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COMPARATIVE STUDY ON ANTIFUNGAL AND ANTIMICROBIAL ACTIVITY OF FORMULATED CLOTRIMAZOLE CREAM INCORPORATED WITH BIOTIC MATERIALS

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ABSTRACT

Cutaneous fungal infections remained a significant health concern, particularly in immunocompromised individuals, demanding the development of more effective topical treatments. This study evaluated the anti-fungal and anti-microbial activities of formulated clotrimazole creams incorporated with Chitosan and Papaya seed extract, which had been extracted using the maceration method with methanol as the solvent. The formulations tested were **C1** (Clotrimazole cream), **C2** (Clotrimazole + Chitosan cream), **C3** (Clotrimazole + Papaya seed extract cream), and **C4** (Clotrimazole + Chitosan + Papaya seed extract cream). The anti-fungal and anti-microbial activities were assessed using the agar well diffusion method against Candida albicans and Staphylococcus aureus. The results showed that all formulations exhibited inhibitory effects against both pathogens, with formulation **C4** demonstrating the largest zones of inhibition, suggesting a synergistic effect from the combination of Chitosan and Papaya seed extract. In addition to microbiological testing, the creams were evaluated for physical properties such as pH, viscosity, spreadability, washability, irritancy, phase separation, and dye solubility tests. The formulations showed improved stability, controlled release, and better skin penetration due to the inclusion of Chitosan and Papaya seed extract, which enhanced both therapeutic efficacy and skin health. These findings indicated that the combination of Chitosan and Papaya seed extract with clotrimazole offered a promising approach to improving topical treatments for fungal and bacterial infections. Future studies should focus on understanding the molecular mechanisms and conducting in vivo evaluations to confirm long-term safety and clinical

applicability.

KEYWORDS: Cutaneous fungal infection, Clotrimazole, Chitosan, Papaya seed extract, Anti-fungal, Anti-microbial, Candida albicans, Staphylococcus aureus, Synergistic effect, Physical evaluation.

1. INTRODUCTION

Fungal infection is one of the most common causes of skin illness worldwide. Fungal infection affects approximately 40 million persons in emerging and impoverished countries. Fungi typically attack the skin's surface first, then penetrate deeper layers by desquamation. Candida species is one of the fungi that cause the most superficial skin infections. Fungal infections exhibited in the deeper layers of the skin are known as cutaneous mycoses. Fungal skin infections are treated with topical antifungal medication. Synthetic antifungal and antimicrobial agents like clotrimazole have been widely used to treat such infections, there is an increasing need for alternative treatments due to growing concerns over drug resistance and adverse side effects. The development of more effective topical formulations that combine both synthetic and natural ingredients offers a promising avenue for enhancing treatment outcomes while minimizing these concerns. Clotrimazole, Chitosan, and Papaya seeds extract's antifungal and antimicrobial properties are evaluated and examined in this study.[1]

Clotrimazole is used to treat fungal skin infections including candidiasis, ringworm, jock itch and athlete's foot. A type of azole antifungal, clotrimazole inhibits the growth of fungi. Clotrimazole inhibits the formation of ergosterol, which is a necessary component of fungal cell membranes. Fungal growth is inhibited in a dose-dependent manner when the preceding processes occur quickly because ergosterol directly stimulates fungal cell proliferation in a hormone-like manner.[2]

Biotic material or biological derived material is any material that originates from living organisms. Chitosan and Papaya seed extract are considered biotic materials because they are derived from living organisms. Both contain bioactive compounds with beneficial biological properties, such as antimicrobial, antifungal, making them valuable in pharmaceutical and cosmetic applications. Their natural origins and therapeutic potential classify them as biotic materials.[3]

Chitosan (CS), a biopolymer produced from chitin which is obtained from the exoskeletons of crustaceans, is known for its potent antifungal properties while remaining biodegradable, biocompatible and nontoxic. Because of its properties, it has been widely utilized in the control of fungal infections. Papaya seed extract is another natural ingredient gaining attention for its potential antimicrobial and antifungal properties. The active compounds present in papaya seeds, such as alkaloids, flavonoids, and phenolics, have been shown to exhibit inhibitory effects against a wide range of pathogenic bacteria and fungi. Papaya seed extract contains several antifungal components, such as benzyl isothiocyanate (BITC), fatty acid-methyl esters, heterocyclic amides and phenolic compounds, which collectively contribute to its ability to inhibit fungal growth.[4,5]

1.1 CLOTRIMAZOLE

Figure 1: Clotrimazole cream.

Clotrimazole is an antifungal medication from the imidazole class, used primarily to treat superficial fungal infections such as oral and vaginal candidiasis, athlete's foot, jock itch, ringworm and pityriasis versicolor. It works by inhibiting the synthesis of ergosterol, a critical component of the fungal cell membrane, which weakens the membrane, leading to cell death and preventing further fungal growth. Clotrimazole is available in various forms, including topical creams, oral lozenges and vaginal tablets, and is typically used for localized infections due to its poor systemic absorption. At higher concentrations, it may also show activity against certain bacteria and protozoa, such as *Trichomonas* spp.

Clotrimazole is generally well-tolerated, with common side effects including mild skin irritation, itching, or a burning sensation, especially with vaginal preparations. Serious side effects are rare but can include allergic reactions or swelling. It is contraindicated in individuals with known allergies to imidazole antifungals. While topical clotrimazole is considered safe during pregnancy, oral and vaginal forms should be used cautiously under medical guidance. It is important to be careful in patients with liver issues or those using the

medication on large skin areas for prolonged periods. When combined with corticosteroids such as betamethasone for treating inflammatory fungal infections, extra attention is needed to prevent worsening of the fungal condition.[6]

1.2 BIOTIC MATERIALS

1. Chitosan
2. Papaya seed extract.

1.2.1 CHITOSAN

Figure 2: Chitosan.

Chitosan is a biopolymer derived from chitin, which is primarily found in the shells of crustaceans, as well as the exoskeletons of insects and fungi. It is produced by deacetylating chitin, a process that introduces amino groups, allowing chitosan to be soluble in acidic solutions. Its chemical structure consists of repeating glucosamine and N-acetylglucosamine units, with its properties, such as solubility and biological activity, influenced by the degree of deacetylation. Chitosan is valued for its biocompatibility, biodegradability, and non-toxicity, making it widely applicable in pharmaceuticals, tissue engineering, food preservation and environmental protection.[7] Chitosan also demonstrates significant antimicrobial and antifungal properties, owing to its positively charged amino groups, which interact with microbial cell membranes, disrupt vital processes and inhibit growth. It is effective against various microorganisms, including bacteria like *Escherichia coli* and *Staphylococcus aureus*, as well as fungi like *Candida albicans*. The antimicrobial action of chitosan involves binding to microbial cell walls, obstructing nutrient flow, and causing leakage of intracellular contents. Its effectiveness can be enhanced by altering its molecular properties, such as its molecular weight or by preparing it in nanoparticle form, positioning it as a promising alternative to synthetic chemicals for infection control and medical applications.[8,9]

1.2.2 PAPAYA SEED

Figure 3: Papaya.

Carica papaya Linn, commonly known as papaya, is a fast-growing tropical tree cultivated in regions such as India, Sri Lanka, the Philippines and South Africa, though it is believed to have originated in southern Mexico and Costa Rica. The tree can reach heights between 2 to 10 meters and features a distinctive grey, hollow trunk. The black, tuberculous seeds of the papaya are known for their medicinal properties, including their use as counter irritants, vermifuges, emmenagogues, and carminatives. Extracts of these seeds are traditionally used to treat conditions like enlarged liver and spleen, bleeding piles and skin disorders such as psoriasis and ringworm.[10]

Papaya seed extract has demonstrated strong antifungal and antimicrobial properties, particularly against pathogens like *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. Its antifungal effects are attributed to the ability of papaya seed compounds to disrupt fungal cell membranes, leading to cell death. The extract also shows broad-spectrum activity against both Gram-positive and Gram-negative bacteria, making it a versatile antimicrobial agent. While studies suggest its effectiveness in treating skin infections caused by fungi and bacteria, including those resistant to antibiotics, further clinical research is needed to confirm its safety and efficacy for human use. The growing interest in papaya seed extract highlights its potential as an alternative treatment, especially in the context of rising antibiotic resistance.[11]

