**A Green Approach to Antifungal Therapy: Formulation and Evaluation of Clitoria ternatea-Based Cream**

**Abstract**

**Introduction:**Clitoria ternatea, a medicinal plant, has been traditionally used for its various therapeutic properties, including antifungal, anti-inflammatory, and antioxidant effects. The present study aimed to evaluate the antifungal potential of Clitoria ternatea seed extract incorporated into a cream formulation, with an emphasis on phytochemical screening, antifungal activity, and formulation characterization.

**Materials and Methods:** The Clitoria ternatea seeds were collected, extracted using water and methanol, and subjected to various phytochemical tests to detect alkaloids, flavonoids, tannins, phenols, and amino acids. The cream formulations were prepared as oil-in-water (O/W) emulsions, incorporating Gokarn and a mixture of plant extracts. Antifungal activity was evaluated by the disc diffusion method, testing against Candida albicans (ATCC 90028). The prepared creams were assessed for parameters such as appearance, pH, homogeneity, spreadability, irritancy, and washability.

**Results:**Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, phenols, and amino acids in the Clitoria ternatea extract. FTIR studies revealed characteristic functional groups in the extract and the formulation. Antifungal testing showed that the cream with Gokarn exhibited a significant zone of inhibition, though smaller than the positive control (marketed antifungal cream). The cream formulations were homogeneous, non-irritating, and easily spreadable, with satisfactory emolliency and washability.

**Conclusion:**The results suggest that Clitoria ternatea seed extract possesses significant antifungal potential. The formulated cream showed promising results, with good physical properties and therapeutic potential for skin-related conditions. Further clinical studies are recommended to optimize the formulation for broader applications.

**Keywords:**

Clitoria ternatea, antifungal activity, Candida albicans, disc diffusion method, spreadability, emolliency, skin application.

**INTRODUCTION**

**Overview of Traditional Herbal Medicine**

India is widely recognized as the cradle of traditional systems of medicine, such as Ayurveda, Siddha, and Unani, which have historically utilized a vast array of medicinal plants to manage human ailments. Globally, approximately 65% of the population continues to rely on traditional medicine for primary healthcare needs.[01] With the resurgence of interest in natural and plant-based remedies, a global "herbal renaissance" is currently underway. Despite the dominance of synthetic pharmaceuticals over the past century, herbal medicines are regaining popularity due to growing concerns over the safety, cost, and side effects associated with modern drugs. [02]

According to the World Health Organization (WHO), traditional medicine encompasses a broad range of health practices based on cultural beliefs, utilizing plant, animal, and mineral-based products as well as spiritual and manual therapies, either alone or in combination, to prevent, diagnose, or treat illness and maintain well-being.[03] The shift back to natural therapies is largely driven by the perception of greater safety and holistic efficacy. Numerous plant-derived compounds are now recognized for their dual roles offering both nutritional benefits and therapeutic effects and are increasingly being explored for treating various diseases. [04]

**Structure and Function of the Skin**

The human skin, the body's largest organ, serves as a critical interface between the body and the external environment. It plays a pivotal role in providing a physical and immunological barrier against pathogens, mechanical damage, ultraviolet radiation, and chemical exposure.[05]Structurally, the skin is composed of three main layers: the epidermis, dermis, and hypodermis. Each of these layers differs in anatomy and function, contributing uniquely to skin integrity and homeostasis.

The epidermis, the outermost layer, comprises several sublayers stratum basale, spinosum, granulosum, lucidum, and corneum each performing specialized functions such as keratin production, barrier formation, and immunological defense.[06] The dermis, located beneath the epidermis, consists of connective tissue and houses critical structures including blood vessels, nerve endings, sweat glands, and hair follicles.[07] Beneath the dermis, the hypodermis or subcutaneous tissue contains adipose tissue and provides insulation and cushioning. [08]

The skin also serves several vital physiological roles. These include the regulation of body temperature and hydration, production of vitamin D, sensory perception, and immunological surveillance. The epidermal water barrier, formed by lipid-rich secretions and specialized proteins, prevents excessive water loss and blocks pathogen entry. Immune defense is particularly supported by Langerhans cells and keratinocytes, which interact with both the innate and adaptive immune systems. [09]

**Fungal Infections and Public Health Relevance**

Fungal infections are an escalating global health concern, particularly among immunocompromised populations. The clinical spectrum ranges from superficial skin infections to life-threatening systemic mycoses. Superficial infections like athlete’s foot, jock itch, ringworm, and cutaneous candidiasis are among the most common forms affecting the skin. [10]These infections are often underdiagnosed or mismanaged in early stages, leading to complications and increased treatment challenges.

