



Extraction And Microbial Evaluation Of Lemmongrass -Tulsi Gel

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➤ Abstract:

Background: Fungal infections, especially those caused by *Candida albicans*, are increasingly difficult to treat due to rising resistance to antifungal drugs. Natural remedies like lemongrass (*Cymbopogon citratus*), Tulsi (*Ocimum sanctum*) show as alternative treatments due to their antifungal properties by evaluating against *Candida albicans*.

Material and Methods: The study was conducted in (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) with the concentrations of 0.1%, 0.3%, and 0.5% of each extract on *Candida albicans* for 30 days. In vitro activity of (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) we have evaluated.

Results and Discussion: Based on the findings of the current study; The study assessed the antifungal efficacy of (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) extracts at concentrations of 0.1%, 0.3%, and 0.5% against *Candida albicans* over 30 days. Results showed that both extracts effectively inhibited fungal growth, with the highest concentration (0.5%) yielding the most significant inhibition. These findings highlight the potential of using these plant extracts as alternative treatments for *Candida albicans*. The formulation of the extracts from (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) was evaluated using the agar gel diffusion method, which demonstrated promising results. Additionally, In vivo evaluations were conducted at temperatures of 8 °C and 40 °C in a thermostatic isobath over periods of 7, 15 and 30 days.

Conclusion: The study concludes that (*Ocimum tenuiflorum*) and *Ocimum sanctum* extracts possess significant antifungal activity against *Candida albicans*, particularly at higher concentrations. The promising results obtained from the agar gel diffusion method, In vivo evaluations conducted at varying temperatures.

➤ Introduction:

Fungal infections, particularly those caused by *Candida albicans*, are becoming increasingly difficult to treat due to the rising resistance to antifungal medications(1). This challenge is especially prevalent in immunocompromised individuals, such as those with weakened immune systems due to conditions like HIV, cancer treatments, or organ transplants. *Candida albicans* is a common fungal pathogen that can cause a range of infections, from superficial infections like oral thrush to more severe systemic infections(2). The growing resistance to standard antifungal therapies has led to the need for alternative treatment approaches.

Natural plant-based remedies, such as lemongrass (*Cymbopogon citratus*) and Tulsi (*Ocimum sanctum*), have shown considerable promise due to their antifungal and antimicrobial properties (3). Lemongrass oil, known for its strong antifungal activity, contains active compounds such as citral, which is effective in fighting infections. Tulsi, or holy basil, is another medicinal plant with a long history of use in traditional medicine, particularly in India. It contains bioactive compounds like eugenol and ursolic acid, which contribute to its antimicrobial effects. Both lemongrass and Tulsi have been studied for their ability to combat a variety of pathogens, including fungi(4).

In this study, a polyherbal formulation that combines lemongrass oil and Tulsi is being evaluated for its antifungal efficacy against *Candida albicans*. The research focuses on assessing the phytochemical content of these plants and identifying the active compounds responsible for their antifungal properties. The effectiveness of this polyherbal formulation in combating *Candida albicans* is also being tested(5). The study aims to determine whether the combination of these natural remedies can provide a potent alternative treatment, especially in cases where conventional antifungal therapies are no longer effective due to resistance. According to the World Health Organization (WHO), herbal medicine plays a crucial role in healthcare, particularly in developing countries. It is estimated that more than two-thirds of the population in these regions rely on herbal remedies as part of their healthcare regimen. This highlights the importance of continuing research into the efficacy of plant-based treatments, as they can provide accessible and cost-effective alternatives to conventional medicines in many parts of the world(6).

The two plants that we have to study in this is that which shows many medicinal benefits is that lemongrass (*Cymbopogon citratus*) and Tulsi (*Ocimum Sanctum*)(7).

➤ Literature Survey:

- I. Aladejana EB October 2023 : Traditional and folkloric medicine has been a key part of healthcare globally, with polyherbal formulations gaining recognition for their medicinal potential due to their optimized therapeutic effects and low toxicity. This study reviewed 99 articles (2010–2022) on the antimicrobial, anti-inflammatory, antioxidant, and toxicological properties of polyherbal formulations using major scientific databases. India leads in publications on this topic, followed by Nigeria and Pakistan. Research validates the traditional use of polyherbal medicines, but further clinical studies are needed to fully explore their therapeutic potential. The growing interest in cost-effective, plant-based remedies underscores their role in drug discovery and the development of potent cures for human diseases.⁵
- II. Claudia Spampinato et.al 26 June 2013 : The genus *Candida* comprises approximately 200 species, although only a few are opportunistic pathogens in humans, causing infections when the host is debilitated or immunocompromised. *Candida* infections may be superficial or invasive. Superficial infections typically affect the skin or mucous membranes and can be effectively treated with topical antifungal drugs. In contrast, invasive fungal infections are often life-threatening, largely due to inefficient diagnostic methods and inadequate initial antifungal therapies. This review summarizes current knowledge about pathogenic *Candida* species and the causes of yeast infections, with a focus on existing antifungal drugs, resistance mechanisms, and emerging therapeutic alternatives for treating *Candida* infections.¹
- III. Moumita Malakar et.al 2024 : Basil (*Ocimum basilicum L.*), derived from the Greek word *basileus* meaning "king," is an aromatic plant in the Lamiaceae family, encompassing numerous herb and shrub species. Known as Tulsi in India and Nepal, it is widely used in folk medicine, Ayurveda, and culinary applications. This herb is rich in medicinal compounds with therapeutic potential, though many phytochemicals remain to be fully explored. Basil exhibits diverse bioactivities, including CNS depressant, anticancer, cardiac stimulant, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulatory, analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, chemomodulatory, and larvicidal effects. Its essential oil, extracted mainly from leaves, is a significant source of compounds like methyl eugenol (78.02%), α -cubebene, and nerol, contributing to 80% of patents involving *Ocimum* species. Advances in genome sequencing and metabolic profiling of this sacred herb could facilitate the development of nutraceuticals and novel drugs targeting emerging diseases.¹⁸
- IV. Roshan Kumar et.al 2022 : Plants have been used medicinally by humans for millennia, with Tulsi being one of the prominent examples. This aromatic plant, found throughout India, emits a distinctive fragrance and is often grown around Hindu temples and places of worship. Its leaves, seeds, and roots are widely used in Ayurvedic medicine. Tulsi is rich in

nutrients and bioactive compounds, with key chemical components including linalool, caryophyllene, oleanolic acid, and rosmarinic acid. Modern science has standardized Tulsi, identifying eugenol (essential oil) and ursolic acid (acidic compound) as its primary active ingredients. The leaves exhibit diaphoretic, anti-periodic, and anti-bronchitis properties. A decoction of the leaves can treat coughs, malaise, and colds, while also providing hydration. Additionally, the oils from the flowers are effective against ringworm. Due to its antibacterial and antifungal properties, *Ocimum sanctum* has been extensively studied in therapeutic trials. This article explores the chemistry and pharmacology of various *Ocimum* species.²⁸

