiasis in critically illpatients in China, but other species are also emerging as important pathogens.

TINOSPORA CORDIFOLIA

Tinospora cordifolia, commonly known as Giloy or Guduchi, is a herbaceous vine nativeto the Indian subcontinent. It has been used in traditional Ayurvedic medicine for centuries due to its various health benefits. Tinospora cordifolia is known for its immunomodulatory, antioxidant, and anti-inflammatory properties.

In Ayurveda, Tinospora cordifolia is often used to boost immunity, improve digestion, and promote overall health and well-being. It is also used in the treatment of various ailments such as fever, diabetes, and skin disorders.

Table 2: Taxonomy of t cordifolia.

Kingdom	Plantae-Plant
Subkingdom	Tracheobionta-Vascular plant
Super division	Spermatophyta-Seed bearing plant
Division	Magnoliophyta – Flowering
Class	Magnoliopsida – Dicotyledons
Sub-class	Polypetalae – Petals are free
Series	Thalamiflorae – Many stamens and flower hypogynous
Order	Ranales
Family	Menispermaceae – The Moonseed family
Tribe	Tinosporeae
Genus	*Tinospora*
Species	*T. cordifolia*

Fig 5 t. Cordifolia

Fig 5a.leaf Fig 5b.stem Fig 5c.leaf Fig 5d. flower Fig 5e. fruit Fig 5f. stem

Morphological Description

Tinospora cordifolia is a large deciduous, extensively spreading climbing shrub with a number of coiling branches. Tinospora cordifolia is a glabrous, succulent, woody climbing shrub native to India. It thrives well in the tropical region, often attains a great height and climbs up the trunk of large trees.

Different parts of Tinospora have following type of morphology

Table 3: morphological description of t.cordifolia.

Stem	Stem of this plant is rather succulent with long, filiform, fleshy and climbing in nature. Aerial roots arise from the branches. The bark is creamy white to grey in colour and deeply left spirally.
Arial Root	Arial roots are present, these aerial roots are characterized by tetra to penta-arch primary structure. However, cortex of root is divided in to outer thick walled and inner parenchymatous zone.
Leaves	Leaves of this plant are simple, alternate, ex-stipulate, long petioled approximately 15 cm, round, pulvinate, heart shaped, twisted partially and half way round. Lamina is ovate, 10-20 cm long, 7 nerved and deeply cordate at the base and membranous.
Flowers	Flowers are unisexual, recemes, greenish yellow in colour, appears when plant is leaf less. Male flowers are clustered and female flowers exist in solitary inflorescence. Sepals are 6 in 2 series of 3 each. Outer ones are smaller than the inner sepals. Petals are also 6, smaller than sepals, free and membranous. Flowering occurs during March to June.
Seed	Curved seed have been reported in this species. Hence this family is named as moonseed family.
Fruit	They are orange-red in colour, fleshy, aggregate of 1-3 and ovoid, smooth, drupelets on thick stalk with a sub terminal style scars. Fruits develop during winter.

Phytoconstituents in Tinospora cordifolia

Alkaloids: Berberine, gilonins, gilosterol, tinosporine, tinosporin.

Glycosides: Tinosporide, tinosporaside.

Steroids: Cordifolide, cordifol.

Other compounds: Heptacosanol, clerodane diterpenes, β- sitosterol Essential oils, fatty acids, polysaccharides.

Stem compounds: Berberine, palmatine, tembertarine, magniflorine, choline, tinosporin.

Microscopic characteristics

Tinospora cordifolia, also known as Guduchi or Giloy, is a well-known medicinal plant used in traditional medicine systems, especially in Ayurveda. When observed under a microscope, different parts of the plant, such as the stem and leaf, exhibit distinct microscopic characteristics. Here's a breakdown of its key microscopic features.

Stem

- **Epidermis:** Small cuboidal/rectangular cells with a cuticle layer.
- **Cortex:** Parenchymatous cells containing starch grains; sclerenchymatous fibers for support.
- **Cork Cells:** Rectangular cells forming the outer layer; phellogen underneath producing corkand phelloderm.
- **Phloem**: Contains sieve elements, companion cells, and fibers.
- **Xylem**: Wide, lignified vessels arranged radially with starch-rich xylem parenchyma.
- **Medullary Rays**: Radial parenchymatous rows connecting pith and periphery.
- **Pith**: Thin-walled parenchyma filled with starch grains.