Fungi such as *Candida*, *Aspergillus*, *Fusarium*, and *Mucorales* have demonstrated significant adaptability and resistance mechanisms, including biofilm formation and immune evasion strategies.[11] Invasive fungal infections are particularly problematic in individuals with HIV, cancer, organ transplants, or those receiving prolonged antibiotic or corticosteroid therapies. The Centers for Disease Control and Prevention (CDC) has recognized the urgency of fungal disease awareness and has emphasized the need for early diagnosis and improved therapeutic options.[12]

The rising antifungal resistance and the limitations of existing drugs highlight the urgent need for alternative and complementary approaches, including those derived from traditional herbal medicine. Plant-based antifungal agents may offer safer and more sustainable therapeutic avenues due to their lower toxicity, reduced risk of resistance, and synergistic effects with existing treatments. [13]

**Herbal Approaches in Antifungal Therapy**

The increasing resistance of fungal pathogens to conventional antifungal drugs has fueled interest in phytotherapeutic approaches as safer and effective alternatives. Among these, plant-based antifungal formulations, especially creams, offer the benefit of topical application, targeted delivery, and reduced systemic side effects. One such promising formulation includes Ajmoda oil (*Apium graveolens*), incorporated into a topical cream using emulsifying wax, stearic acid, and distilled water. Preformulation and evaluation studies confirmed its stability, desirable pH, rheology, and potent antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum.* [14]

Medicinal plants are known to contain a wide range of bioactive phytochemicals with antimicrobial and antifungal properties. For instance, Clitoria ternatea, commonly known as butterfly pea, has shown significant antifungal activity in studies using its methanolic seed and root extracts. These extracts were effective against fungal pathogens such as *A. niger*, *A. ochraceus*, and *C. albicans*, potentially due to the presence of quercetin and a low-molecular-weight cysteine-rich protein known as finotin. [15,16]

Asparagus racemosus (Shatavari), a key medicinal herb in Ayurveda, has long been used for its immunomodulatory, anti-inflammatory, and antimicrobial effects. While it is primarily known as a female reproductive tonic, recent pharmacological studies support its antifungal, antioxidant, and antiulcer properties. These effects are largely attributed to its steroidal saponins (Shatavarins I–IV), which have shown activity against T-cell dependent antigens, suggesting immune-enhancing and infection-preventive capabilities. [17,18]

Ferula asafoetida (Shudha Hingu), a sulfur-rich oleo-gum-resin, has been traditionally used in Ayurveda and Unani medicine for treating gastrointestinal and respiratory disorders. More recent pharmacological studies have identified its antifungal, antimicrobial, and anti-inflammatory effects. These benefits are associated with volatile oils and organosulfur compounds that inhibit fungal growth, making it a potential component in antifungal topical formulations. [19]

Another potent Ayurvedic ingredient is Shuddha Gandhak, a purified form of sulfur known for its antifungal, antibacterial, and detoxifying actions. It supports skin health by promoting keratinocyte regeneration and inhibiting microbial proliferation. Traditionally processed by melting and quenching in milk or ghee, Shuddha Gandhak is commonly included in Ayurvedic skin preparations for its effectiveness in treating fungal skin infections, dermatitis, and acne. [20]

The combination of these herbs into a single formulation provides a multi-targeted mechanism of action disrupting fungal cell walls, inhibiting spore germination, and enhancing the host immune defense. Their synergistic effects and relatively low toxicity make them ideal candidates for developing herbal antifungal treatments. As fungal resistance continues to rise and the efficacy of synthetic antifungals declines, research into such herb-based therapies offers a sustainable and holistic alternative for combating superficial and systemic mycoses.

**Materials**

The following materials were procured for the formulation and evaluation of the antifungal herbal cream. Shatavari (*Asparagus racemosus*) and Shuddha Gandhak (purified sulfur) were obtained from *Green Pharmacy, India*. Clitoria ternatea seeds were collected from the *Biological Garden of Jaysingpur College, Jaysingpur*. Shuddha Hingu (purified *Ferula asafoetida*) was procured from *NHSC Foods Private Limited*. Karanj oil was sourced from *Shree Samarth Enterprise*, while Kumkumadi oil was acquired from *Nisha Herbal Products*. Excipients and other formulation agents including stearic acid, cetyl alcohol, glyceryl monostearate, propylene glycol, methyl paraben, and rose water were purchased from *Loba Chemie Pvt. Ltd.* Additionally, a marketed formulation, Fungiwin cream, was obtained from *Inducare Pharma Pvt. Ltd.* for comparative analysis.