- **BHINGE, Somnath D. et.al 2019** : In Ayurveda, *Azadirachta indica*, *Adhatoda vasica*, *Piper betle*, *Ocimum tenuiflorum*, and *Pongamia pinnata* are recognized for treating infectious diseases. This study formulated and evaluated a polyherbal gel containing ethanolic extracts of these plants in three concentrations (0.1%, 0.3%, and 0.5%) across formulations A, B, and C. The gels were assessed for physical properties, skin irritation, and antimicrobial activity against *S. aureus*, *B. subtilis*, *A. niger*, and *E. coli*. All formulations showed significant antimicrobial activity, with formulation C demonstrating the highest efficacy, attributed to its higher extract concentration. The results suggest that the polyherbal gel's antimicrobial properties are due to the synergistic effects of its plant constituents⁶³

➤ **History:**

Lemongrass, a perennial plant commonly grown in the subtropics and tropics, refers to two different species: West Indian lemongrass (*Cymbopogon citratus*) and East Indian lemongrass (*Cymbopogon flexuosus*)(8). Various species of lemongrass are native to Southeast Asia, South Asia, and Australia, which is why it is called tropical Asian East Indian lemongrass. East Indian lemongrass (*Cymbopogon flexuosus*), also known as Cochin or Malabar grass, is native to Sri Lanka, India, Thailand, and Burma. West Indian lemongrass (*Cymbopogon citratus*) is also widely cultivated today. In the Philippines, lemongrass was distilled for export as early as the 17th century. Closely related citronella oil was first showcased at the World's Fair in London's Crystal Palace in 1851. In India, lemongrass oil, locally known as "choomana polu," has been popular for many years and refers to the red grass stem. Indigenous Australians used citrus fruits to make a drink and for washing skin cuts and eyes(9). Lemongrass is commercially cultivated in India, Guatemala, Paraguay, China, Sri Lanka, England, and other parts of Africa, Indochina, South America, and Central America. The plants grow in dense clumps, reaching up to 2 meters in diameter, with leaves as long as 1 meter. This genus is native to South Asia, Australia, and Southeast Asia(10).

LEMONGRASS:

I. Introduction of Lemongrass:

Lemongrass, popularly known as citronella grass is a member of the Poaceae family and belongs to the genus *Cymbopogon*. The genus *Cymbopogon* comprises approximately 140 species that are widely distributed across the semi-temperate and tropical regions of Asia, the Americas, and Africa. In contrast, only a few species of lemongrass are found in Australia and Europe(11). Commonly referred to as "Squinant" in English, lemongrass is known by various other colloquial names around the world. Members of the *Cymbopogon* genus produce volatile oils, which is why they are also referred to as aromatic grasses.



Fig No 4.1: Lemongrass Plant.

The strong lemon fragrance, a characteristic feature of lemongrass, is attributed to the high citral content in its essential oil(12). This distinct aroma makes the oil a popular ingredient in soaps, detergents, and other fragrant products. Due to its rich citral content, lemongrass is also widely used in the perfumery and food industries. Additionally, it serves as a starting material for the production of ionones, which are essential compounds in the synthesis of Vitamin A(13).

II. Taxonomical and Botanical Description:

Lemongrass is a perennial monocotyledonous grass that can grow up to 6 feet in height and 4 feet in width, typically growing in clusters(14). It features long, slender, drooping, bright green leaves that range from 1.3 to 2.5 cm in width and can reach up to 3 feet in length. The leaves are simple, with entire margins. The flowers grow on spikes, with a long inflorescence that measures between 30 and 60 cm. This floral arrangement gives the grass its name, *Cymbopogon*. *Cymbopogon citratus* is commonly found in Southeast Asia(15).

<u>Taxonomic details of this herb are as follows:</u> Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	Cymbopogon
Species	Citrates

Table No.4.1:Taxonomical Description.**III. Morphological description of *Cymbopogon citratus*:**

PARTS	DESCRIPTION
Leaves	The strap-like leaves are 0.5-1 in (1.3-2.5cm) wide, about 3 ft (0.9 m) long and have gracefully drooping tips. The evergreen leaves are bright bluishgreen and release a citrus aroma. When crushed leaf arrangement: most emerge from the soil, usually without a stem Leaf Type: simple Leaf Margin: Entire Leaf shape: Linear Leaf Venation: Parallel Leaf type and Persistence: Fragrant Leaf blade Length: 18-36 Leaf colour: Green Fall Characteristic: Showy
Leaf Type: simple	The lemongrass plants that you are likely to encounter are cultivars and do not typically produce flowers.
Leaf Margin: Entire	Inflorescences are 30-60-cm-long and nodding the partial inflorescence are paired racemes of spikelets subtended by spathes

Table No.4.2: Morphological description of *Cymbopogon citutus*.

IV. Chemical Composition:

Composition of essential oil in *Cymbopogon citratus*[25–28]

Essential Oil Composition	Percentage Of Components
Citral α	40.8
Citral β	32
Nerol	4.18
Geraniol	3.04
Citronellal	2.10
Terpinolene	1.23
Geranyl acetate	0.83
Myrecene	0.72
Terpinol	0.45
Methylheptenone	0.2
Borneol	0.1-0.4
Linalyl acetate	0.1
α Pinene	0.07
B Pinene	0.4
Limonene	Traces
B-caryophyllene	Traces

Table No.4.3: Nutritional Content of Lemongrass.