1.3 ANTI-FUNGAL ACTIVITY

Antifungal activity refers to a substance's ability to inhibit the growth of fungi, including yeasts, molds, and other pathogenic species. These agents, which can be synthetic, plant-derived, or naturally occurring, target specific components of fungal cells such as the cell wall, membrane, or intracellular structures to prevent fungal proliferation. The effectiveness of antifungal agents is typically assessed through diffusion tests (measuring the zone of

inhibition) or broth dilution methods (determining the minimum inhibitory concentration, MIC). Antifungal agents are essential in treating infections caused by fungi like *Candida*, which can lead to conditions such as candidiasis, particularly in immunocompromised individuals.[14]

Candida species, especially *Candida albicans*, are commonly used in antifungal susceptibility testing. Techniques such as the disk diffusion method or agar well diffusion method are employed, where antifungal agents are applied to an agar plate inoculated with Candida cultures. The zone of inhibition around the agent indicates its effectiveness. This testing is crucial for selecting the right antifungal treatment, especially as resistance to commonly used antifungals is on the rise. Monitoring antifungal susceptibility is vital in clinical settings to ensure effective therapy and combat the growing issue of antifungal resistance.[15]

1.4 ANTI-MICROBIAL ACTIVITY

Antimicrobial activity refers to a substance's ability to inhibit the growth of microorganisms, including bacteria, fungi, viruses, and parasites. These substances can be either naturally derived, such as plant extracts, or synthetic, like antibiotics. The antimicrobial effect can be bactericidal (killing microorganisms) or bacteriostatic (inhibiting growth). Mechanisms of action include disrupting microbial cell walls, inhibiting protein synthesis, or interfering with DNA replication. With the rise of antibiotic-resistant bacteria, researchers are exploring new antimicrobial agents to combat infections and prevent the spread of resistant pathogens.[12]

The Agar Well Diffusion Method is a common technique used to assess the antimicrobial activity of substances. In this method, an agar plate is inoculated with a microbial culture and wells are created in the agar. The antimicrobial agent is introduced into the wells and as it diffuses radially, the effectiveness is indicated by a zone of inhibition where microbial growth is prevented. The size of the inhibition zone correlates with the strength of the antimicrobial activity. This method is simple, cost- effective, and useful for screening multiple agents, though it is semi-quantitative and may require further testing for precise potency measurements.[13]

CUTANEOUS FUNGAL INFECTION

Dermatomycoses, another name for cutaneous fungal infections, are prevalent illnesses that affect about one billion individuals globally. Human skin, hair, and nails are most commonly affected by these illnesses. Three main categories of fungi are responsible for them: yeasts,

non-dermatophyte molds and dermatophytes. Numerous fungi can infiltrate different parts of the human body and cause superficial fungal diseases. These diseases include *Malassezia sp*., which needs lipids and a humid milieu to develop and dermatophytes, which infect keratinized epithelium, hair follicles and nail apparatus. Clinical features are usually sufficient to determine the diagnosis. In some circumstances, a mycological evaluation may be required prior to initiating antifungal medication.[16]

Types of CFI

1. Ringworm (dermatophytosis)
2. Onychomycosis
3. Tinea versicolor/pityriasis versicolor
4. Candidiasis.[17]

1.5 TOPICAL FORMULAIONS FOR CFI

Topical formulations are directly applied to the skin. Topical formulations are created in a vehicle, or base, that can be tailored to a specific body spot or skin condition. The product may be intended to moisturize or to maximize the penetration of an active substance, typically a medicine, into or through the skin.

Advantages

- A higher concentration of medication is directed specifically to the area that requires treatment.
- This approach minimizes side effects and reduces the risk of toxicity to other organs compared to treatments that affect the whole body.

Disadvantages

- The process of applying them can take a significant amount of time.
- The treatment plan may become complex, particularly when multiple formulations are required.
- The applications might be inconvenient or cause discomfort, and they can be messy at times.[18]

1.6 CREAMS

Cream is a preparation usually for application to the skin. Creams are semi-solid emulsions of oil and water. They are divided into two types: Oil-in-water (O/W) creams contain small oil

droplets dispersed in a continuous water phase, while water-in-oil (W/O) creams have small water droplets dispersed in an oily phase. Water-in-oil creams are more difficult to handle, but hydrophobic medicines are more easily freed from them compared to oil-in-water creams.[19]

Advantages

- Creams are more acceptable to the patients because they are less greasy and are easier to apply.
- They interfere less with skin functions.
- O/W type of creams (superior to w/o type) can be rubbed onto the skin more readily and are easily removed by washing. w/o can be spread more evenly.
- O/W type of cream are less likely to soil clothes.
- Evaporation of water from o/w type of cream causes a cooling sensation.
- O/W creams absorb the discharges from the wound (liquid exudate) very quickly.
- W/O creams (e.g. cold creams) restricts evaporation from the skin, it can be used on non-weeping surfaces to prevent dehydration (in the dry season), restore suppleness (softness).[19]

Creams for CFI

Topical dose formulations are intended to deliver the therapeutic influence at specific skin regions while minimizing the likelihood of side effects. Topical formulations are effective for treating a wide range of illnesses and are easy to apply and transport. The efficacy of the sequential processes listed below determines the onset, rate, and degree of therapeutic response for any topical formulation: The active component is released from the dosage form, and the medicine penetrates and diffuses through the stratum corneum (SC) and other layers of the skin, producing the desired pharmacological effect. changes in these distinct pathways cause changes in the safety and effectiveness of formulations. Although there are many other topical formulations available on the market, semisolids—such as ointments, lotions, and gels—are most frequently used for this purpose. Antifungal creams work by either killing the fungus or inhibiting its growth. The cream should be applied to the infected area and the surrounding skin as directed. This ensures that the medication effectively targets the fungus at the site of infection locally. Antifungal medications are used to treat fungal infections of the skin, nails, lungs, and other organs. Some fungal infections go away in a few weeks. Some patients may need care for months. Long-term usage of antifungal drugs or insufficient treatment plans can lead to the development of antifungal resistance.[20]

Marketed dosage forms

Table 1: Marketed Brand names of antifungal drugs.[20]

SI.no	DRUGS	BRAND NAMES
1.	Clotrimazole	Lotrimin AF, Trivagizole 3, Clotrimazole 3, Candid
2.	Miconazole	Monistat, Vagistat, Micatin
3.	Terbinafine	Lamisil AT
4.	Tolnaftate	Tinactin, Lamisil AF
5.	Butenafine	Lotrimin Ultra

2. REVIEW OF LITERATURE

1. **El-Araby A *et al.*, (2024)** evaluated the antifungal properties of chitosan extracted from shrimp shell waste (*Parapenaeus longirostris*) against post-harvest spoilage fungi in strawberries (*Fragaria×ananassa*). The physicochemical characteristics of the extracted chitosan, including yield, degree of deacetylation (83.50%), molecular weight (180 kDa), and solubility (80.10%), were determined. The antifungal activity was notably dose-dependent, indicating that chitosan presents a viable alternative to synthetic antimicrobials for the preservation of strawberries.[21]

2. **Panusa A *et al.*, (2024)** focused on *Carica papaya* Linn., a common tropical plant valued for its fruit and traditional medicinal uses, particularly its seeds, which are rich in glucotropaeolin (benzyl glucosinolate). When damaged, this compound is hydrolysed by myrosinase to produce benzyl isothiocyanate, known for its anticancer properties. This study utilized UHPLC-PDA ESI/MS to analyse the extracts, revealing the presence of 4-hydroxybenzoic acid—previously undetected in papaya seeds—alongside glucotropaeolin and tryptophan. The research also identified mono-, di-, and triglycosides of 4-hydroxybenzoic acid and found that these compounds were more effectively recovered following myrosinase deactivation using an oven compared to microwave treatment.[22]

3. **Bhargava S *et al.*, (2023)** conducted a study on rising burden of superficial fungal infections in India and the role of clotrimazole for optimal management. The review provided an overview of the use of topical clotrimazole, available in cream and powder forms, and its application in combination with topical steroids. This approach was deemed optimal for managing skin fungal infections, underscoring the efficacy of clotrimazole in clinical settings.[23]

4. **Poznanski P *et al.*, (2023)** focused on chitosan (CS) and chitosan nanoparticles (CSNPs) as antifungal agents, highlighting their biodegradable, biocompatible and non-toxic

properties. Various studies demonstrated that the antifungal activity of CS was influenced by parameters such as molecular weight, degree of deacetylation and acetylation pattern. The impact of concentration and pH of CS solutions on their biological efficacy was also examined. Additionally, the characteristics of CSNPs, including particle size and zeta potential, were shown to correlate with their antifungal effectiveness. This review synthesized findings from diverse studies to establish a clearer understanding of how CS and CSNP parameters shaped their antifungal properties.[4]