**Plant Material Collection and Extraction**

Healthy and disease-free seeds of *Clitoria ternatea* (blue-flowered variety) were collected from the Botanical Garden of Jaysingpur College, Jaysingpur. Further it is authenticated from Department of Botany Jaysingpur College Jaysingpur. Among the two commonly found variants white and blue the seeds from the blue-flowering plant were chosen for further studies due to their higher therapeutic potential and phytochemical content. [21]

**Collection and Preparation**

Fresh, mature leaves and seeds were harvested. The seeds were manually decorticated to remove their seed coats. Both the leaves and seeds were washed under tap water for 10 minutes to remove surface impurities, followed by rinsing with sterile distilled water to reduce microbial load. The plant material was then air-dried completely under shade at ambient temperature to preserve heat-sensitive phytoconstituents. [22]

**Grinding**

Dried plant materials were finely powdered using a mechanical grinder and stored in airtight containers at room temperature until extraction. This ensured minimal degradation of active phytochemicals. [23]

**Extraction Procedure**

The powdered samples were subjected to extraction using two different solvents: distilled water and 60% methanol. A quantity of 10 g of each powdered sample was mixed with 100 ml of the respective solvent in conical flasks. The flasks were kept on a rotary shaker at 100 rpm for 72 hours at room temperature to ensure complete extraction of bioactive components. [24]



**Fig No 01: Clitoria ternatea**

**Filtration and Storage**

After the extraction period, the mixtures were filtered through Whatman No.1 filter paper to remove particulate matter. The obtained filtrates were stored in clean, airtight glass containers at 4 °C in a refrigerator until further use for phytochemical screening and antifungal activity studies. [25]

**Phytochemical Screening of Clitoria ternatea Seed Extracts**

Phytochemical screening of the Clitoria ternatea seed extracts (aqueous and methanolic) was performed to identify the presence of bioactive compounds. The following tests were carried out:

**Screening for Alkaloids**

To detect alkaloids, 1–2 mL of the extract was mixed with a drop of Mayer’s reagent at the side of the test tube. The appearance of a white and creamy precipitate indicated the presence of alkaloids. [26]

**Flavonoids**

To 1 mL of the extract, a few drops of dilute sodium hydroxide were added. The formation of an intense yellow color that became colorless upon addition of dilute acid confirmed the presence of flavonoids.[27]

**Tannins**

For tannin detection, 5 mL of the extract was combined with a few drops of 1% lead acetate solution. The formation of a yellow precipitate was indicative of tannins. [28]

**Phenols**

To 2 mL of the extract, 2 mL of ferric chloride (FeCl₃) was added. The development of a deep bluish-green color indicated the presence of phenolic compounds. [29]

**Amino Acids**

To 1 mL of the extract, a few drops of Ninhydrin reagent were added. The appearance of a purple color suggested the presence of amino acids. [30]

The phytochemical screening of Clitoria ternatea seed extracts revealed the presence of several important bioactive compounds. The alkaloid test, using Mayer’s reagent, indicated the presence of alkaloids, which are known for their diverse pharmacological activities, including antimicrobial and anti-inflammatory effects. [26] The flavonoid test, based on the color change upon adding sodium hydroxide and acid, confirmed the presence of flavonoids, which are potent antioxidants with potential anticancer, anti-inflammatory, and cardioprotective properties.[27] The tannin test, using lead acetate, revealed the presence of tannins, compounds known for their antimicrobial, antiviral, and antioxidant properties. [28]The phenol test, using ferric chloride, showed the presence of phenolic compounds, which are widely recognized for their antioxidant and anti-inflammatory effects, crucial in reducing oxidative stress and preventing various diseases. [29] Lastly, the amino acid test, using Ninhydrin reagent, indicated the presence of amino acids, which are essential for protein synthesis and have potential neuroprotective properties. [30] These findings support the pharmacological potential of Clitoria ternatea seed extracts, highlighting their therapeutic promise in various medicinal applications.

|  |  |  |  |
| --- | --- | --- | --- |
| **Phytochemical Test** | **Reagent/Method** | **Observation/Result** | **Reference** |
| **Alkaloids** | Mayer’s reagent (few drops) added to extract | White, creamy precipitate indicates presence of alkaloids | [26] |
| **Flavonoids** | Dilute sodium hydroxide (few drops) + dilute acid | Intense yellow color turns colorless on addition of acid | [27] |
| **Tannins** | 1% lead acetate solution (few drops) added to extract | Yellow precipitate indicates presence of tannins | [28] |
| **Phenols** | Ferric chloride (FeCl₃) (2 mL) added to extract | Deep bluish-green color indicates presence of phenols | [29] |
| **Amino Acids** | Ninhydrin reagent (few drops) added to extract | Purple color indicates presence of amino acids | [30] |

**Table No. 01: Phytochemical Tests**

**Method of Preparation: O/W Cream**

The **Oil-in-Water (O/W) emulsion** is a semi-solid preparation used for topical application on the skin and mucous membranes. It consists of oil droplets dispersed in a continuous water phase, which provides a less greasy texture, making it cosmetically appealing and easily washable with water. O/W creams are often utilized in both pharmaceuticals and cosmetics for the treatment of various skin conditions due to their therapeutic properties and non-greasy formulation.