V. Nutritional component:

S. No.	Mineral	Quantity (mg/100g)
1.	Na	54.8
2.	K	59.5
3.	Ca	39.5
4.	Mg	70
5.	Fe	0.024
6.	Mn	0.952
7.	Zn	121
8.	P	89.3
9.	Phytate	11860

Table No.4.4: Mineral Content of Lemongrass.

TULSI:

I. Introduction of Tulsi :



Fig No.5.1: Tulsi Plant.

Plants are one of the most important sources of medicine, and a large number of drugs currently in use are derived from plants(16). The therapeutic uses of plants are considered safe, economical, and effective due to their ease of availability. Among plants known for their medicinal value, those of the genus *Ocimum*, belonging to the family Lamiaceae, are highly regarded for their therapeutic potential(17). *Ocimum sanctum*, commonly known as Tulsi, has two varieties: black (Krishna Tulsi) and green (Rama Tulsi), both of which have similar chemical constituents. *Ocimum sanctum* is widely distributed across the Indian subcontinent, extending up to altitudes of 1800 meters in the Himalayas and reaching as far as the Andaman and Nicobar Islands(18). The word "Tulsi" in Sanskrit means "the incomparable one" and holds a special place in Hindu culture. Numerous medicinal properties have been attributed to the Tulsi plant, not only in Ayurveda and Siddha but also in Greek, Roman, and Unani systems of medicine(19). *Ocimum sanctum* Linn., popularly known as holy basil or Tulsi in India, is a traditional home remedy for various illnesses(20). Historically, the fresh fruit and leaf juice have been commonly used to treat cough as a demulcent, mild upper respiratory tract infections, general stress syndrome, worm infestations, superficial fungal infections, and as a diuretic. This plant has been pharmacologically evaluated for its immunomodulatory, antistress, antimicrobial, anti-inflammatory, antiasthmatic, hypoglycemic, hypotensive, and analgesic activities, and has shown varying degrees of effectiveness in animal models(21).

The phytoconstituents isolated from various parts of the *Ocimum sanctum* plant include eugenol, cardinene, cubenol, borneol, linoleic acid, linolenic acid, oleic acid, palmitic acid, stearic acid, vallinin, vicenin, vitexin, vallininic acid, orientin, circineol, gallic acid, vitamin A, vitamin C, phosphorus, and iron . *Ocimum sanctum* is a plant known for its wide range of medicinal properties, including analgesic, anti-ulcer, anti-arthritis, immunomodulatory, antiasthmatic, antifertility, anticancer, anticonvulsant, antidiabetic, antihyperlipidemic,

anti-inflammatory, antioxidant, antistress, as well as memory-enhancing and neuroprotective activities. This review article summarizes the various phytoconstituents present in *Ocimum sanctum* and lists its numerous biological activities(22).

Many plants are used to manufacture medications, with medicinal plant secondary metabolites and therapeutic oils being well known(23). Medicinal plants offer several advantages over pharmaceuticals, including lower costs, higher efficacy, and greater global accessibility(24). The use of plants for medicinal purposes has a long history; for instance, the Chinese utilized herbs as medicine as early as 4000-5000 B.C. The Rigveda, dating from 3500 to 1600 B.C., is one of the earliest texts to record the use of plants for medicinal purposes. Additionally, the medicinal properties of plants have been extensively studied in the context of Ayurveda(25).

Tulsi is considered sacred in India. Known scientifically as *Ocimum sanctum*, it is an aromatic plant used in Ayurveda and belongs to the Labiateae family. This plant is cultivated in several tropical and subtropical countries and can be found in almost every field across India. Tulsi is a tall, slender, and fragrant plant, and its name means "incomparable" in Sanskrit. The entire plant is recognized for its medicinal properties(26).

In India, Tulsi is classified into two varieties: dark or Shyama (Krishna) and light or Rama. Other related species found in India include *O. canum*, *O. basilicum*, *O. kilimandscharicum*, *O. americanum*, *O. camphora*, and *O. micranthum*. Toxicological studies have indicated that this plant possesses analgesic and antibacterial properties. Tulsi is also regarded as a valuable source of vitamins for animals(27).

The essential oil contains several compounds, including carvacrol, methylchavicol, limatol, and caryophyllene. The seeds are rich in fatty acids and sitosterols, while the roots contain sitosterol and triterpenes A-C. N-Triacontanol is found in the leaves. The oil also includes caryophyllene, terpinene, 4-decylaldehydes, selinene, pinenes, and camphene, as well as nerol. Additionally, thymol and citral are among the chemical components present(28).

II. SOURCES:

Biological Source:

The Lamiaceae family includes *Ocimum* species, such as *Ocimum sanctum* L. and *Ocimum basilicum*. To prepare Tulsi, both fresh and dried leaves from these plants are used(29).

The origin of the location:

The plant can be found growing from the Andaman and Nicobar Islands to the Himalayas, at elevations of up to 1,800 feet above sea level. It is also present in countries such as Malaysia, Australia, West Africa, and several Arab nations(30). In India, this multi-branched, herbaceous annual plant is treasured by Hindus for its therapeutic virtues. It is popular among both gardeners and temple-goers and is propagated from seeds. The volatile oil extracted from Tulsi is now being cultivated for use in commercial products(31).

III. CLASSIFICATION:

In the taxonomic classification of plants, there are kingdoms, sub-kingdoms, superdivisions, divisions, and phyla. As a result, Tulsi, also known as the "Queen of Herbs," has a specific designation. Its genus and species are clearly identified in its binomial nomenclature. Tulsi belongs to the Lamiales order and the Lamiaceae family(32). Here is a brief classification:

IV. Taxonomical Classification of Tulsi Kingdom:

Taxonomic details of this herb are as follows:

Kingdom	Plantae
Sub kingdom	Tracheobionta
Superdivision	Spermatopta
Division	Magnoliophyta
Class	Magnolipsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Species	O. Sanctum