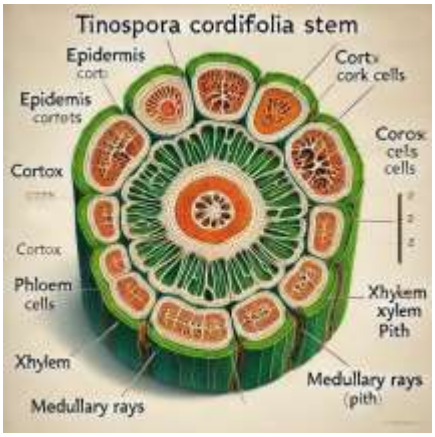


Fig 6: microscopic view of t. Cordifolia.

Tinospora cordifolia, also known as Giloy or Guduchi, possesses several beneficial properties

1. Immunomodulatory: Tinospora cordifolia is known for its ability to modulate and strengthen the immune system, helping the body defend againstinfections and diseases
2. Antioxidant: It exhibits antioxidant properties, which help in combatingoxidative stressand reducing damage caused by free radicals in the body.
3. Anti-inflammatory: Tinospora cordifolia has anti-inflammatory properties that canhelp reduce inflammation in the body, making it beneficial for conditions involving inflammation.
4. Antipyretic: It also has antipyretic properties, meaning it can help reducefever andalleviate associated symptoms.

These properties make Tinospora cordifolia a valuable herb in traditional medicine systemslike Ayurveda for promoting health and treating various health condition.

ANTI FUNGAL PROPERTY OF GILOY

Antifungal property of Giloy or Guduchi refers to its ability to combat fungal infections by inhibiting the growth of fungi, particularly Candida species that can cause skin infections. This property makes Guduchi a valuable herb in traditional medicine for treating various fungal skin conditions and promoting skin.

MECHANISM OF GILOY

The mechanism of action of Tinospora cordifolia (Guduchi) as an antifungal agent involvesits bioactive compounds, such as berberine, palmatine, and giloin. These compounds have been found to inhibit the growth of various fungi, including Candida species.

Berberine, one of the key components of Guduchi, disrupts the cell membrane of fungi, leading to leakage of cellular contents and ultimately causing fungal cell death. Palmatine, another compound in Guduchi, exhibits antifungal activity by interfering with fungal cell wall synthesis. Giloin, found in Guduchi, also contributes to its antifungal properties by inhibiting fungal growth and reproduction.

Overall, the combination of these bioactive compounds in Guduchi works synergistically to combat fungal infections by targeting different aspects of fungal cell structure and function, making it an effective natural antifungal agent.

OTHER USES OF GILOY INCLUDE

- Boosting immunity
- Treating respiratory problems
- Managing diabetes
- Improving digestion
- Reducing stress and anxiety
- Treating arthritis and joint pain

AIM AND OBJECTIVE

AIM

To FORMULATION AND EVALUATION OF TOPICAL GEL OF TINOSPORA CORDIFOLIA AS ANTI FUNGAL AGENT.

OBJECTIVE

Inhibition of fungal growth

To prevent the growth and proliferation of fungal pathogens.

Treatment of fungal infections

To effectively treat various fungal infections, such as skin infections, respiratory tract infections, and systemic mycoses.

Alternative to synthetic antifungals

To provide a natural, safe, and effective alternative to synthetic antifungal agents, which can have adverse effects and promote drug resistance.

Enhancement of immune system

To boost the immune system, enabling the body to fight off fungal infections more effectively.

Prevention of fungal-related diseases

To prevent diseases caused by fungal infections, such as allergic reactions, asthma, and autoimmune disorders.

Development of new antifungal drugs

To isolate and characterize bioactive compounds from Tinospora cordifolia, leading to the development of new antifungal drugs.

Topical applications

To explore the use of Tinospora cordifolia in topical formulations for skin and mucous membrane infections.

FORMULATIONAND PREPARATION

INGREDIENTS

Topical gel was formulated as per described and the ingredients used in the formulation of topical gel are.

1. GILOY STEM EXTRACT
2. CARBAPOL
3. TRIETHANOLAMINE.
4. METHYL PARABEN
5. DISTILLED WATER
6. GLYCERINE

GILOY STEM EXTRACT

The Giloy stem extract, which comes from the Tinospora cordifolia plant, is well-known for itsmedicinal benefits.

It is used in traditional medicine for its antifungal properties, anti-inflammatory effects, and ability to boost the immune system. This extract is commonly used to treat fungal infections and promote overall health and wellness.

Fig 7: stem.