**Preparation of O/W Cream:**

* **Cream Description:**
* **Type:** Oil-in-Water (O/W) emulsion.
* **Characteristics:** Less greasy, easily washable with water, and cosmetically acceptable.
* **Ingredients:**
* **Oil Phase (A):** The oil phase consists of oils and emulsifiers, which are mixed and heated to 70°C with constant stirring in a china dish placed on a water bath.
* **Aqueous Phase (B):** The aqueous phase consists of water-soluble ingredients, which are mixed and heated separately to the same temperature as the oil phase in a beaker.
* **Preparation Steps:**
* **Step 1:** The aqueous phase is added to the oil phase gradually, drop by drop, with continuous stirring using an emulsifier.
* **Step 2:** Therapeutically active Chlorphenesin is dissolved in distilled water and added to the mixture, which is then stirred continuously until a homogeneous cream forms.
* **Step 3:** Preservatives propylparaben and methylparaben are added after the mixture cools down to 40°C to maintain the stability and shelf-life of the cream.

This method ensures the formation of a stable, homogeneous cream with the desired consistency and therapeutic properties.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Ingredients** | **F1 (gm)** | **F2 (gm)** | **F3 (gm)** |
| **Water Phase** |  |  |  |  |
| 1 | Gokarn | - | 2.8 | 4 |
| 2 | Shatavari | 4 | 1.2 | 0 |
| 3 | Shudha Hingu | 0.8 | 0.8 | 0.8 |
| 4 | Shudha Gandhak | 1.2 | 1.2 | 0.8 |
| 5 | Rose Water | q.s. | q.s. | q.s. |
| 6 | Water | q.s. | q.s. | q.s. |
| **Oil Phase** |  |  |  |  |
| 7 | Karanj Oil | 1 | 1 | 1 |
| 8 | Kumkumadi Oil | 0.6 | 0.6 | 0.6 |
| 9 | Glyceryl Monostearate | 0.2 | 0.2 | 0.2 |
| 10 | Cetyl Alcohol | 4.6 | 4.6 | 4.6 |
| 11 | Propylene Glycol | 1.8 | 1.8 | 1.8 |
| 12 | Stearic Acid | 2 | 2 | 2 |
| 13 | Methyl Paraben | 0.8 | 0.8 | 0.8 |

**Table No. 2: Ingredients for O/W Cream Formulation (20 g)**

**IR Spectroscopy Analysis**

IR Spectroscopy (Infrared Spectroscopy) is a vital technique used for the identification of functional groups in a compound by analyzing the absorption of infrared radiation. In this study, IR spectroscopy was employed to analyze the antifungal cream and confirm the presence of specific functional groups within the formulation.

**Antifungal Screening by Disc Diffusion Method**

The antifungal activity of the formulated creams was evaluated using the Disc Diffusion Method, which is commonly employed to assess the effectiveness of antimicrobial agents by measuring the zone of inhibition formed around the disc containing the agent. In this study, the antifungal activity was tested against *Candida albicans* (ATCC 90028), a common yeast pathogen.

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**Diffusion Method Procedure:**

* **Preparation of Culture:**
* A 35 μl homogeneous culture of *Candida albicans* was spread evenly on sterile yeast peptone dextrose (YPD) agar plates using a pre-sterilized L-shaped glass rod [32].
* **Placement of Discs:**
* Sterile discs with a diameter of 2–3 mm were placed on the surface of the YPD agar plate.
* **Application of Formulated Creams:**
* Formulated creams were prepared in concentrations of 1 mg/ml and 2 mg/ml in dimethyl sulphoxide (DMSO). A 20 μl volume of each cream formulation was applied onto the sterile discs.
* **Positive and Negative Controls:**
* A standard antifungal cream, Fungiwin, was used as a positive control, while sterile distilled water (D/W) served as the negative control.
* **Incubation:**
* The agar plates were incubated at 35°C for 24 hours to allow sufficient time for fungal growth and activity of the cream formulations.
* **Observation of Zone of Inhibition:**
* After incubation, the plates were observed for the zone of inhibition (clear area around the disc) formed by the antifungal cream. The zone of inhibition is a critical parameter for determining the effectiveness of the antifungal agent.
* **Measurement:**
* The diameter of the zone of inhibition was measured using a scale, divider, or Vernier calipers. The average of the two diameters of each zone was calculated for precise evaluation.