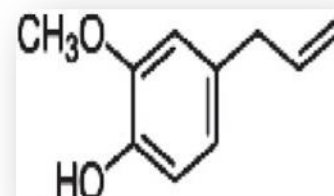
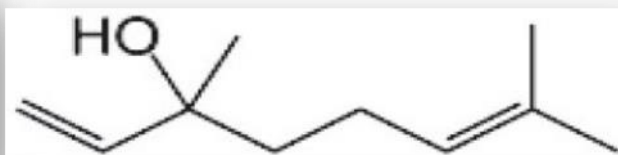
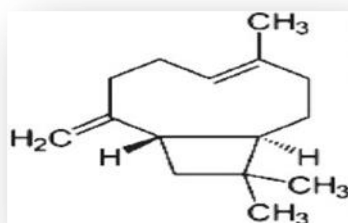
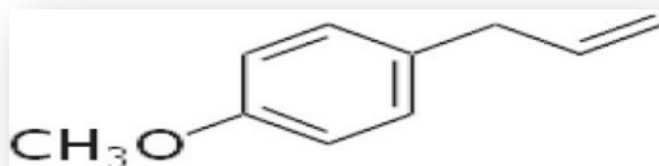
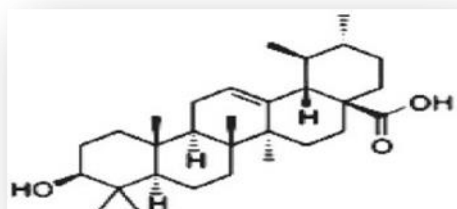
Table No.5.5: Taxonomical detail of Tulsi.

V. Chemical constituents:

Tulsi's chemical composition is highly complex, containing numerous nutrients and biologically active compounds. The proportions of these compounds can vary significantly between different strains and even among plants within the same field. Additionally, the quantities of many of these constituents are greatly influenced by variations in growing, harvesting, processing, and storage conditions, which are not yet fully understood(33).

The nutritional and pharmacological properties of the whole Tulsi herb, as traditionally used in its natural form, result from the synergistic interactions of numerous active phytochemicals. Consequently, the overall effects of Tulsi cannot be fully replicated with isolated compounds or extracts(34). Due to its inherent botanical and biochemical complexity, Tulsi standardization has thus far eluded modern science. The volatile oil from the leaves contains eugenol (1-hydroxy-2-methoxy-4-allylbenzene), euginal (also known as eugenic acid), ursolic acid (2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid), carvacrol (5-isopropyl-2-methylphenol), linalool (3,7-dimethylocta-1,6-dien-3-ol), limatrol, caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene), and methyl chavicol (also called estragole: 1-allyl-4-methoxybenzene)(35).

The seed volatile oil contains fatty acids and sitosterol, while the seed mucilage contains sugars, and anthocyanins are present in the green leaves. The sugars consist of xylose and polysaccharides(36).



Although Tulsi is known as a general vitalizer and enhances physical endurance, it contains no caffeine or other stimulants. The stem and leaves of holy basil contain various constituents that may have biological activity, including saponins, flavonoids, triterpenoids, and tannins(37). Additionally, several phenolic compounds with antioxidant and anti-inflammatory properties have been identified, such as rosmarinic acid ((2R)-2-[[[(2E)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]] oxy]-3-(3,4-dihydroxyphenyl) propanoic acid), apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone), isothymusin (6,7-dimethoxy-5,8,4'-trihydroxyflavone), and isothymonin. Two water-soluble flavonoids, orientin (8-C-beta-glucopyranosyl-3',4',5,7-tetrahydroxyflav-2-en-3-one) and vicenin (6-C-beta-D-xylopyranosyl-8-C-beta-D-glucopyranosyl apigenin), have been shown to protect human blood lymphocytes from radiation-induced chromosomal damage(38).

VI. Nutritional components :

Contents	Quantity
Carbohydrate	2.3gm
Calcium	25mg
Carotene	2.5µg
Chromium	2.9µg
Copper	0.4µg
Fat	0.5mg
Iron	15.1mg
Nickel	0.73µg
Phosphorus	287mg

Table No. 5.6: Nutritional Components.

VII. Advantages:

Tulsi offers several advantages due to its phytoconstituents. Apigenin is known for its neurogenesis-stimulating activity. Bisabolol possesses multiple benefits, including wound healing, anti-inflammatory, antimicrobial, anti-irritant, and anti-cancer properties. Borneol contributes insect repellent activity, thanks to the essential oils in Tulsi. Caffeic acid exhibits anti-cancer, antioxidant, and antifungal activities. The presence of calamine provides dermatological benefits. Additionally, Tulsi is utilized as an anticoagulant, in sunscreen formulations, and for medicinal diagnostics due to the presence of esculetin(39).

VIII. Disadvantages:

The excessive consumption of Tulsi and its products can negatively impact the reproductive health of both males and females. This may lead to decreased testicular weight, sperm mortality, and reduced sperm count. Additionally, Tulsi exhibits antiestrogenic activity, which can cause contractions and potentially lead to abortion in pregnant women. It can also affect lactation. Women may experience side effects such as cramps, backache, diarrhea, and bleeding. Scientific studies have identified various acute side effects associated with Tulsi(40). Extraction:

I. Extraction of Lemongrass :

CLAVENGER APPARATUS:



Fig No 6.1:CLAVENGER APPRATUS (Assembly of Lemongrass).

II. Parts of clavenger apparatus:

1. Heating mantle
2. Round bottom flask
3. Condenser
4. Clevenger part
5. Water inlet and water outlet (10)

Essential oils are extracted from various plant materials, including flowers, herbs, and trees. These oils contain a mixture of chemical compounds, primarily terpenes associated with aldehydes, alcohols, and ketones. Citronella oil, derived from lemongrass, is used not only in the manufacture of perfumes, soaps, cosmetics, and detergents but also in the pharmaceutical industry. The extraction of this oil is considered a clean technology. Lemongrass contains 1-2% essential oil on a dry weight basis. Traditional methods of extraction, such as steam and hydro distillation, are time-consuming. However, an innovative method called Microwave-Assisted Hydrodistillationessential (MAHD) significantly reduces extraction time while preserving the quality of the oil(41).

In solvent extraction, a hydrocarbon solvent (usually n-hexane) is added to the plant material to dissolve the essential oil. After filtering the solution and concentrating it by distillation, a substance containing resin (resinoid) or a combination of wax and essential oil remains. This method is quite efficient for extracting lemongrass essential oil (Schoenberg & Khan, 2002) and is relatively simple, but it generally requires high volumes of solvent and may yield unsatisfactory reproducibility. After the extraction process, the sample is concentrated by evaporation, which can reduce volatiles. Additionally, there is a risk of contaminating the essential oil with solvent residues(42).