5. **Anilkumar A and Bhanu PA., (2022)** study aimed to evaluate the methanolic extract of papaya seeds (MPB) for its cytotoxic effects against human liver cancer Hep G2 cell lines. The half maximal inhibitory concentration (IC50) was determined using the MTT assay This emerging evidence highlights the need for further exploration into the medicinal properties of papaya seeds, which have historically been overlooked in contemporary herbal medicine. Recent research has shifted focus to the anticancer potential of papaya black seeds.[24]

6. **Khattak RZ *et al.*, (2022)** demonstrated that topical drug delivery, particularly through chitosan-decorated bacitracin-loaded cream, significantly improved drug permeation across the skin, increased bioavailability, and extended the residence time of the drug. In this study, researchers prepared and assessed chitosan-coated bacitracin cream, investigating its synergistic antibacterial effects compared to a simple bacitracin-loaded cream. Various in vitro characteristics, including rheology, pH, viscosity, drug content, and antibacterial activity, were evaluated. The formulations were stable regarding colour, liquefaction, and phase separation under accelerated conditions, although notable variations in pH were observed over time.[25]

7. **Elshaer EE *et al.*, (2021)** demonstrated that chitosan nanoparticles loaded with clotrimazole and Egyptian Thompson Seedless *Vitis vinifera* extract (NCs/VJ/Cz) exhibited significant antifungal effects, with average inhibition zone diameters of 74 mm against *Candida albicans* and 72 mm against *Aspergillus niger*. The integration of chitosan nanoparticles with clotrimazole and Egyptian *Vitis vinifera* juice extract was evaluated to enhance antifungal activity while minimizing side effects. Ex vivo and in vivo evaluations on experimental rats with skin fungal infections indicated that the NCs/VJ/Cz ointment facilitated sustained drug release and demonstrated complete wound healing and tissue repair after 7 days of administration. Overall, the findings suggested

that NCs/VJ/Cz ointment could serve as a novel anti-dermatophytic agent with notable wound healing properties.[26]

8. **Sundar S *et al*., (2021)** studies on papaya seed extracts, obtained using ethanol, methanol, and chloroform solvents, revealed significant antimicrobial properties. The antimicrobial activity was tested against three Gram-positive and three Gram- negative bacteria, as well as two fungal species, using the agar disc diffusion method. The results showed that methanol and chloroform extracts exhibited stronger antibacterial effects against Gram-negative bacteria and more potent antifungal activity, especially against *Candida albicans* and *Aspergillus niger*. The ethanol and chloroform extracts also demonstrated superior antibacterial effects against Gram-positive bacteria. These findings suggest that *Carica papaya* seed extracts possess promising antimicrobial potential.[27]

3. AIM AND OBJECTIVE

3.1 AIM

This study aims to conduct a comparative analysis of the antifungal and antimicrobial activities of clotrimazole cream formulations incorporated with biotic materials, specifically chitosan and papaya seed extract (*Carica papaya* Linn). The study will evaluate the synergistic effects of these biotic materials on the efficacy of clotrimazole against common fungal and microbial pathogens.

3.2 OBJECTIVE

- To prepare papaya seed extract by maceration extraction using methanol as solvent.
- To formulate four different clotrimazole cream formulations by incorporating chitosan and papaya seed extract.
- To evaluate the antifungal activity of the formulated clotrimazole creams incorporated with chitosan and papaya seed extract against the common fungal pathogen *Candida albicans*.
- To evaluate the antimicrobial activity of the formulated clotrimazole creams incorporated with chitosan and papaya seed extract against the common bacterial pathogen *Staphylococcus aureus*.
- To compare the antifungal and antimicrobial activities of the formulated clotrimazole creams incorporated with chitosan and papaya seed extract with those of a standard formulated clotrimazole cream.
- To analyse the potential synergistic effects of combining chitosan and papaya seed extract

with clotrimazole in enhancing the overall antifungal and antimicrobial potency of the creams.

- To characterize the physicochemical properties of the formulated creams, including pH, viscosity, spreadability, greasiness, washability, irritancy, phase separation, and dye solubility.

4. MATERIAL USED

4.1 DRUG PROFILE

4.1.1 CLOTRIMAZOLE

Clotrimazole is a broad-spectrum antifungal discovered in the 1960s, available in various forms for treating fungal infections like oral candidiasis, vaginal candidiasis, and dermatomycoses.

Table 2: Basic details of clotrimazole.

Brand name	Canesten, Mycelex, Clotrimaderm
IUPAC name	1-[(2-chlorophenyl) diphenylmethyl]-1H-imidazole
Molecular formula	$C_{22}H_{17}ClN_2$
Molecular weight	344.837 g/mol
CAS number	23593-75-1

Table 3: Physiochemical properties of clotrimazole.

Appearance	White to off-white solid powder
Solubility	Poorly soluble in water, soluble in methanol, sparingly soluble in ether, slightly soluble in benzene and toluene.
Melting point	147–149°C (297–300°F)
pH	1.2–7.5

- **Mechanism of action**

Clotrimazole primarily inhibits ergosterol biosynthesis in fungal cell membranes by targeting lanosterol 14-demethylase (CYP51), leading to impaired membrane integrity and inhibition of fungal growth. In addition to its antimycotic effects, clotrimazole also affects sarcoplasmic reticulum Ca2+-ATPase, depletes intracellular calcium and blocks calcium-dependent potassium channels and voltage-dependent calcium channels, contributing to other pharmacological actions beyond its antifungal activity.

- **Pharmacodynamics**

Clotrimazole is a broad-spectrum antifungal agent that inhibits yeast growth by altering cell membrane permeability. It is typically fungistatic at concentrations up to 20 mcg/mL but can

be fungicidal against *Candida albicans* and other Candida species at higher concentrations. While generally considered fungistatic, clotrimazole may exhibit fungicidal properties at elevated doses, though resistance to the drug is increasingly common.

➢ **Pharmacokinetics**

- **Absorption:** As clotrimazole is typically not well absorbed, it does not pose significant risks for drug interactions.
- **Distribution:** The topical form of clotrimazole is minimally absorbed into the bloodstream and tissues.
- **Metabolism:** Metabolized in the liver to inactive metabolites.
- **Elimination:** The primary route of elimination is through the liver.

➢ **Dosage**

Apply twice daily to the affected area, and if there's no improvement after 4 weeks, the diagnosis should be re-evaluated. For vaginal candidiasis, use the 1% cream for 7-14 days or the 2% cream for 3 consecutive days, preferably at bedtime. For superficial dermatologic infections, apply twice daily for 7 days.

➢ **Contraindications**

- Topical clotrimazole is ineffective for onychomycosis, requiring oral antifungals.
- During pregnancy, only topical use is recommended, as it has poor absorption and does not cross the placenta.
- Clotrimazole should be used with caution during breastfeeding, as topical use poses little risk, but oral lozenges should be limited.
- Patients should avoid sexual intercourse and tampons during treatment, as clotrimazole can affect contraceptive devices.
- Those with azole hypersensitivity should avoid clotrimazole.

➢ **Drug interaction**

- Acenocoumarol
- Capmatinib
- Clindamycin
- Dicoumarol
- Fluindione.

➢ **Adverse drug reaction**

When clotrimazole is applied locally and topically, toxic effects such as:

- Pelvic cramps
- Hives
- Skin rash
- Occasional headache
- Itching and irritation of the vulva and vagina may be observed.[2,6]

4.2 CHITOSAN PROFILE

Chitosan is a linear polysaccharide derived from chitin, a naturally occurring biopolymer found in the exoskeletons of crustaceans, the cuticles of insects and the cell walls of certain fungi. Chitosan is produced by the deacetylation of chitin, which involves removing acetyl groups to convert chitin into a more active, cationic form.

➢ **Origin**

Chitosan is obtained by the deacetylation of chitin, which is abundant in crustaceans (such as crabs, shrimp, and lobsters), arthropods, mollusks and some fungi. The chemical extraction of chitin includes several steps: deproteinization, demineralization and discoloration. Once chitin is isolated, it is chemically modified to form chitosan by using sodium hydroxide (NaOH) in excess and water as a solvent. This method of extraction is commonly used in commercial production, though biological methods involving microorganisms can also be employed to obtain chitosan.