**Evaluation Parameters**

The formulated creams were evaluated based on several parameters to determine their quality and effectiveness. The appearance of the cream was judged by assessing its color, pearlescence, and roughness. These visual and tactile properties were used to assign a grade to the cream, ensuring its uniformity and aesthetic appeal.

pH measurement was conducted by calibrating a pH meter and then using it to measure the pH of a 20 mg sample of the cream placed in a beaker. This step helps determine the cream's compatibility with the skin and ensures it is within an acceptable pH range. The homogeneity of the cream was assessed through both visual inspection and touch. Visual inspection focused on ensuring uniformity in appearance, while the touch test was performed to assess the cream’s texture and consistency.

The spreadability of the cream was evaluated using a standardized procedure. A 500 mg sample of the cream was placed between two glass slides. A 100 g weight was placed on the upper slide, and after removing the weight, any excess cream was scraped off. The lower slide was fixed while a 20 g weight was tied to the upper slide. The time it took for the upper slide to slip off was recorded. The test was repeated three times, and the average of the three readings was recorded.

The patch test involved applying 1–3 grams of the cream to a piece of fabric or funnel, which was then placed on a sensitive area of the skin, such as behind the ear. Control patches of a known commercial cosmetic were also applied for comparison. The site was inspected after 24 hours, and if no reaction occurred, the test was repeated two more times. If no reaction was observed after the third application, the person was considered not hypersensitive.

The smear type was observed by applying the cream to the skin and noting whether the smear formed was oily or aqueous. This helped assess the texture and finish of the cream. The emolliency of the cream was determined by checking its slipperiness and the amount of residue left after applying a fixed amount of cream. These characteristics are important for evaluating the cream's moisturizing properties. The washability of the cream was tested by applying it to the skin and removing it under tap water with minimal force. The ease of removal is an indicator of how the cream will perform under normal conditions.

The irritancy test involved applying 1 square centimeter of cream to the dorsal side of the left hand. The site was observed for up to 24 hours for signs of irritancy, redness, or edema. This helped assess the cream’s potential for causing adverse reactions. Lastly, accelerated stability studies were conducted by storing the cream at room temperature for 20 days. During this period, parameters like homogeneity, viscosity, physical changes, pH, and smear type were checked at regular intervals to evaluate the cream's stability over time.

**Pre-formulation Studies: IR Analysis**

The IR spectrum of the individual ingredients, namely Gokarna, Shatavari, and the final antifungal cream formulation, was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with the Attenuated Total Reflection (ATR) sampling method. FTIR spectroscopy is an essential technique used in Pre-formulation studies to understand the functional groups and molecular interactions within the compounds, ensuring the correct chemical profile of each ingredient and the formulation.

The IR spectrum of Gokarna exhibited characteristic peaks corresponding to various functional groups. Specifically, the O-H stretching vibrations were observed in the region around 3200–3400 cm⁻¹, indicating the presence of hydroxyl groups, which are common in plant-based materials. Additionally, peaks around 1600 cm⁻¹ were attributed to C=O stretching vibrations, suggesting the presence of carbonyl groups, which play a crucial role in the antifungal activity of certain compounds.

For Shatavari, the IR spectrum revealed prominent peaks corresponding to C-H stretching in the 2900 cm⁻¹ region, indicative of the presence of aliphatic hydrocarbons. The spectrum also showed C=O stretching at approximately 1720 cm⁻¹, a key feature of plant-based flavonoids, which are known for their antioxidant and antifungal properties. These findings align with the known phytochemical composition of Shatavari, which contains saponins and flavonoids that contribute to its therapeutic properties.

The IR spectrum of the antifungal cream formulation was analyzed to confirm the presence of the active ingredients and ensure the proper blending of the ingredients. Peaks corresponding to the functional groups of Gokarna and Shatavari were evident, along with additional peaks due to the excipients and emulsifiers used in the cream formulation. Notably, the C-H stretching (2900 cm⁻¹) and C=O stretching (1720 cm⁻¹) were well represented, confirming the presence of the plant-derived compounds. The spectrum also showed the characteristic O-H stretching vibrations around 3400 cm⁻¹, indicative of the water content in the cream.

The absence of any new peaks or significant shifts in the functional groups suggested that the active ingredients were not chemically altered during the formulation process. This is an important finding as it confirms the stability of the ingredients and the preservation of their therapeutic properties. Furthermore, the absence of any undesirable peaks indicates that no chemical degradation occurred during the cream preparation.

The FTIR analysis thus confirmed that the formulation retained the expected chemical characteristics of the individual components, and there were no interactions that would compromise the cream’s efficacy. These results are consistent with the standard procedures for creating stable and effective pharmaceutical formulations, reinforcing the overall integrity of the antifungal cream.

Fig 2: IR SPECTRUM OF Clitoria ternatea seeds extract (Gokarn)

Fig 3: IR spectrum of Shatavari 

Fig 4: IR spectrum of Mix formulation:

Fig 5: IR spectrum of Gokarna formulation

**FTIR Studies of Clitoria ternatea Seeds Extract and Antifungal Cream**

The FTIR spectra of Clitoria ternatea seeds extract and the antifungal cream formulation were obtained in the wavenumber range of 4000–400 cm⁻¹ to identify the characteristic functional groups and to evaluate the interaction between the active ingredients and the excipients used in the formulation.