Fig.No.6.2: Extraction of lemongrass oil.

The Soxhlet apparatus is sometimes used for the solvent extraction of lemongrass essential oil (Alhassan et al., 2018). In Soxhlet extraction, the plant material is in continuous contact with the refluxing liquid phase, resulting in increased extraction efficiency(43). However, a significant drawback of Soxhlet extraction compared to other conventional methods is the long heating period at high temperatures (usually close to the boiling point of the solvent), which may lead to the thermal degradation of delicate compounds. Both solvent extraction by maceration and Soxhlet extraction require careful selection of the solvent to achieve a good extraction yield and to prevent the loss of volatiles(44).

In recent years, lemongrass essential oil has been successfully extracted from dry and fresh lemongrass leaves using solvent extraction techniques by Suryawanshi et al. (2016) and Alhassan et al. (2018). The oil yield obtained by Suryawanshi's group was 1.85%, while Alhassan's group achieved a yield of 4.5%, with n-hexane used as the solvent in both experiments(45). Furthermore, Schaneberg and Khan (2002) reported that

sonication-assisted n-hexane extraction produced lemongrass essential oil with comparable levels of the main compounds to those obtained by steam distillation(46).

Currently, steam distillation is the most popular method for extracting lemongrass essential oil. This method is primarily used for temperature-sensitive materials such as oils, resins, and hydrocarbons, which are insoluble in water and may decompose at their boiling points. It is also commonly employed to extract other essential oils (47)(Fernandes et al., 2019).

In steam distillation, steam passes through the dried or fresh plant material, softening the cells and allowing the essential oil to escape in vapor form. The steam temperature should be sufficiently high to vaporize the oil, but not so high that it damages the plant material or burns the essential oils(48). The released essential oil, along with steam molecules, is subsequently cooled in a condenser and collected. This process allows for the extraction to occur below the boiling points of the individual components. Essential oils consist of chemical compounds whose boiling points often exceed 200°C (Berk, 2013)(49). In the presence of steam, these substances can be extracted at temperatures close to 100°C under atmospheric pressure. The primary advantages of steam distillation are its simplicity and the low cost of the apparatus. The properties of the essential oil remain unchanged, as its constituents do not decompose(50). This technique can also be performed under pressure. Lemongrass can be distilled fresh or after wilting; wilting the plant material before distillation reduces moisture content and increases oil yield (Skaria et al., 2012). Literature reports on the yields of essential oil obtained using this method vary significantly, ranging from 0.24% (Anggraeni et al., 2018) to 0.3% (Santin et al., 2009), 0.6% (Boukhatem et al., 2014a), and 0.71% (Kpoviessi et al., 2014). Steam distillation remains the leading technique for extracting lemongrass oil. Although newer methodologies, such as subcritical carbon dioxide extraction, are promising, they are more expensive and have not yet surpassed this simple distillation method(51).

In hydrodistillation (HD), the plant material is completely submerged in water, which is boiled using direct heat. The essential oil distills alongside the water molecules and is separated after condensation. This method is relatively straightforward and does not involve high setup costs(52). However, the process is slow, which may lead to the degradation of some compounds through hydrolysis or polymerization reactions due to prolonged heat exposure. The yield depends on various factors, including the size and weight of the raw material, the nature of the herb, and the volume of water used. A typical HD of lemongrass is conducted in a Clevenger-type apparatus for three hours, following the method recommended by Guenther in 1950 (Guenther, 1950). Recent reports indicate that the yields of essential oil obtained via HD range between 0.43% (Marongiu et al., 2006) and 1.80% (Desai & Parikh, 2015). Ajayi et al. (2016) demonstrated that the composition of the essential oil extracted from lemongrass leaves by HD varies significantly when using neutral, weakly acidic, or basic mediums. The total yield of volatile fractions was 0.73% for HD, 0.64% for microwave-assisted hydrodistillation (MAHD), 0.7% for acid-distillation, and 0.45% for base-distillation(53). The main component in the obtained essential oil was citral, with contents of 72.6% for HD, 44.7% for MAHD, 30.07% for acid-distillation, and 78.61% for base-distillation. Significant differences were observed in the contents of major components (geranial, neral, myrcene) as well. The low concentration

of citral in the essential oil distilled in acidic conditions resulted from chemical transformations that can occur in an acidic solution (Ajayi et al., 2016). Citral contains an aldehyde moiety that can react with the hydroxyl group of other terpenoids in the presence of acid molecules, forming acetals(54). Other reactions, such as polymerization, disproportionation, and cyclization, are also likely. A basic medium provides the best environment for the extraction of lemongrass essential oil with a high citral (55).

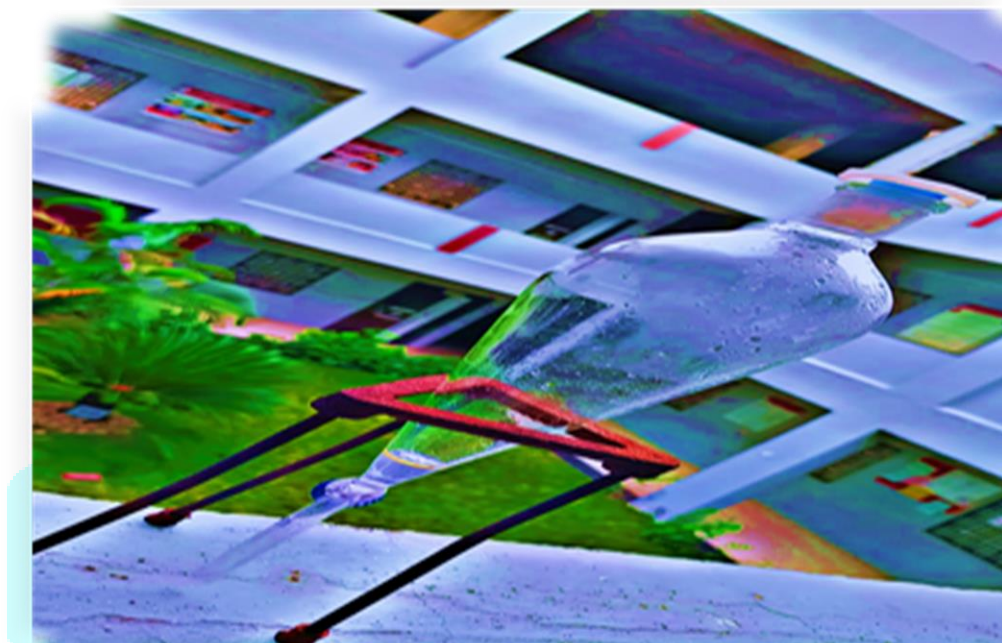


Fig.No.6.3: Extracted Oil from Lemongrass.