➢ **Synonyms**

Chitosan, also known by various names such as Poliglusam, Deacetylchitin, Poly (D) glucosamine, BC, Chitopearl, Chitopharm, Flonac and Kytex.

➢ **Biological Source**

Chitosan is not naturally occurring and must be synthesized from chitin, which is found in various natural sources, including marine, terrestrial and microbial environments. Marine sources, particularly the exoskeletons of crustaceans like lobsters, shrimp, and krill, as well as mollusks such as squid, clams, and oysters, are the most common and widely utilized for chitosan production due to established extraction methods. Chitin is also present in certain algae, such as brown algae and diatoms. Its composition can vary depending on factors like age, season and environmental conditions influencing the quality and yield of chitosan.

➢ **Chemical Constituents**

Chitosan is a copolymer consisting of two main chemical units:

- N-acetyl-D-glucosamine (GlcNAc): An acetylated unit.
- D-glucosamine (GlcN): A deacetylated unit.

These units are linked by β-(1-4)-glycosidic bonds and are randomly distributed throughout the polymer chain. Typically, more than 80% of chitosan consists of GlcN units, making it the predominant component.

➢ **Uses**

Chitosan has diverse applications, including enhancing plant defense and yield in agriculture, serving as a natural biocontrol agent, aiding in water filtration by removing sediments and contaminants, improving wine quality and stability during winemaking and promoting faster wound healing by forming adhesive layers in dressings.[28]

4.3 PAPAYA PLANT PROFILE

➢ **Scientific classification**

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dilleniidae
Order : Brassicales
Family : Caricaceae
Genus : Carica
Species : *Carica papaya*.

➢ **Synonyms**

Sanskrit : Madhukarati
English : Papaw
Hindi : Papita
Telugu : Boppayi
Tamil : Pappali
Malayalam : Omakkay, Kappalanga.[29]

➢ **Orgin and cultivation**

Papaya (*Carica papaya L.*), a tropical and subtropical fruit from the Caricaceae family, is native to southern Mexico and Central America. Introduced to India in the late 16th century, it is known for its high nutritional and medicinal value. Papaya is easy to cultivate, produces fruit in under a year, and is profitable due to its ability to flower and bear fruit year-round. The plant grows up to 30 feet tall, with large leaves reaching up to 3 feet in diameter.

India is the world's largest papaya producer, cultivating approximately 70,100 hectares and producing around 17.67 lakh tons annually. The fruit is grown in 24 Indian states, with significant production in Assam, Karnataka, Gujarat, Andhra Pradesh, Maharashtra and other regions such as Orissa, West Bengal and Uttar Pradesh.[30]

➢ **Biological source**

Papaya (*Carica papaya L.*) is a commercially valuable fruit crop that belongs to the Caricaceae family and is native to the Mesoamerica region of the Americas. It is cultivated widely in tropical and subtropical areas across the world.[29]

➢ **Botanical description**

Papaya is a tropical fruit available year-round. The fruit is commonly eaten fresh as a dessert or made into juice. Its seeds are black, embedded within the pulp. Typically, the seeds of a ripe papaya constitute about 16% of the fresh fruit's weight and are considered a by-product. Due to the abundant availability of papaya throughout the year and the low economic value of the seeds, nutritionists have explored utilizing papaya seeds as a protein-rich feed ingredient, particularly for poultry, as well as a functional feedstuff.[30]

➢ **Chemical constituents**

Papaya (*Carica papaya L.*) contains bioactive compounds such as papain, carpaine flavonoids, alkaloids, tannins, steroids, and saponins, which contribute to its medicinal properties and enhance its therapeutic potential.

➢ **Uses**

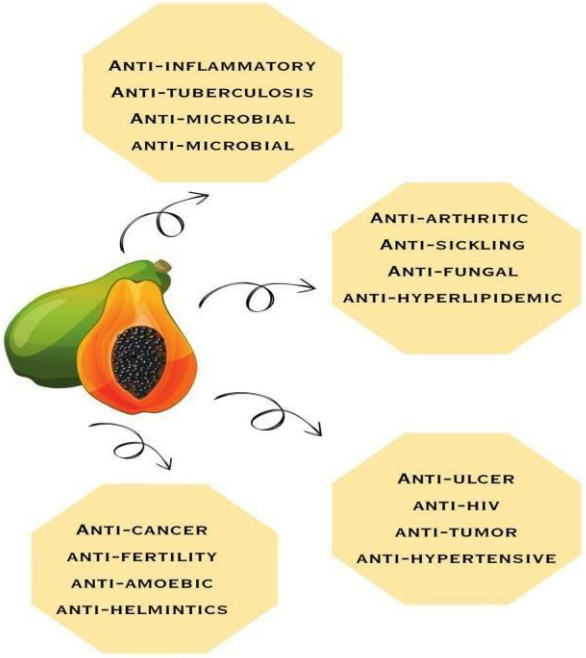


Figure 11: Uses of *Carica papaya* Linn.

4.4 EXCIPIENT PROFILE

Excipients	Synonyms	Source	Uses	Figure
Glycerine	Glycerol	Derived from plants, animals, and petroleum.	Humectant, skin care, pharmaceutical.[31]	 **Figure 12**
Liquid Paraffin	Paraffinum liquidum	Derived from petroleum distillation.	Hydrating agent, lubricant, after-wax wipes.[32]	 **Figure 13**
Beeswax	Paraffin wax, Turpentine	Produced by honeybees from glandular secretions.	Cosmetics (moisturizing), industrial (candles, metal casting), pharmaceuticals.[33]	 **Figure 14**

White Soft Paraffin	White petroleum, Vaseline	Byproduct of petroleum refining.	Moisturizing agent, skin barrier, wound care.[34]	 **Figure 15**
Cetyl Alcohol	1- exadecanol	Derived from palm or coconut oil, or synthetically produced.	Emollient, emulsifier, thickener in cosmetics, water-binding, industrial uses.[35]	 **Figure 16**
Borax	Disodium borate	Naturally occurring in evaporated lake deposits.	Household cleaner, cosmetics (antiseptic), paint, ceramics.[36]	 **Figure 17**
Methyl Paraben	Methyl-4-Hydroxybenzoate	Naturally occurring in fruits, synthesized from methanol.	Preservative, antimicrobial in cosmetics, pharmaceuticals, and food products[37].	 **Figure 18**

5. METHODOLOGY

5.1 CLOTRIMAZOLE

5.1.1 COLLECTION

The study on Clotrimazole was conducted using a sample obtained from Dhamtec Pharma, and Consultants, Mumbai. Clotrimazole was synthesized by reacting 2 chlorotri phenyl methylchloride with imidazole in the presence of triethylamine. The Clotrimazole appeared as a powder of white colour and was stored in a cool place for further use.

Figure 19: Clotrimazole powder.

5.1.2 HPLC ANALYSIS

High-Performance Liquid Chromatography (HPLC) method was done to analyse clotrimazole in pharmaceutical formulations, using an LC-20AD system with a SPD- 20A UV-VIS detector and a C18 column for separation. The mobile phase consists of methanol and K_2HPO_4 in an 85:15 ratio, with a flow rate of 1.5 mL/min and a 20 µL injection loop. The method was validated following ICH guidelines, demonstrating selectivity, reproducibility and the ability to separate excipients while accurately quantifying clotrimazole. A calibration curve was constructed, showing a linear relationship between peak area and concentration, ensuring accurate drug quantification. The validation of accuracy, precision and specificity underscores the method's reliability for consistent and reproducible clotrimazole analysis in pharmaceutical products.[38]

5.2 CHITOSAN

5.2.1 COLLECTION

The study conducted on Chitosan. The sample was obtained from ISF Chitin and marine products LLP, Cochin. Test result prepared by Roshin Poly, QC Chemist and test result approved by Sameer Saga MP, QC Head. Chitosan was obtained by first extracting chitin from crustacean shells through demineralization with acid (to remove minerals) and alkaline treatment (to remove proteins). Chitosan are seen in powder, flakes, semi grind of offwhite colour. They are stored in sealed bags away from water and direct heat in room temperature for further use.

Figure 20: Chitosan.