1. CARBOPOL

Carbomer 940 forms a gel-like network, thanks to its cross-linked polymer structure. It's this unique formation that gives Carbomer 940 its stability and viscosity properties, making it an invaluable ingredient in various formulations. Carbomer has good gelling, viscosity, thickening, emulsifying, suspending and film-forming properties, and its chemical properties are stable and safe, without irritation or allergic reactions. Carbomer 940 forms a gel-like network, thanks to its cross-linked polymer structure. It's this unique formation that gives Carbomer 940 its stability and viscosity properties, making it an invaluable ingredient in various formulations.

Fig 8: carbopol 940.

2. TRIETHANOLAMINE

Triethanolamine is a compound commonly used in various cosmetic and personal care products. It acts as a surfactant, emulsifier, and pH adjuster inproducts like lotions, creams, and soaps. It helps to stabilize the product, adjust its pH, and enhance its texture.

Fig 9: Triethanolamine.

3. GLYCERINE

Glycerin, also known as glycerol, is a simple polyol compound. It is a colorless, odorless, viscousliquid that is sweet-tasting and non-toxic. Glycerin is widely used in pharmaceutical formulations, food products, cosmetics, and personal care items due to its moisturizing and emollient properties. It is also used as a humectant, solvent, and preservative in various products.

Fig 10: glycerin.

4. DISTILLED WATER

Distilled water is water that has been boiled into vapor and condensed back into liquid in aseparatecontainer. Impurities in the original water that do not boil below or near the boiling pointof waterremain in the original container. Thus, distilled water is a type of purified water. Distilled water is steam from boiling water that's been cooled and returned to its liquid state. Some people claim distilled water is the purest water you can drink.

Fig 11 distilled water.

5. METHYL PARABEN

Methyl paraben is a preservative commonly used in cosmetics, personal care products and food toprevent the growth of bacteria and fungi. It helps extend the shelf life of products by inhibiting microbial growth.

Fig 12: methyl paraben.

TABULAR COLUMN: 01 FORMUALTION OF TOPICAL GEL

Table 4: formulation of topical gel.

SLNO.	INGREDIENTS	PROPERTIES	QUANTITY
1.	GILOY STEM EXTRACT	Antifungal agent	1gm
2.	CARBOPOL	Thickening and gelling agent	1gm
3.	METHYL PARABEN	Preservative	0.02gm
4.	DISTILLED WATER	Purity	100ml
5.	TRIETHANOLAMINE	Ph adjuster, emulsifier andsurfactant	q.s
6.	GLYCERINE	Humectant	q.s

METHODOF

PREPARATION

The method of preparation involves two primary steps: Giloy Stem Extract and Gel Preparation. Here's a detailed breakdown of each step.

1. Giloy Stem Extract

Selection of Stems: Fresh Guduchi stems (Tinospora cordifolia), commonly known as Giloy, wereselected based on their health and maturity.

Peeling the Stems: The outer layer of the selected stems was carefully removed to ensure that only the inner stem, which contains the most beneficial compounds, was used.

Smashing and Washing: After peeling, the stems were smashed or crushed to break them down into smaller pieces, which increases the surface area for extraction. These crushed stems were thenthoroughly washed using distilled water to remove any impurities or contaminants.

Filtration: The washed stems were subjected to filtration using a muslin cloth, which served to separate the larger solid plant particles from the liquid extract. This process ensures a cleaner extract, devoid of large debris.

Sedimentation: The filtered liquid was left undisturbed for 5-6 hours, allowing any fine particles in the extract to settle at the bottom. During this period, sedimentation occurs, leading to the separation of the water and solid components.

Separation of Powder: After the sedimentation period, the excess water was carefully removed, leaving behind the sedimented powder. This powder, which contains the active compounds fromthe Giloy stem, was collected.

Drying: The collected sediment was then dried to remove any residual moisture, resulting in a fine, dry Giloy stem extract powder that could be stored for further use.

2. Gel Preparation

Carbopol Dissolution: For the gel base, 1 gm of Carbopol (a thickening agent commonly used ingel formulations) was dissolved in 100 ml of distilled water. The dissolution process ensures thatthe Carbopol is fully hydrated, creating a uniform gel base.

Magnetic Stirring: The mixture was then placed on a magnetic stirrer for approximately 15 minutes. The continuous stirring helps achieve an even distribution of Carbopol within the water,ensuring the formation of a smooth gel without lumps.