➤ Extraction of Eugenol from Tulsi:

The extraction process has recently been employed commercially, both in large-scale industrial applications and smaller-scale laboratory procedures. It is now considered an essential unit operation in the advanced toolkit of modern chemical engineering(56). Extraction is fundamentally a process of separating one or more components from a mixture based on their physical and chemical properties. For example, acetic acid can be removed from a water solution either through distillation or extraction, using organic solvents such as isopropyl ether (Geankoplis, C.J., 2003)(57). Additionally, herbal plant extracts have recently gained significant commercialization, particularly in the food, pharmaceutical, and cosmetic industries (Wang & Weller, C.L., 2006). In the pharmaceutical industry, products like penicillin, which are produced in complex fermentation mixtures, can be effectively separated through liquid extraction(58).

A method that has gained significant commercial popularity nowadays is ultrasonic extraction, which is one of the easiest ways to naturally obtain pure compound extracts. Additionally, there are various extraction techniques available, including Soxhlet extraction (Soxtec),(59) ultrasonic-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, and many others. The choice of method depends on factors such as relative cost, benefits, environmental impact, and the specific parameters used(60).

The medicinal plants used for the experiment included the leaves of *Ocimum sanctum* (Tulsi). The plant parts were identified based on various sources, including Medicinal Plants of Nepal by HMG/N (1976) and other relevant taxonomic literature(61).

I. Soxhlet apparatus :



Fig.No.7.1: Soxhlet Apparatus(Extraction).

Five grams of plant powder were placed in the thimble of a Soxhlet apparatus. The apparatus was fitted with a round-bottom flask containing 250 ml of absolute ethanol, while the upper part was connected to a condenser. A Mantox heater provided

constant heat for solvent recycling. After the extraction was complete, the extract in the round-bottom flask was transferred into clean, pre-weighed universal tubes. The tubes containing the extracts were weighed, and

the weights were recorded. Finally, the percentage yield was calculated by dividing the final weight of the extract by the initial weight of the raw material(6

II. Extract extracted from Tulsi:



Fig.No.7.2:Extract of tulsi.

Material and Methods:

I. Preparation of polyherbal gel:

Formulations A, B, and C were prepared, each comprising ethanolic extracts of, *Ocimum tenuiflorum*, and *ocimum sanctum* in concentrations of 0.1%, 0.3%, and 0.5%, respectively, as shown in Table 8.1. To prepare 100 g of gel, Carbapol-940, propylene glycol 400, ethanol, methyl paraben, propyl paraben, EDTA, and triethanolamine were used, along with a sufficient quantity of distilled water.

The water required for these formulations was divided into two parts. In the first part, the exact amount of the extract was dissolved, and the calculated quantities of propylene glycol 400 and ethanol were added. In the second part, Carbapol-940 was dissolved, followed by the addition of methyl paraben, propyl paraben, and EDTA. Both solutions were then mixed in a beaker, and triethanolamine was added dropwise to the mixture to achieve gel consistency. The mixture was stirred using a propeller at 500 rpm for 2 hours to obtain a homogeneous gel, free from any entrapped air bubbles.

Various formulation batches were prepared as outlined in Table 8.1. A gel base was also	Qty.Taken in % for gel base	Qty. Taken in % for formulation A	Qty. Taken in % for formulation B	Qty taken in %for Formulation C	Role

prepared by excluding the plant extracts. The prepared gel formulations and base were left at room temperature for 24 hours.					
Ingredient					
Carbopol 90	1.0	1.5	1.0	1.0	Gelling agent
Extract of each drug	-	0.1	0.3	0.5	Antifungal agent
Propylene glycol 400	4.0	4.0	4.0	4.0	Base
Ethanol	3.0	3.0	3.0	3.0	Solvent
Methyl paraben	0.2	0.2	0.2	0.2	Preservatives
Propyl paraben	0.02	0.02	0.02	0.02	Preservatives
EDTA	0.03	0.03	0.03	0.03	Chelating agent
Triethanolamine	1.2	1.2	1.2	1.2	PH advancement
Water	Q.S(100ml)	Q.S(100ml)	Q.S(100ml)	Q.S(100ml)	Base

Table No.8.1:Formulation Table.



Fig.No.8.2: Formulation A.



Fig.No.8.3 :Formulation B.



Fig.No.8.4:Prepared herbal Gel.

➤ **EVALUATION OF POLYHERBAL GEL:**

I. Physical analysis of the prepared polyherbal gel:

The freshly prepared formulations were observed to range from off-white to yellow in color . Regarding the base and formulations, A, B, and C, no changes in color, odor, or appearance were noted throughout the 30-day observation period(66).

Appearance and Homogeneity:

The prepared gels and the control (base) were evaluated for physical appearance and homogeneity through visual observation .

II. pH Measurement:

A digital pH meter (Digital PH meter

) was used to determine the pH of the prepared formulations and the control (base) . Three grams of gel were accurately weighed, dispersed in 30 mL of distilled water, and stored for two hours before the pH was measured separately . The pH measurement for each formulation was performed in triplicate, and the average values are presented in (67).

III. Viscosity and rheological studies:

Brookfield viscometer instrument was used to determine the rheological characteristics of control (base) and Formulations at 25o C . The measurement was made over the whole range of speed settings from 10 rpm to 100 rpm with 30 seconds between two successive speeds and then in a descending order [68].