5.2.2 DEGREE OF DEACETYLATION

Degree of deacetylation of chitosan was determined by the titration method by dissolving a known amount of chitosan in an acidic solution (e.g., 0.1 M acetic acid) and then titrating it with a standardized NaOH solution. The free amino groups in chitosan, resulting from the removal of acetyl groups, react with the base. Using phenolphthalein as an indicator, the endpoint of titration is reached when the solution turns from colourless to pink, indicating neutralization of the free amines. The volume of NaOH consumed is used to calculate the DD using a specific formula, which relates the amount of NaOH to the number of free amino groups, thus determining the extent of deacetylation.[39]

5.3 PAPAYA SEED EXTRACT

5.3.1 COLLECTION AND DRYING

The study was conducted on seeds of Raw papaya. The sample of raw papaya fruits were obtained from cultivated local farmland Thrissur, Kerala during November 2024. Identified and authenticated by Dr. M Bheemalingappa, scientist-B, forest botany department, KSC CSTE-Kerala Forest research institute, Peechi. The seeds were separated from papaya plant, washed for removing the impurities. Raw papaya seeds are the small, round, white coloured seeds found inside the papaya fruit (*Carica papaya* Linn). They are encased in a jelly-like, translucent pulp. The seeds were dried under shade for 1 week and ground into a fine powder using a mechanical grinder. Store for further use.

Figure 22: Stages of Drying.

5.3.2. MACERATION EXTRACTION

In order to identify the phytochemicals, *Carica papaya* Linn seed was extracted using methanol. The maceration technique was used for extraction. Maceration was a simple

extraction process in which plant material, either coarse or powdered, was soaked in solvents such as methanol, ethanol, ethyl acetate, acetone, or hexane. This technique was popular and economical for extracting bioactive compounds from plants. The process of maceration involved soaking plant material in a solvent to enable the solvent to dissolve certain chemicals and permeate the sample. The polarity of the solvent and the kind of plant matter were significant variables that impacted the extraction efficiency.[40]

Chemical Used

1. Methanol

Procedure

- Weigh 80.65 g of fine powder (*Carica papaya* Linn).
- Immerse the 80.65 g of fine powder into 300 mL of methanol. Allow the mixture to stand for 7 days at room temperature to enable the solvent to penetrate the plant material and extract bioactive compounds.
- After 7 days, perform filtration using Whatman No. 1 filter paper to separate the liquid (filtrate) from the solid plant residue.
- Pour the filtrate into a clean and dry conical flask.
- Place the conical flask containing the filtrate on a hot plate set at 30°C and evaporate the solvent until the liquid becomes semi-solid.[41]

Figure 23: Raw papaya seed Extract.

5.3.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Methanolic extract was subjected to qualitative phytochemical test for detection of different constituent such as:

- Alkaloids
- Tannins
- Flavonoids

- Saponins
- Proteins
- Carbohydrates
- Fixed oil.[42,43,44]

➢ **Chemical test for alkaloids**

Table 5: Chemical test for alkaloids.

Test	Procedure
Dragendorff's test	The acidified extract (2ml) was treated with 1 ml of Dradendorff's reagent (Pottasium bismuth iodide) and observe for the presence of orange precipitate.
Hager's test	The acidified extract (2ml) was treated with 1 ml of Hager's reagent (Saturated picric acid solution) and observe for the presence of yellow precipitate.

➢ **Chemical test for tannins**

Table 6: Chemical test for tannins.

Test	Procedure
Lead acetate test	The extract dissolved in water was treated with 10% lead acetate solution. Note the presence of bulky white precipitate.
Gelatin test	The extract dissolved in water was filtered. To the filtrate, 2% solution of gelatin containing 10% sodium chloride was added. Note the presence of milky white precipitate.

➢ **Chemical test for flavonoids**

Table 7: Chemical test for flavonoids.

Test	Procedure
Aqueous sodium hydroxide test	The extract is added to the aqueous sodium hydroxide (NaOH) solution and observe the presence of orange or yellow coloration.

➢ **Chemical test for saponins**

Table 8: Chemical test for saponins.

Test	Procedure
Foam test	1ml solution of extract was diluted with distilled water to 20ml and shaken in graduated cylinder for 15 min to produce foam.

- **Chemical test for carbohydrates**
- **Table 9: Chemical test for carbohydrates.**

Test	Procedure
Molisch test	Add 2 drops of Molisch's reagent to 2ml extract and observe the purple ring coloration junction.
Iodine test	Add a few drops of iodine solution to extract and observe blue-black coloration.

- **Chemical test for proteins**
- **Table 10: Chemical test for proteins.**

Test	Procedure
Biuret test	The acidified extract (2ml) was treated with 1 ml of Biuret reagent (Copper sulphate) and observe the purple coloration..
Millon's test	The acidified extract (2ml) was treated with 1 ml of Millon's reagent (Mercuric nitrate) and observe the red coloration.

- **Chemical test for fat and oil**
- **Table 11: Chemical test for fat and oil.**

Test	Procedure
Solubility test	Oils are soluble in ether, benzene and chloroform but insoluble in ethanol and water.
Sudan red III test	And few drops of Sudan red III solution to the extract and observ the sample for red coloration.
Fat test	Add a few drops of extract to a piece of filter paper and observe if translucent stain forms.

5.3.4 GC-MS ANALYSIS

GC-MS technique was done to analyse liquid, gaseous, or solid samples, using a SH-I- 5Sil MS column of 30.0 m in length, 0.25 mm in inner diameter and 0.25 µm in film thickness for separation. The process began by vaporizing the sample in a gas chromatograph, where it was separated into individual components using a capillary column coated with a stationary phase. An inert carrier gas, such as helium, hydrogen, or nitrogen, moved the sample through the column, with each compound eluting at a different time based on its boiling point and polarity, known as its retention time. After separation, the compounds were ionized and fragmented in the mass spectrometer, typically using electron or chemical ionization. The resulting ions were sorted based on their mass-to-charge (m/z) ratios. GC-MS data were collected in full scan mode, covering a wide range of m/z ratios, or in selected ion monitoring (SIM) mode, focusing on specific masses. The fragmented ions were detected and analysed, with peak areas correlating to the amount of each compound. The resulting chromatogram

produced multiple peaks, each corresponding to a unique mass spectrum that helped identify compounds. By comparing these spectra with commercial libraries, unknown compounds and target analytes were identified and quantified.[45]

6. FORMULATION OF CREAMS

➢ **Objective**

To prepare 20g creams of following:

C1 (Clotrimazole cream)

C2 (Clotrimazole + Chitosan cream)

C3 (Clotrimazole + Papaya seed extract cream)

C4 (Clotrimazole + Chitosan + Papaya seed extract cream)

➢ **Formula**

➢ **Table 12: Formula of clotrimazole based creams (C1, C2, C3, C4).**

SI. no	INGREDIENTS	WEIGHT TAKEN (gm)			
		C1	C2	C3	C4
1.	Clotrimazole	0.2	0.2	0.2	0.2
2.	Chitosan	-	0.2	-	0.2
3.	Papaya seed extract	-	-	0.2	0.2
4.	Beeswax	3.6	3.6	3.6	3.6
5.	Liquid paraffin	4.0	4.0	4.0	4.0
6.	Cetyl alcohol	0.8	0.8	0.8	0.8
7.	Distilled water	qs	qs	qs	qs
8.	White soft paraffin	7.4	7.4	7.4	7.4
9.	Glycerine	1	1	1	1
10.	Borax	0.02	0.02	0.02	0.02
11.	Methyl paraben	0.02	0.02	0.02	0.02

➢ **Procedure**

1. Preparation of C1 Cream (Clotrimazole Cream)

The ingredients were accurately weighed. The aqueous phase was prepared by mixing distilled water, glycerine, borax and methyl paraben in a beaker. In another beaker, the oil phase was prepared by melting beeswax, liquid paraffin, cetyl alcohol, and white soft paraffin in a water bath at 70°C. Clotrimazole was dissolved in methanol and added to the oil phase. Both the aqueous and oil phases were heated at 70°C for 30 minutes. The aqueous phase was then slowly combined with the oil phase while stirring continuously to emulsify the mixture. Afterward, the mixture was allowed to cool, stirred occasionally, and then packaged into a labelled container.[25]

Figure 24: Clotrimazole cream (C1).