Triethanolamine Addition: To achieve the desired gel consistency, triethanolamine was added dropwise. Triethanolamine acts as a neutralizing agent, converting the Carbopol solution into a gelby adjusting its pH. This process continued until a transparent gel was formed, indicating thatthe gel base was ready for further ingredients.

3. Incorporating the Extract into the Gel

Adding Giloy Stem Extract: Once the gel base was prepared, 1 ml of the previously prepared Giloy stem extract was carefully added to the gel. This amount of extract is sufficient to impart thebeneficial properties of the Giloy stem to the gel formulation.

Preservative Addition: To ensure the stability and shelf-life of the gel, 0.02 gm of methylparaben, a commonly used preservative in cosmetic and pharmaceutical preparations, was added.

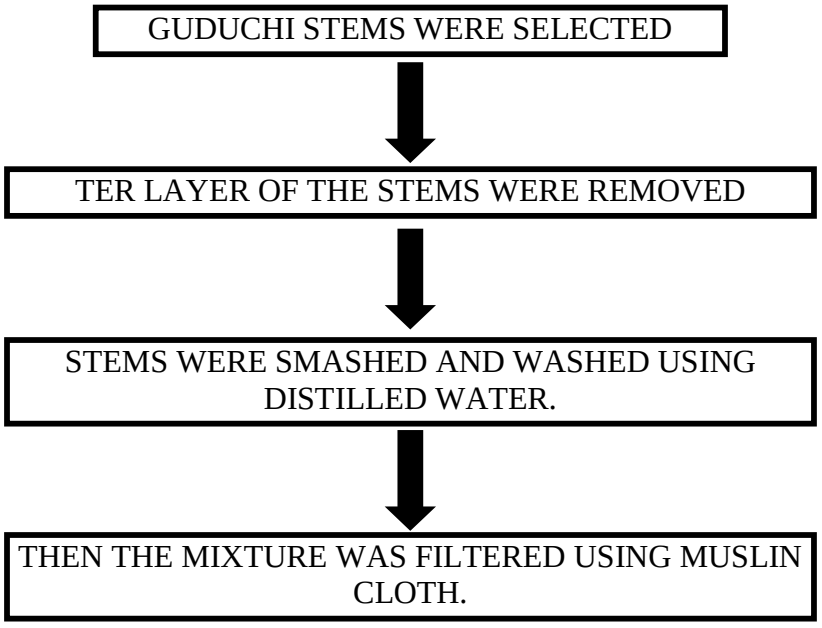
Methylparaben helps prevent microbial growth in the gel.

Glycerine Addition: Finally, glycerine was added as required. Glycerine acts as a humectant, providing moisture-retaining properties to the gel, making it more hydrating and soothing on the skin. The final gel formulation was then mixed thoroughly to ensure uniform distribution of all theingredients.

This method results in a Giloy-infused gel that can be used for topical applications, providing themedicinal benefits of Giloy in a convenient and easy-to-apply form.

Fig 13: Prepared Gel.

1. GILOY STEM EXTRACT



THE FILTRATE WAS THEN LEFT UNDISTURBED FOR 5-6 HOURS.

THEN THE WATER WAS REMOVED AND THE SEDIMENTED POWDER EXTRACT WAS SEPARATED.

THE POWDER WAS DRIED AND COLLECTED

2. GEL PREPARATION

1GM OF CARBOPOL WAS DISSOLVED IN 100ML DISTILLED WATER.

THE MIXTURE WAS KEPT IN MAGNETIC STIRRER FOR ABOUT 15MINUTES

ADD TRIETHANOLAMINE DROPWISE UNTILTRANSPARENT GEL IS FORMED.

3. ADDING EXTRACT TO GEL PREPARATION

ADD 1ML OF GILOY STEM EXTRACT INTO THE GEL PREPARATION.

ADD 0.02GM OF METHYL PARABEN AS PRESERVATIVE

ADD GLYCERINE AS REQUIRED.

Fig 14 a. Selected guduchi stems.

Fig 14 b. Peeled outer layer.

Fig 14 c. Stems were crushed.

Fig 14 d. Thoroughly washed stems.

Fig 14 e. Filtered and removed waste using muslin cloth.

Fig 14 f. Filtrate left for sedimentation.

Fig 14 g. Extracted liquid.

Fig 14 h. Carbopol gel preparation.

Fig 14 i. Final product.

EVALUATION

Evaluation parameter is divided into

Part 01: Pre formulation studies

Part 02: In vitro studies

PART 01: PRE FORMULATION STUDIES

Pre formulation studies for topical gels are essential steps in the development of pharmaceutical formulations. These studies help in understanding the properties of the active ingredients and excipients, ensuring that the final product is safe, effective, and stable.