IV. Spreadability :

A special apparatus, as suggested by Mutimer et al. (1956), was designed to determine the spreadability of the prepared gel formulations . An excess amount of gel sample was placed between two glass slides, and a 1000 mg weight was applied for 5 minutes to compress the sample to a uniform thickness . A weight of 50 g was then added to the pan. The time required for the two slides to separate was recorded as a measure of spreadability (69). The shorter the separation time, the better the spreadability. Spreadability (S) was calculated using the formula:

$$S = M.L/t$$

where M is the weight (g) tied to the upper glass slide, L is the length (cm) moved on the glass slide, and t is the time (seconds) taken for the slides to separate.

V. Washability:

The formulations were applied to the skin, and the ease and extent of washing with water were manually determined (70).

Parameter	8°C	40°C	8°C	40°C	8°C	40°C
Appearance Formulation and B	A Semisolid B Semisolid	A Semisolid B Semisolid	A Semisolid B Semisolid	A Semisolid B Semisolid	A Semisolid B Semisolid	B Semisolid Liquid
Color Formulation A and B	A White B Pale Yellow	A White B Pale Yellow	A White B Pale Yellow	A White B Pale Yellow	A White B Pale Yellow	A White B Pale Yellow
Odure Formulation A and B	A Characteristics B Characteristics	A Characteristics B Characteristics	A Characteristics B Characteristics	A Characteristics B Characteristics	A Characteristics B Characteristics	A Character istics B Character istics
PH Formulation A and B	A)6.63 ± 0.22 B)6.90 ± 0.17	A)6.91 ± 0.17 B)6.91 ± 0.19	A)6.87 ± 0.91 B)6.80 ± 0.35	A)6.93 ± 0.23 B)6.82 ± 0.54	A)6.80 ± 0.38 B)6.91 ± 0.42	A)7.08 ± 0.14 B)6.81 ± 0.98
Viscosity Formulation A and B	A)7968 ± 3.92 B)7939 ± 2.67	A)7962 ± 2.33 B)7934 ± 3.65	A)7958 ± 3.50 B)7958 ± 4.01	A)7952 ± 4.01 B)7918 ± 3.65	A)7945 ± 2.90 B)7910 ± 4.06	A)7939 ± 3.09 B)7903 ± 3.56
Spreadability Formulation A and B	A)8.4 ± 0.01 B)9.8 ± 0.02	A)8.4 ± 0.04 B)9.8 ± 0.03	A)8.3 ± 0.09 B)9.8 ± 0.08	A)8.4 ± 0.01 B)9.7 ± 0.06	A)8.3 ± 0.06 B)9.8 ± 0.08	A)8.3 ± 0.10 B)9.7 ± 0.07

Table No.9.1:Evaluation of polyherbal gel.

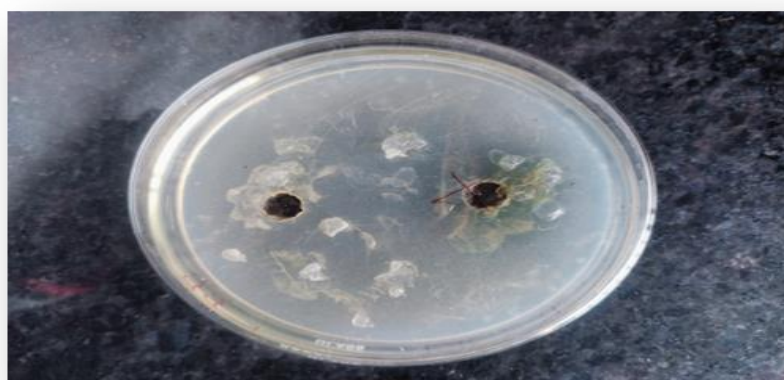


Fig.No.9.1:A. Zone of inhibition for formulation A&B Against E.coli.

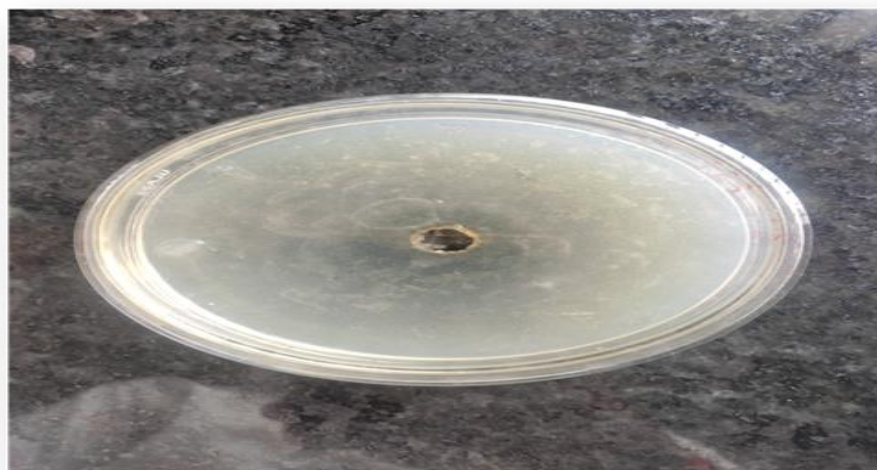


Fig.No. 9.2:B.Zone of inhibition for formulation A+B Against E.coli.

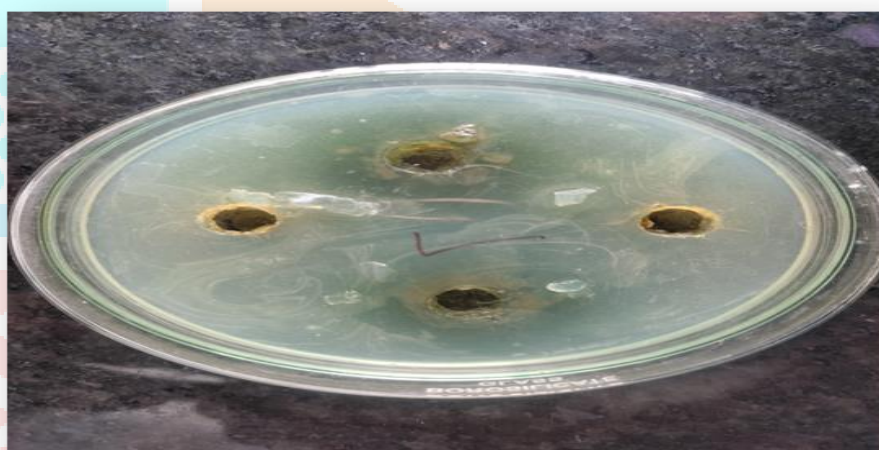


Fig.No. 9.3:C:Zone of inhibition for formulation A&B Against E.coli.& S.Aureus.