**Clitoria ternatea Seeds Extract:**

The FTIR spectrum of Clitoria ternatea seeds extract exhibited prominent peaks that correspond to key functional groups present in the plant's bioactive compounds. The spectrum revealed a broad O-H stretching vibration around 3300–3400 cm⁻¹, indicating the presence of hydroxyl groups, which are typically associated with phenolic compounds, flavonoids, and other phytochemicals known for their antioxidant and antimicrobial activities.

A sharp peak around 1600 cm⁻¹ was observed, which is characteristic of C=C stretching vibrations, commonly found in aromatic compounds, including flavonoids and alkaloids. This peak indicates the presence of aromatic rings that contribute to the biological activity of the extract. Additionally, C=O stretching was observed near 1700 cm⁻¹, suggesting the presence of carbonyl groups, which are often found in saponins and other bioactive molecules in plants.

Further peaks around 1200 cm⁻¹ and 1100 cm⁻¹ were attributed to C-O stretching vibrations, which are indicative of the presence of glycosidic bonds in flavonoids and saponins, both of which are abundant in Clitoria ternatea.

**Antifungal Cream Formulation:**

The FTIR spectrum of the antifungal cream formulation showed similar peaks to those observed in the Clitoria ternatea extract, which is consistent with the incorporation of the extract into the formulation. The O-H stretching peak around 3300–3400 cm⁻¹ remained prominent, confirming the presence of water and hydroxyl groups in the cream base. The peak at 1600 cm⁻¹ was also retained, reflecting the continued presence of aromatic compounds from the Clitoria ternatea extract.

However, the C=O stretching band at 1700 cm⁻¹ was slightly altered, likely due to interactions between the active compounds in the extract and the emulsifying agents in the cream base. These interactions are expected and suggest that the formulation process did not degrade the bioactive components, but rather allowed for proper dispersion and interaction within the cream matrix.

Additionally, new peaks associated with excipients such as glycerol monostearate and cetyl alcohol appeared around 1200 cm⁻¹ and 1100 cm⁻¹, corresponding to C-O stretching vibrations typical of emulsifiers and stabilizers used in cosmetic formulations. The presence of these peaks confirms the integration of the excipients into the formulation without interfering with the key active ingredients.

The absence of any new peaks or significant shifts from the original extract spectrum suggests that the Clitoria ternatea seeds extract remained chemically stable and that the formulation process did not induce any unwanted chemical reactions that would compromise its efficacy.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No** | **Functional Group** | **Range of Functional Group (cm⁻¹)** | **Clitoria ternatea Extract (cm⁻¹)** | **Formulation (cm⁻¹)** |
| 1 | OH (Stretching) | 3200–3600 | 3429.78 | 3398.92 |
| 2 | CH (Stretching - Aliphatic group) | 2850–2950 | 2924.52 | 2920.66 |
| 3 | CH₂ (Stretching - Aliphatic group) | 2850–2900 | 2855.1 | 2852.2 |
| 4 | C=O (Inorganic Carbonate) | 1650–1680 | 1644.02 | 1645.95 |
| 5 | NO₂ | 1500–1600 | 1543.74 | 1549.52 |
| 6 | Nitrosamine | 1440–1470 | 1457.93 | 1463.71 |
| 7 | C-O | 1380–1030 | 1240.00 | 1279.54 |
| 8 | CO Ethers | 1030–1120 | 1057.76 | 1009.83 |
| 9 | Alkyl Halide | 515–850 | 582.4 | 573.72 |

Table No. 3- Summarizes the characteristic functional groups identified in both Clitoria ternatea seeds extract and its antifungal cream formulation. The FTIR analysis was performed to identify and compare the functional groups present in both samples, which are crucial for determining their chemical composition and understanding the interaction between the active ingredients and excipients.