It includes.

1. Appearance
2. Colour
3. Odour
4. pH
5. Spreadability
6. Irritability

1. Appearance

Topical gels are semisolid dosage forms that are applied to the skin or mucous membranes for localized effects. They are typically clear or translucent in appearanceand have a smooth, gel-like consistency.

2. Colour

The colour of a topical gel can vary depending on the ingredients used in its formulation.Our topical gels are clear or translucent, while others may have a specificcolor due to thepresence of active ingredients or additives.

3. Odour

Odour in the context of evaluation, especially for topical gels or other pharmaceutical and cosmetic products, refers to the scent or smell emitted by the product. It is an important parameter in assessing the overall user experience and acceptability of theproduct.

4. Ph

Topical gels are formulated to have a pH that is close to the pH of the skin, which is around 4.5 to 5.5. This slightly acidic pH range helps maintain the skin's natural barrier functionand is

considered optimal for skin health. However, the pH of a specific topical gel can be adjusted based on the active ingredients and their intended effects on the skin.

5. Spreadability

The spreadability of a topical gel refers to how easily it can be spread on the skin's surface. It is an important characteristic as it affects the application process and the coverage of the gel on the skin. Topical gels are formulated to have good spreadability toensure even distribution of the active ingredients over the skin area being treated.

Factors that influence the spreadability of a topical gel include its viscosity, consistency, and the presence of additives that affect the texture. A gel with good spreadability will glide smoothly over the skin, allowing for easy and uniform application. Formulators often consider the spreadability of a gel during the development process to ensure that itprovides a pleasant application experience for the user.

6. Irritability

Irritabilit y in the context of antifungal topical gels refers to the tendency of the gel to cause adverse reactions on the skin, such as redness, itching, burning, or inflammation. It is an important evaluation parameter that helps determine the safety and tolerability of the gel for users. High levels of irritability can lead to discomfort and may affect patient compliance with the treatment.

Part 02: In vitro studies

To evaluate the antifungal effect on C.albicans our sample was submitted to the Averin biotech lab.

Table 5: details of sample and microbial ssp used for study.

Sl. No.	Sample Name/Code	Concentration used	Microbial Sps
1	Sample	Dilution: 1 (1mg/ml)	Fungi: *Candida albicans*

Anti-fungal Test by disc diffusion method

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method. Fungal strain viz., Candida albicans was swabbed using sterile cotton swabs on SDA agar plate. Up to 100µl of given test compound with desired concentration was introduced in the sterile discs (6 mm) using sterile pipettes. The standard drug Fluconazole with 150ug

concentration disc was used (6 mm) as a positive control and empty sterile disc was used for negative control. The disc was then placed on the surface of SDA medium and the plate was kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zone was examined around the disc and measured with transparent ruler in millimeters. Same experiment repeated for 3 times for the reproducibility of results.

RESULT AND DISCUSSION

Part 01: Performulation studies

1. APPEARANCE

Topical gels are semisolid dosage forms that are applied to the skin or mucous membranesfor localized effects. They are typically clear or translucent in appearance and have a smooth, gel- like consistency.

The formulated gel was found to be translucent

2. COLOUR

The colour of a topical gel can vary depending on the ingredients used in its formulation. Our topical gels are clear or translucent, while others may have a specific colordue to the presence of active ingredients or additives.

The formulated gel was found to be whitish grey

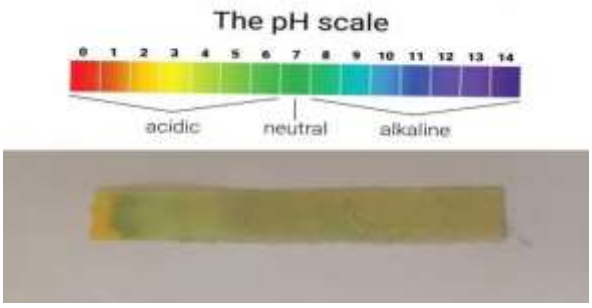
3. ODOUR

Odour in the context of evaluation, especially for topical gels or other pharmaceutical and cosmetic products, refers to the scent or smell emitted by the product. It is an important parameter in assessing the overall user experience and acceptability of the product.