2. Preparation of C2 Cream (Clotrimazole + Chitosan Cream)

The ingredients were accurately weighed. The aqueous phase was prepared by dissolving chitosan with distilled water, glycerine, borax and methyl paraben. The oil phase was prepared by melting beeswax, cetyl alcohol, liquid paraffin, and white soft paraffin at 70°C. Clotrimazole was dissolved in methanol and added to the oil phase. Both phases were heated at 70°C for 30 minutes. The aqueous phase was then slowly mixed with the oil phase while stirring continuously to emulsify the mixture. Finally, the cream was allowed to cool and was then packaged in a labelled container.[25]

Figure 25: Clotrimazole + Chitosan cream (C2).

3. Preparation of C3 Cream (Clotrimazole + Papaya Seed Extract Cream)

The ingredients were accurately weighed. The aqueous phase was prepared by mixing distilled water, papaya seed extract, glycerine, borax and methyl paraben. The oil phase was prepared by melting beeswax, cetyl alcohol, liquid paraffin, and white soft paraffin at 70°C. Clotrimazole was dissolved in methanol and added to the oil phase. Both phases were heated at 70°C for 30 minutes. The aqueous phase was then combined with the oil phase while stirring continuously to emulsify the mixture. Finally, the mixture was allowed to cool and was packaged in a labelled container.[25]

Figure 26: Clotrimazole + Papaya seed extract cream (C3).

4. Preparation of C4 Cream (Clotrimazole + Chitosan + Papaya Seed Extract Cream)

The ingredients were accurately weighed. The aqueous phase was prepared by dissolving chitosan with distilled water, papaya seed extract, glycerine, borax and methyl paraben. The oil phase was prepared by melting beeswax, cetyl alcohol, liquid paraffin, and white soft paraffin at 70°C. Clotrimazole was dissolved in methanol and added to the oil phase. Both phases were heated at 70°C for 30 minutes. The aqueous phase was then slowly mixed with the oil phase while stirring continuously to emulsify the mixture. Finally, the cream was allowed to cool and was transferred into a labelled container.[25]

Figure 27: Clotrimazole + Chitosan + Papaya seed extract cream (C4).

7. EVALUATION OF CREAMS

7.1 PHYSICAL EVALUATION

7.1.1 Organoleptic evaluation

The formulated clotrimazole creams were further evaluated based on the following physical parameters:

- **COLOUR:** The colour of the creams was assessed visually, ensuring consistency and uniformity in appearance.
- **ODOUR:** The characteristic odour of the creams was evaluated, ensuring it aligns with the expected fragrance of the formulation.

- **STATE:** The physical state (e.g., smooth, homogeneous) of the creams was visually examined to ensure no separation or unusual texture.
- **CONSISTENCY:** The consistency of the cream was tested by manually rubbing a small amount on the hands to assess its smoothness, spreadability, and texture.[46]

7.1.2 Irritancy

To assess irritancy, 1 cm^2 area was marked on the left-hand dorsal surface of the skin. A small amount of the cream was applied to this marked area, and the time of application was noted. The skin was monitored for any signs of irritancy, erythema (redness), or oedema (swelling) over a period of up to 24 hours.[46]

7.1.3 Washability

Small amount of each cream was applied to the hand, and the ease of removal was evaluated by washing it with tap water. The cream's washability was assessed based on how easily it was removed without leaving a greasy residue.[46]

7.1.4 pH

0.5 g sample of each cream was taken and dispersed in 50 ml of distilled water. The pH was measured using a digital pH meter, ensuring the formulation's pH falls within the acceptable range for skin compatibility.[46]

7.1.5 Viscosity

The viscosity of each cream was measured using a Brookfield viscometer at a temperature of 25°C, using spindle No. 64. The viscosity measurement provided insights into the cream's flow properties and spreadability.[46]

7.1.6 Phase Separation

Prepared creams were stored in closed containers at temperatures ranging from 25°C to 100°C, away from light. The creams were observed for phase separation at intervals over a period of 24 hours to 30 days, noting any changes in their stability or structure.[46]

7.1.7 Spreadability

The spreadability of each cream was evaluated using the parallel plate method. Two glass slides were used: one fixed to a wooden block, and 1.0 g of cream placed on it. A second slide was placed on top, and a 1 kg weight was applied for 5 minutes to ensure a uniform film and to remove air bubbles. Excess cream was scraped off the edges. A string was attached to the

top slide, and a 20 g weight was used to pull the top slide, separating it from the bottom. The distance covered (7.5 cm) was recorded to calculate spreadability using the formula:

S=ML/T

Were,

S = Spreadability

M = Weight tied to the upper slide L = Length moved on the glass slide

T = Time taken to separate the slides completely.[46]

7.1.8 Greasiness

Each cream was applied in the form of a smear on the skin surface, and the greasiness was assessed visually. If the smear left an oily or greasy residue, it was noted as indicative of the cream's greasiness level.[46]

7.1.9 Dye Test

Sudan III dye was mixed with the cream and a drop of the mixture was placed on a microscopic slide. A cover slip was applied, and the sample was examined under a microscope. If the dispersed globules appeared red with a colourless background, the cream was classified as O/W (Oil-in-Water) type. Conversely, if the dispersed globules appeared colourless with a red background, the cream was classified as W/O (Water- in-Oil) type.[47]

7.2 BIOLOGICAL ACTIVITY STUDIES

7.2.1 ANTI-FUNGAL STUDY

- **Clinical fungal cultures and culture media**

The antifungal activity of the formulated clotrimazole creams examined against clinical *Candida albicans* species. Clinical Microbial cultures were procured from Center for research on molecular & applied science Pvt Ltd, Thiruvananthapuram. Potato Dextrose Agar Medium, Potato Dextrose Broth used as the culture media for the antifungal study.

- **Agar-Well diffusion method Principle**

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres.

Materials required

1. Potato Dextrose Agar Medium (1 L)

   Dissolve 39 g of Potato Dextrose Agar (HiMedia) in 1000 ml distilled water. Autoclave at 15 lbs pressure, 121°C for 15 minutes. Mix well and pour 25-30 ml per 100 mm petri plate while molten.
2. Potato Dextrose Broth (1 L)

   Dissolve 24 g of Potato Dextrose Broth (HiMedia) in 1000 ml distilled water, boil to dissolve. Dispense and autoclave at 15 lbs pressure, 121°C for 15 minutes.
3. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5% *Candida albicans* (ATCC 10231).

Procedure

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Candida albicans* were swabbed. Wells of approximately 10mm was bored using a well cutter and 1000µg of samples were added in each wells. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (**C1**).[48]

7.2.2 ANTI-MICROBIAL STUDY

➢ **Agar-Well diffusion method Principle**

The Agar Well Diffusion Method is a commonly used laboratory technique to test the antimicrobial activity. It involves creating wells in an agar plate that is inoculated with the microorganism to be tested. The antimicrobial agent is introduced into these wells, and the effectiveness of the agent is determined by observing the zone of inhibition— an area around the well where microbial growth is prevented due to the agent's activity.

Materials required

Preparation of Agar Plate

Prepare a sterile agar plate (Nutrient Agar for bacteria). Inoculate the surface of the agar plate with a gram-positive bacterium, *Staphylococcus aureus*. This is typically done using a sterile swab to spread the microorganism evenly across the entire surface, ensuring a uniform lawn of growth.

Procedure

Wells of approximately 10 mm were created in an agar plate using a well-puncher in four

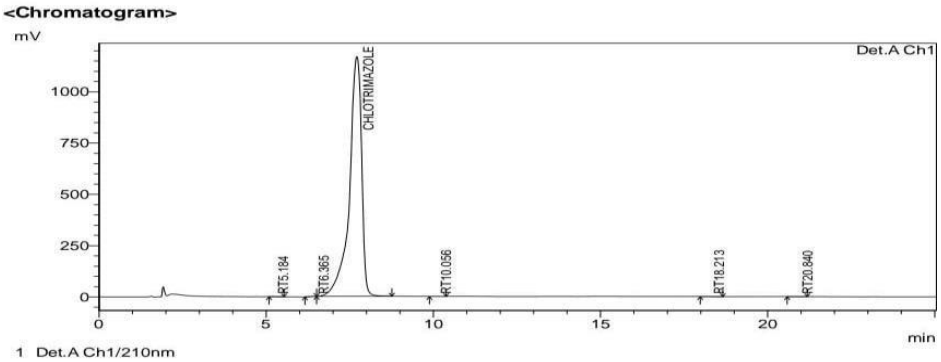
plates, and 0.1g of formulated creams were then carefully added to the wells using a sterile pipette, ensuring the creams stayed within the well. The plates were incubated at the optimal temperature for the microorganism being tested (37°C) for a period of 18–72 hours. After incubation, the plates were examined for clear zones of inhibition around the wells, and the diameters of these zones were measured and compared with that of standard formulated clotrimazole cream (**C1**) to assess the antimicrobial activity.[13]

8. RESULTS

8.1 EVALUATION OF CLOTRIMAZOLE

8.1.1 HPLC ANALYSIS

The purity of clotrimazole powder by HPLC analysis was found to be 99.85%.