**ANTIFUNGAL ACTIVITY:**

**Determination of Zone of Inhibition:**

The antifungal activity of the formulated creams was evaluated using the disc diffusion method against Candida albicans (ATCC 90028). The following observations were made regarding the zone of inhibition produced by different samples:

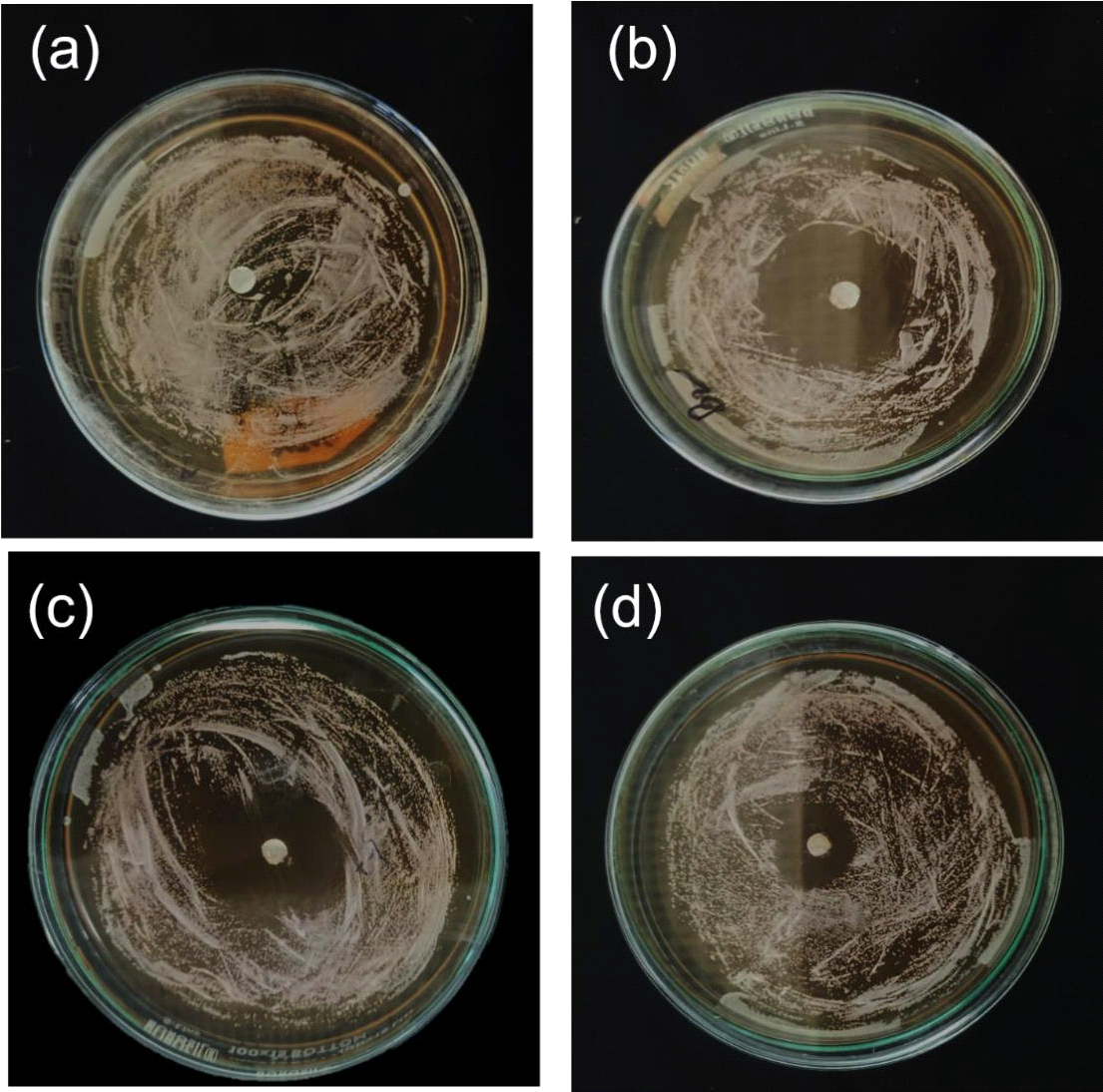
**(a) Negative Control (Distilled Water - D/W):** The negative control, consisting of distilled water (D/W), showed no zone of inhibition around the disc. This indicates that the negative control had no antifungal activity, as expected.

**(b) Positive Control (Marketed Antifungal Cream):** The positive control, a marketed antifungal cream, produced a significant zone of inhibition, confirming its potent antifungal effect. This result served as a benchmark for comparing the efficacy of the formulated creams.

**(c) Cream Formulated with Only Gokarn:** The cream containing only Gokarn produced a zone of inhibition, which was smaller than the positive control. This confirms that Gokarn has some antifungal activity, though its effect is less pronounced than the marketed cream. The size of the zone of inhibition suggests a moderate antifungal effect, but not as strong as the positive control.

**(d) Cream Formulated with Mixture of Ingredients:** The cream formulated with a mixture of ingredients, which likely included other bioactive compounds, produced a zone of inhibition smaller than both the positive control and the cream containing only Gokarn. This indicates that the antifungal effect of the mixture is relatively weaker compared to the single-component formulation containing Gokarn. The lack of a significant zone of inhibition suggests that the combination of ingredients did not enhance the antifungal activity as expected.