The formulated gel was found to have a characteristic odour

4. Ph

Topical gels are formulated to have a pH that is close to the pH of the skin, which is around 4.5 to 5.5. This slightly acidic pH range helps maintain the skin's natural barrier function and is considered optimal for skin health. However, the pH of a specific topical gel can be adjusted based on the active ingredients and their intended effects on the skin.



The formulated gel has a ph between 4.5 to 5.5It was found using ph paper

5. Spreadability

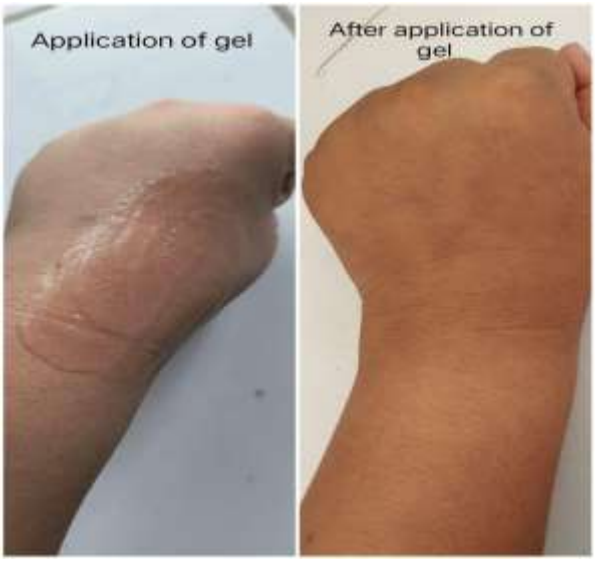
The spreadability of a topical gel refers to how easily it can be spread on the skin surface. It is an important characteristic as it affects the application process and the coverage of the gel on the skin. Topical gels are formulated to have good spreadability toensure even distribution of the active ingredients over the skin area being treated.

Factors that influence the spreadability of a topical gel include its viscosity, consistency, and the presence of additives that affect the texture. A gel with good spreadability will glide smoothly over the skin, allowing for easy and uniform application. Formulators oftenconsider the spreadability of a gel during the development process to ensure that it provides a pleasant application experience for the user.

The formulated gel has a even spreadability

6. Irritability

Irritability in the context of antifungal topical gels refers to the tendency of the gel to cause adverse reactions on the skin, such as redness, itching, burning, or inflammation. It is an important evaluation parameter that helps determine the safety and tolerability of the gel for users. High levels of irritability can lead to discomfort and may affect patient compliance with the treatment.



The formualed gel was found to be a non irritant on skin surface.

Part 02: In vitro studies

Anti-fungal Test by disc diffusion method

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method. Fungal strain viz., *Candida albicans* was swabbed using sterile cotton swabs on SDA agar plate. Up to 100µl of given test compound with desired concentration was introduced in the sterile discs (6 mm) using sterile pipettes. The standard drug Fluconazole with 150ug

concentration disc was used (6 mm) as a positive control and empty sterile disc was used for negative control. The disc was then placed on the surface of SDA medium and the plate was kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zone was examined around the disc and measured with transparent ruler in millimeters. Same experiment repeated for 3 times for the reproducibility of results.

RESULTS: ANTI-FUNGAL ACTIVITY

Table 6: fungal zone of inhibition.

Fungal Zone of Inhibition ±SD (mm)	
Fungal sps/NPs	*C.albicans*
Negative control	0
Fluconazole-150ug	19±1
Sample	15±2

Table: Diameter of Zone of inhibition (mm) of Sample against the *C.albicans* after the incubation period of 48hrs. The presented values were the average of 3 independent individual experiments (N=3). Keys: Positive Control for Fungi: Fluconazole with 150ug and Negative Control-Distilled water

Table 7: zone of inhibition of c.albicans by disc diffusion method.

DISC DIFFUSION METHOD-*Candida albicans*							
	Zone of inhibition (mm)						
Culture condition	Exp-1	Exp-2	Exp-3	Average	SD	SE	ZOI±SD
Control	0	0	0	**0.00**	0.00	0.00	0
Fluconazole-150ug	18	20	19	**19.00**	1.00	0.58	19±1
Sample	13	15	17	**15.00**	2.00	1.15	15±2