PeakTable

Detector A Ch1 210nm

Peak#	Name	Ret. Time	Area	Height	Area %
1	RT5.184	5.184	11232	888	0.038
2	RT6.365	6.365	9347	1243	0.031
3	CHLOTRIMAZOLE	7.708	29687593	1164783	99.859
4	RT10.056	10.056	5683	455	0.019
5	RT18.213	18.213	7562	466	0.025
6	RT20.840	20.840	7978	526	0.027

Figure 28: HPLC chromatogram of Clotrimazole.

8.2 EVALUATION OF CHITOSAN

8.2.1 DEGREE OF DEACETYLATION

Degree of deacetylation of chitosan was determined by titration method and the result was found to be 91.00%.

8.3 EVALUATION OF PAPAYA EXTRACT

8.3.1 PHYTOCHEMICAL STUDIES

Percentage yield of maceration of seeds of *Carica papaya* Linn obtained as given below:

Table 13: Percentage yield of extract.

Extract	Method of extraction	Physical nature	Percentage yield (% w/w)
Methanol	Maceration	Semi solid	6

8.3.2 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of extract was carried out to identify the presence of various phytoconstituents. The results were summarised in Table below:

Table 14: Qualitative phytochemical analysis of extract.

SI.no	CHEMICAL CONSTITUENTS	ETHANOLIC EXTRACT	INFERENCE
1.	ALKALOIDS Dragendorff's test Hager's test		Presence of alkaloids.
2.	TANNINS Lead acetate test Gelatin test		Presence of tannins.
3.	FLAVONOIDS Aqueous sodium hydroxide test		Presence of flavonoids.
4.	SAPONINS Foam test		Presence of saponins.
5.	CARBOHYDRATES Molisch test Iodine test		Presence of carbohydrate
6.	PROTEINS Biuret test Millon's test		Presence of proteins.
7.	FAT & OILS Solubility test Sudan red III test Fat test	Soluble in ether	Presence of fats and oils.

Note: Positive (+) indicates presence of various phytoconstituents.

8.3.3 GC-MS ANALYSIS

GC-MS analysis of the extract identified 24 components including benzyl isothiocyanate, hexadecenoic acid, octadecanoic acid, imidazolidine-4-one and pyrrolidine-2-carboxylic acid, all of which exhibit antifungal and antimicrobial properties, enhancing the extract's potential therapeutic efficacy against microbial infections.

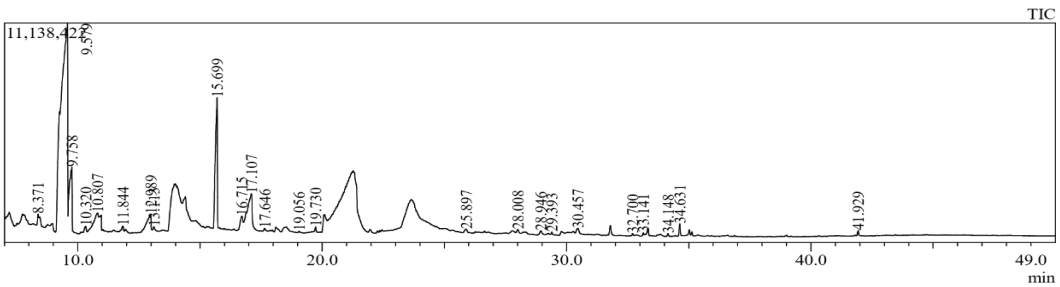


Figure 29: GC-MS chromatogram of methanolic extract of *Carica papaya* Linn.

Table 15: GC-MS Analysis report.

Peak	R.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.371	1747367	0.64	544771	1.96	3.21	MI	N-Acetyl-3-pyrroline
2	9.579	146746645	53.64	9621246	34.70	15.25	MI	Benzyl nitrile
3	9.758	24941649	9.12	3248600	11.72	7.68	MI	4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6- methyl-
4	10.320	1489585	0.54	307946	1.11	4.84	MI	Benzeneace c acid, methyl ester
5	10.807	10554891	3.86	859843	3.10	12.28	MI	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
6	11.844	1128406	0.41	258743	0.93	4.36	MI	2-Oxepanone, 7-methyl-
7	12.989	8336840	3.05	797945	2.88	10.45	MI	Benzeneace c acid
8	13.113	897727	0.33	201172	0.73	4.46	MI	Dicyclohexyl-, ethylphosphonate
9	15.699	37620332	13.75	6638550	23.94	5.67	MI	Benzene, (isothiocyanatomethyl)-
10	16.715	5517461	2.02	712216	2.57	7.75	MI	Benzeneacetamide
11	17.107	22307681	8.15	1733712	6.25	12.87	MI	Benzeneacetamide
12	17.646	606362	0.22	139997	0.50	4.33	MI	Acetamide, N-(phenylmethyl)-
13	19.056	225058	0.08	73550	0.27	3.06	MI	2-naphthalenemethanol, decahydro-6-methyl-
14	19.730	878754	0.32	268254	0.97	3.28	MI	Thieno[2,3-d]pyrimidin-4(1H)-one
15	25.897	1345220	0.49	184811	0.67	7.28	MI	Cyclobutane, 1,2:3,4-di- O-ethylboranediyl-
16	28.008	1036393	0.38	193806	0.70	5.35	MI	Phenylace c acid, 2-dimethylaminoethyl ester
17	28.946	1144038	0.42	189150	0.68	6.05	MI	Fumaric acid, 2-dimethylaminoethyl heptyl ester
18	29.393	401276	0.15	132464	0.48	3.03	MI	n-Hexadecanoic acid
19	30.457	2179584	0.80	297048	1.07	7.34	MI	9H-Pyrido[3,4-b]indole
20	32.700	400965	0.15	125544	0.45	3.19	MI	Oleic Acid
21	33.141	585888	0.21	163640	0.59	3.58	MI	Octadecanoic acid
22	34.148	485754	0.18	143543	0.52	3.38	MI	Verimol K
23	34.631	2163096	0.79	631626	2.28	3.42	MI	Pyrrolidine-2-carboxylic acid, 1- benzylaminothiocarbonyl-
24	41.929	831931	0.30	257611	0.93	3.23	MI	Imidazolidin-4-one, 5- benzyl-2-thioxo-3-p-tolyl-
		273572903	100.00	27725788	100.00			

8.4 EVALUATION OF FORMULATED CLOTRIMAZOLE CREAMS

8.4.1 ORGANOLEPTIC EVALUATION

Physical appearance like colour, odour, state and consistency of the following creams are given in table.

Table 16: Organoleptic evaluation.

SI. no	Parameters	C1	C2	C3	C4
1	Colour	Off White	Off White	Off White	Off White
2	Odour	Mild	Mild	Mild	Mild
3	State	Semisolid	Semisolid	Semisolid	Semisolid
4	Consistency	Smooth	Smooth	Smooth	Smooth

8.4.2 IRRITANCY

The prepared formulations **(C1, C2, C3, C4)** were applied to the skin of the hand. These formulations did not show any irritation, redness, oedema, or inflammation during the study, indicating that they are safe to use.

8.4.3 WASHABILITY

Cream formulations **(C1, C2, C3, C4)** were applied on the skin can be washable with water.

8.4.4 pH

The pH of creams **(C1, C2, C3, C4)** were measured by using digital pH meter.

Table 17: pH of creams.

Parameter	C1	C2	C3	C4
pH	6.2	5.7	6.10	5.5

According to the result, these creams are suitable for the skin's pH, so they can be safely used on the skin.

8.4.5 VISCOSITY TEST

Viscosity measurements were performed using a Brookfield viscometer at a speed of 5 rpm and a temperature of 25°C, with spindle No. 64. The viscosity of the prepared creams (**C1, C2, C3, C4**) was as follows:

Table 18: Viscosity of creams.