The antifungal testing results suggest that the cream formulated with Gokarn exhibits antifungal activity, though it is less potent than the standard marketed antifungal cream. The mixture of ingredients, however, showed reduced antifungal efficacy, indicating that the combination may not have been as effective as the single bioactive ingredient (Gokarn). Further optimization of the formulation may be necessary to improve its antifungal potential.



**Figure No.06 Antifungal activity of formulated creams against C. albicans (ATCC90028)**

The evaluation of the formulated creams (**F1, F2, F3**) was carried out using a series of parameters to assess their physical and functional properties. The results of these evaluations are summarized below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Parameters** | **F1** | **F2** | **F3** |
| 1 | **Appearance** | Smooth | Smooth | Smooth |
| 2 | **Color** | Pale yellow | Greenish yellow | Greenish yellow |
| 3 | **pH** | 6.8 | 7.1 | 6.9 |
| 4 | **Homogeneity** | Homogenous | Homogenous | Homogenous |
| 5 | **Emolliency** | No residue | No residue | No residue |
| 6 | **Washability** | Easily | Easily | Easily |
| 7 | **Irritancy** | None | None | None |
| 8 | **Smear** | Non-greasy | Non-greasy | Non-greasy |
| 9 | **Spreadability** | 4 cm | 3.8 cm | 3.8 cm |
| 10 | **Wetness** | Moisturizes | Moisturizes | Moisturizes |

**Table 4: Evaluation parameters for formulated Cream**

The formulations (F1, F2, F3) exhibited favorable characteristics such as smooth appearance, homogeneity, appropriate pH, excellent spreadability, and good moisturizing properties. None of the formulations showed any irritancy, and all were easily washable, indicating their potential for safe and effective skin application. The minor differences in spreadability and color may be attributed to the varying concentrations of active ingredients or excipients in each formulation. Further studies could focus on optimizing these parameters for improved efficacy and patient comfort.

|  |  |  |
| --- | --- | --- |
| **Tests** | **Observations** | **Inference** |
| **Screening for Alkaloids** | Creamy precipitate | Alkaloids Present |
| **Flavonoids** | Colourless | Flavonoids Present |
| **Tannin** | Yellow precipitate | Tannins Present |
| **Phenol** | Green color | Phenols Present |
| **Amino Acid** | Dark blue color | Amino Acids Present |

Table 5: Results of Phytochemical tests



**Figure No. 7: Phytochemical Tests evaluation**

**Alkaloids:** The presence of a creamy precipitate upon adding Mayer’s reagent to the extract indicates the presence of alkaloids, which are often associated with medicinal properties such as anti-inflammatory and analgesic effects.

**Flavonoids:** The appearance of a yellow color that turns colorless upon the addition of dilute acid confirms the presence of flavonoids. These compounds are known for their antioxidant and anti-inflammatory properties.

**Tannins:** A yellow precipitate formed upon adding lead acetate indicates the presence of tannins, which are known for their antimicrobial and astringent properties.

**Phenols:** The formation of a deep bluish-green color upon adding FeCl₃ confirms the presence of phenols, which have antimicrobial, antioxidant, and potential anticancer effects.

**Amino Acids:** The formation of a dark blue color upon treating the extract with Ninhydrin reagent indicates the presence of amino acids, which are essential for various biological functions, including protein synthesis and enzyme activity.

**Conclusion:**

The Clitoria ternatea seed extract and its formulated creams were subjected to a comprehensive evaluation, including phytochemical screening, FTIR analysis, antifungal activity, and various evaluation parameters. The results suggest that the extract contains a range of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and amino acids, all of which contribute to its potential therapeutic properties.

The FTIR analysis revealed characteristic functional groups such as OH (stretching), CH (stretching), C=O, and others, which confirm the presence of key bioactive constituents in both the extract and the cream formulation. These functional groups align with known compounds that exhibit antioxidant, antimicrobial, and anti-inflammatory properties, further supporting the plant’s therapeutic potential.

The antifungal screening by the disc diffusion method demonstrated the presence of a zone of inhibition, indicating the antifungal potential of the cream formulations. The cream formulated with Gokarn showed antifungal activity, though less pronounced compared to the positive control. The formulation containing a mixture of plant extracts exhibited relatively lower antifungal activity, suggesting that combinations of extracts may have varying effects.

Evaluation parameters such as appearance, pH, homogeneity, spreadability, and emolliency confirmed that the formulated creams were smooth, homogeneous, and easily washable with no signs of irritation. Spreadability was found to be within acceptable limits, and the creams were effective in moisturizing the skin, further supporting their cosmetic and therapeutic potential.

Overall, the results indicate that the Clitoria ternatea seed extract possesses significant antifungal and pharmacological potential, which can be utilized in the development of topical creams for skin-related ailments. However, further studies are needed to optimize the formulation and assess its efficacy in clinical settings for broader applications.

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