OBSERVATIONS

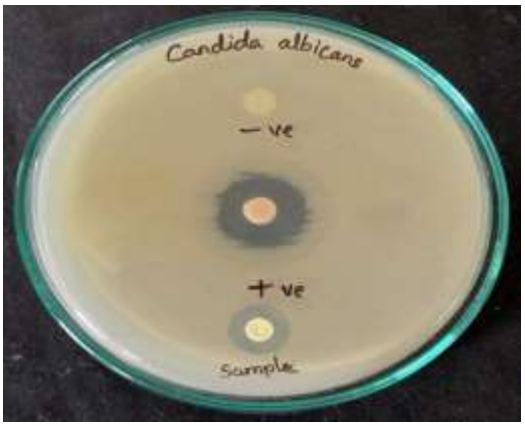
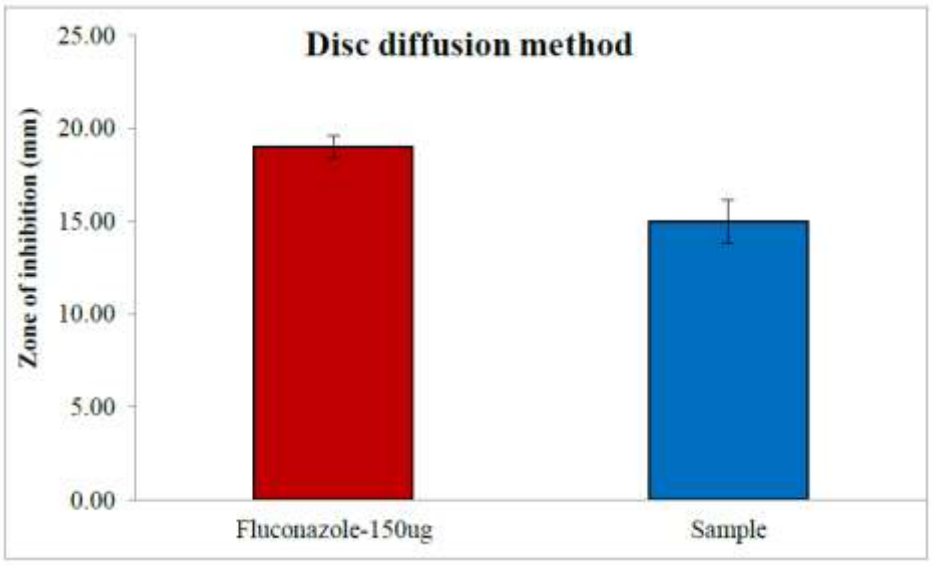


Fig.1: Anti-fungal activity of CD Gel with 1 dilution (1mg/ml) against the *Candida albicans* in comparison to Positive control (Fluconazole with 150ug) and Negative

control (Distilled water) and found that the CD gel may have effective anti-fungal activity.

OVERLAID GRAPH



Graph-1: Overlaid bar graph depicted the anti-microbial activity of Sample against the *C.albicans* in comparison to Positive control (Fluconazole-150ug) and Negative control (Distilled water).

PROJECT REQUIREMENT

- To evaluate the antimicrobial effect (Disc diffusion method) on *C.albicans*, 1 sample was received.
- The sample named as follows.

Table 1: Details of sample and microbial sps used for the study.

Sl. No.	Sample Name/Code	Concentration used	Microbial Sps
1	Sample	Dilution: 1 (1mg/ml)	**Fungi:** *Candida albicans*

BACKGROUND OF THE STUDY

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. Antimicrobial activity refers to the process of killing or inhibiting the disease causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti- fungal or antiviral. They all have different modes of action by which they act to suppress the infection.

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and fungus testing.

MATERIALS

1) Test Organisms: *Fungi: Candida albicans* (MTCC, Chandigarh) MTCC No: 227
2) SDA agar (Cat No: M063, Himedia)
3) Sterile discs (Cat No: SD067, Himedia)
4) Fluconazole 150ug disc (Cat No:SD232, Himedia)
5) Double distilled water (Nice chemicals)
6) Sterile Cotton swabs
7) Sterile Petriplates (Tarsons)
8) Laminar Air Flow (Alpha Linear)
9) Personal Protective Equipment (PPE) i.e., Gloves, Mouth Mosque, Head Cap and Lab Coat etc.
10) Pipettes (10ul, 200ul and 1ml Pipettes)

STEPS FOLLOWED FOR THE STUDY

The microorganisms used for antimicrobial analysis were purchased from Microbial Type Culture Collection and Gene Bank (*MTCC*), Chandigarh, India. The Fungal strains were maintained on SDA Agar (SDA) media.

Fungi growth conditions

Pure cultures from the plate were inoculated into SDA agar plate and sub cultured at 37ºC for 24h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L salinetube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a fungal growth.