Parameter	C1	C2	C3	C4
Viscosity (Centipoise)	70320 cP	65250 cP	78420 cP	80220 cP

That indicates the formulation has the desired viscosity required for semi solid formulation for proper packaging.

8.4.6 PHASE SEPARATION

According to the observation no phase separation was observed in the cream formulations

(**C1, C2, C3, C4**).

8.4.7 SPREADABILITY

If the spreadability of a topical cream increase, it indicates that the cream is more effective, as it will be easier to apply to the skin. Ideally, a cream should spread smoothly without excessive drag, ensuring minimal friction during the rubbing process. To evaluate this characteristic, the parallel plate method was employed, and the average spreadability obtained is as follows:

Table 19: Spreadability of creams. This indicate the formulations show desired spreadability.

Formulation	Weight tied to upper slide(M)	Length of glass slide(L)	Time taken(T)	Average spreadability(S)
C1	20g	7.5cm	24 sec	6.2 g.cm/s
C2	20g	7.5cm	22 sec	6.8 g.cm/s
C3	20g	7.5cm	26 sec	5.7 g.cm/s
C4	20g	7.5cm	27 sec	5.5 g.cm/s

8.4.8 GREASINESS

Here each cream (**C1, C2, C3, C4**) was applied on the skin surface in the form of smear and checked if the smear was oily or grease-like. According to the result we can say that the cream formulations were greasy.

8.4.9 DYE SOLUBILITY TEST

Sudan III dye was mixed with each cream (**C1, C2, C3, C4**). Place a drop of the cream on a microscopic slide cover it with a cover slip, and examine it under microscope. The dispersed globule appears colourless in the red background i.e. W/O type cream.

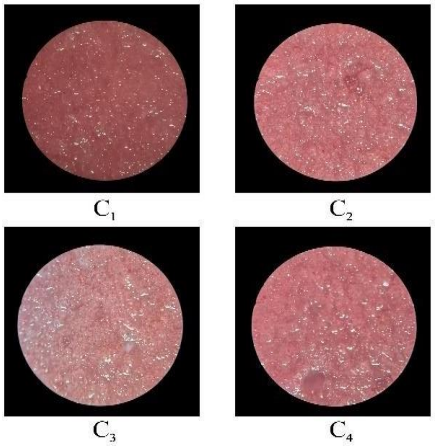


Figure 30: Microscopic examination of globules.

8.4.10 ANTI-FUNGAL ACTIVITY

The anti-fungal activity of the formulated clotrimazole creams (**C1, C2, C3, C4**) was evaluated using the agar well diffusion method against clinical *Candida albicans*. The results indicate that the fungal strain doesn't showed resistance to the formulated clotrimazole creams. A comparative study of the anti-fungal activity of the creams was also conducted.

Table 20: Anti-fungal activity of formulated creams (C1, C2, C3, C4) against clinical organism *Candida albicans*.

SAMPLE	ZONE OF INHIBITION (mm)
C1	24
C2	25
C3	25
C4	26

NOTE: Concentration of sample 10mg/mL in DMSO

Figure 31: Anti-fungal activity of formulated creams (C1, C2, C3, C4) against clinical organism *Candida albicans*.

8.4.11 ANTI-MICROBIAL ACTIVITY

The anti-microbial activity of the formulated clotrimazole creams (**C1, C2, C3, C4**) was evaluated using the agar well diffusion method against clinical *Staphylococcus aureus*. The results indicate that the microbial strain doesn't showed resistance to the formulated clotrimazole creams. A comparative study of the anti-microbial activity of the creams was also conducted.

Table 21: Anti-microbial activity of formulated creams (C1, C2, C3, C4) against clinical organism *Staphylococcus aureus*.

SAMPLE	ZONE OF INHIBITION (mm)
C1	21
C2	23
C3	23
C4	24

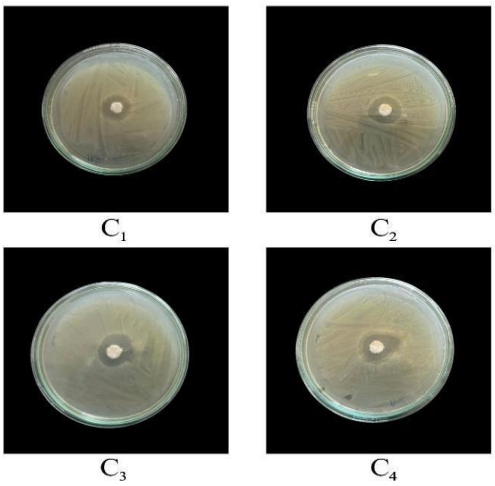


Figure 32: Anti-microbial activity of formulated creams (C1, C2, C3, C4) against clinical organism *Staphylococcus aureus*.

9. DISCUSSION

In this study, the anti-fungal and anti-microbial activities of different formulations of prepared clotrimazole creams were evaluated using the agar well diffusion method. The formulations tested included **C1** (Clotrimazole cream), **C2** (Clotrimazole + Chitosan cream), **C3** (Clotrimazole + Papaya seed extract cream), and **C4** (Clotrimazole + Chitosan + Papaya seed extract cream). The results were measured by the zone of inhibition (in mm) against *Candida albicans* for anti-fungal activity and *Staphylococcus aureus* for anti-microbial activity.

The anti-fungal test demonstrated that all formulations exhibited inhibitory effects against *Candida albicans*. The results indicated that the incorporation of Chitosan (**C2**) or Papaya seed extract (**C3**) into the clotrimazole formulation slightly improved its anti- fungal activity compared to the base clotrimazole cream (**C1**). Furthermore, the combination of both Chitosan and Papaya seed extract in C4 resulted in the largest zone of inhibition, suggesting a possible synergistic effect. The enhanced anti-fungal activity of **C4** could be attributed to the

combined properties of Chitosan and Papaya seed extract, which may have enhanced the penetration and efficacy of clotrimazole against fungal cells.

Similarly, the anti-microbial test against *Staphylococcus aureus* revealed that all formulations displayed anti-microbial activity. The results indicated that the addition of Chitosan and Papaya seed extract to clotrimazole enhanced its antimicrobial activity. The formulation **C4**, which contained both Chitosan and Papaya seed extract, exhibited the largest zone of inhibition, suggesting that the combination of these two ingredients with clotrimazole enhanced its ability to inhibit bacterial growth. The bioactive compounds in Papaya seed extract, along with Chitosan, likely contributed to the improved antimicrobial effect.

On the basis of the comparative analysis, the addition of Chitosan and Papaya seed extract to clotrimazole enhanced its effectiveness against both fungal and bacterial strains. In both anti-fungal and anti-microbial tests, the combination of these additives in formulation **C4** showed the greatest inhibition, indicating a synergistic effect that improved clotrimazole's efficacy against fungal and bacterial growth.

10. CONCLUSION

This study highlighted the significant enhancement of anti-fungal and anti-microbial activities in clotrimazole cream when combined with Chitosan and Papaya seed extract. The formulation **C4**, which included both Chitosan and Papaya seed extract, demonstrated the greatest zone of inhibition against *Candida albicans* and *Staphylococcus aureus*, suggesting a possible synergistic effect that improved the overall efficacy of the cream. Chitosan contributed to the improved formulation by enhancing the penetration of clotrimazole into fungal and bacterial cells. The inclusion of Chitosan in the formulation also contributed to better stability, controlled release, and protection against external contaminants, making the cream more effective and stable over time. Papaya seed extract, when incorporated into cream formulations, offered several skin benefits, including its ability to soothe irritation, promote healing, and support skin regeneration due to its rich content of bioactive compounds that enhanced skin health.

Given the increasing prevalence of fungal infections, particularly in individuals with weakened immune systems, the demand for more effective topical treatments had been rising. Clotrimazole remained a cornerstone in the treatment of fungal infections, especially against *Candida* species. However, the emergence of resistant strains called for new strategies to

improve its effectiveness. The addition of Chitosan and Papaya seed extract in clotrimazole formulations provided a promising approach. These biotic materials improved the penetration and bioavailability of clotrimazole, thereby enhancing its therapeutic outcomes.

Future research should focus on exploring the molecular mechanisms behind the synergistic effects observed, as well as conducting in vivo studies to evaluate the long- term safety, efficacy, and clinical applicability of these formulations. Additionally, investigating other natural compounds with antifungal and antimicrobial properties could further enhance the development of more comprehensive, effective treatments for fungal and bacterial infections, particularly in the face of growing antimicrobial resistance.

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