Anti-fungal Test by disc diffusion method:

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method. Fungal strain viz., *Candida albicans* was swabbed using sterile cotton swabs on SDA agar plate. Up to 100µl of given test compound with desired concentration was introduced in the sterile discs (6 mm) using sterile pipettes. The standard drug Fluconazole with 150ug concentration disc was used (6 mm) as a positive control and empty sterile disc was used for

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RESULTS: ANTI-FUNGAL ACTIVITY

Table 2: Diameter of Zone of inhibition (mm) of Sample against the *C.albicans* after the incubation period of 48hrs. The presented values were the average of 3 independent individual experiments (N=3). Keys: Positive Control for Fungi: Fluconazole with 150ug and Negative Control-Distilled water.

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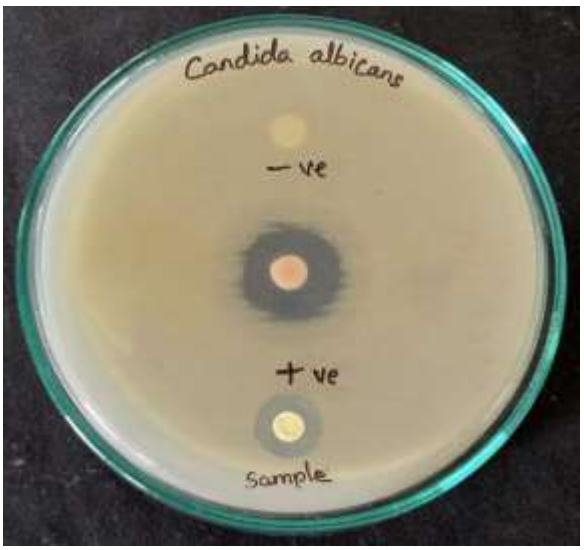

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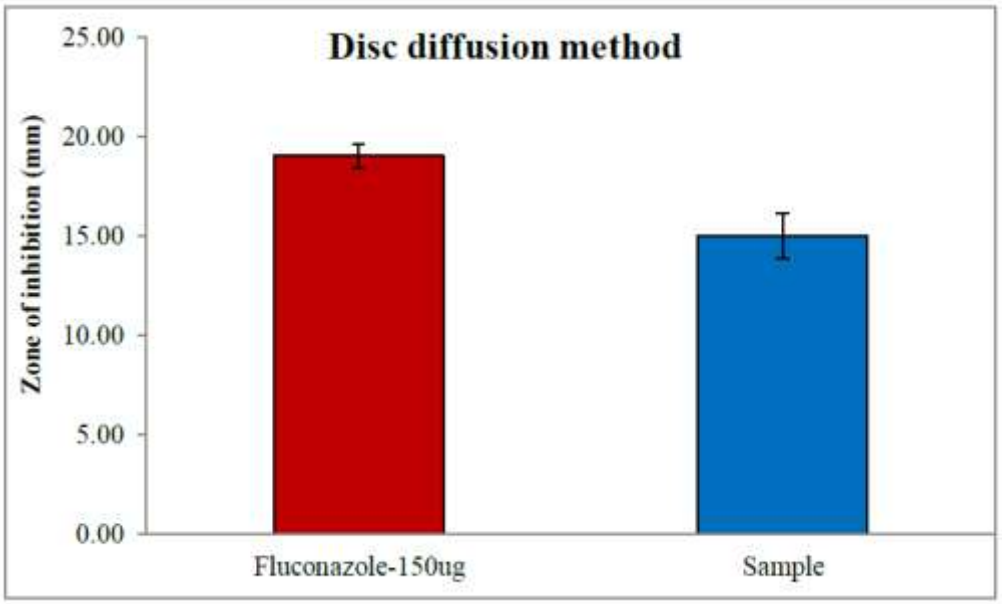

Graph-1: Overlaid bar graph depicted the anti-microbial activity of Sample against the *C.albicans* in comparison to Positive control (Fluconazole-150ug) and Negative control (Distilled water).

CONCLUSION OF THE STUDY

In this study, anti-fungal activity of Sample was assessed by disc diffusion method against the *C.albicans*. Anti-fungal activity results revealed the satisfactory anti-fungal effect of Sample against the *C.albicans* similar to the Fluconazole with 150ug was used as a std control for the anti-fungal activity.

In summary, overall the observed results concluded that Sample caused the effective anti-fungal activity with satisfactory ZOI and confirmed the anti-fungal effect.

REFERENCES OF THE STUDY

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