VI. Antifungal activity:

The antifungal activity of *Ocimum sanctum* was demonstrated by methyl chavicol and linalool, which were obtained from its essential oil. These compounds showed significant antifungal effects against *Candida*, including azole-resistant strains. Their fungicidal action was attributed to the formation of extensive lesions on the plasma membrane and a marked reduction in ergosterol levels. The antifungal activity of *Ocimum sanctum* leaves was also tested against clinically isolated dermatophytes. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of various extracts and fractions of *Ocimum sanctum* leaves were determined against the dermatophytic fungi tested.

The antifungal activity of lemongrass essential oil (LEO) has been reported against multiple fungal species. Volatile compounds in LEO, such as phenols, flavonoids, and flavones, have been shown to be effective

against numerous fungal strains. Helal et al. reported that LEO disrupts the plasma membrane and disorganizes mitochondria, resulting in the leakage of ions such as Ca^{2+} , K^{+} , and Mg^{2+} . This ion loss can impair signal transduction and fungal germination. Additionally, Alviano et al. observed that LEO components reduce cell size and inhibit spore germination in *Candida albicans*. Due to its volatile and lipophilic nature, LEO can directly interact with the fungal lipid bilayer, forming a charge-transfer complex that destabilizes the membrane, inhibits further membrane synthesis, and impedes fungal spore formation and cellular respiration. Boukhatem et al. found that the vapor form of LEO is more effective at inhibiting fungal growth and development than the liquid phase, likely because LEO vapors directly accumulate on fungal mycelium. Table 3 presents the minimum inhibitory concentration (MIC) values of essential oils from different *Cymbopogon* species against various fungal strains.

Lemongrass essential oil (LEO) contains components such as citral, geraniol, myrcene, limonene, and linalool, which exhibit significant antifungal activity. Geraniol increases the rate of potassium ion leakage, while citral damages microtubules and demonstrates cytotoxicity in fungi. Linalool, a monoterpene alcohol, possesses numerous fungicidal properties, inhibiting the overall development and spread of various fungi by restricting the respiration of their aerial mycelia. Additionally, other aldehydes in LEO contribute to its antifungal activity by forming cross-linkages within the fungal membrane.

Essential oils (EOs) can remain effective against fungal spore production for extended periods, thereby improving the shelf life of food products. It has also been suggested that lemongrass oil induces the production of reactive oxygen species (ROS) in fungi, causing severe oxidative damage that ultimately leads to cell death. This makes EOs a sustainable alternative for use in the food preservation and packaging industries. Edible coatings of EOs, including LEO, applied to stored fruits, meat, and dairy products, can prevent fungal attacks and spoilage by inhibiting fungal growth and reproduction. These edible coatings have greater antimicrobial potential than free EOs due to their modified surface charge and enhanced action on multiple targets within the fungal membrane. This helps extend the edibility and maintain the physicochemical qualities, including the taste and aroma, of such products.

However, since different EOs operate through various antimicrobial mechanisms, microbes are likely to develop resistance to the wide array of compounds found.

Results:

The study thoroughly assessed the antifungal efficacy of (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) extracts at concentrations of 0.1%, 0.3%, and 0.5% against *Candida albicans* over a comprehensive duration of 30 days. The experimental results demonstrated that both extracts were effective in inhibiting fungal growth across all tested concentrations. However, the highest concentration of 0.5% yielded the most significant inhibition of *Candida albicans*, indicating a clear dose-response relationship. The formulation of the extracts from (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) was evaluated using the agar gel diffusion method, which showed promising results with a distinct zone of inhibition around the wells containing the

plant extracts. Additionally, in vivo evaluations were conducted to assess the effectiveness of the extracts under physiological conditions at varying temperatures of 8 °C and 40 °C in an isothermal water bath over periods of 15 and 30 days. The favorable outcomes observed during these evaluations suggested that the extracts maintained their antifungal efficacy even when subjected to different temperature ranges.

➤ Discussion:

The substantial antifungal activity of (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) extracts suggests that these plants contain potent bioactive compounds capable of targeting and disrupting the growth and proliferation of *Candida albicans*. The agar gel diffusion method not only confirmed the antifungal properties of the extracts but also provided a straightforward approach for screening other potential therapeutic agents in future research. The study emphasizes the growing importance of plant-based remedies as accessible and cost-effective antifungal options, particularly in regions facing increasing issues of antifungal resistance. As traditional antifungal therapies become less effective due to resistance mechanisms, integrating natural alternatives like (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) into treatment protocols could provide significant benefits. This research lays the groundwork for further exploration into the pharmacological properties of these herbs and highlights the need for additional research to fully understand their mechanisms of action, optimal dosages, and potential synergies with conventional antifungal medications. By expanding the range of available treatments, we can enhance patient care and address the critical challenge of fungal infections in modern medicine.

Conclusion:

The study concludes that (*Ocimum tenuiflorum*) (commonly known as Holy Basil) and (*Ocimum sanctum*) extracts exhibit significant antifungal activity against *Candida albicans*, especially at higher concentrations of 0.5%. The results obtained from the agar gel diffusion method demonstrated a clear inhibitory effect on fungal growth, indicating that these extracts possess potent bioactive compounds effective in combating this common fungal pathogen. Moreover, the favorable outcomes from the In vivo evaluations, conducted at varying temperatures of 8 °C and 40 °C over 7-15 and 30 days, further support the efficacy of these extracts under different environmental conditions, which is crucial for their potential application in real-world scenarios.

These findings suggest that (*Ocimum tenuiflorum*) and (*Ocimum sanctum*) could serve as viable alternative treatments for *Candida albicans* infections, particularly in cases where traditional antifungal medications have become less effective due to rising resistance. This situation has become increasingly concerning in clinical settings, making the need for new therapeutic options more urgent.

Furthermore, the research underscores the importance of continuing to explore and validate plant-based remedies, as they can provide accessible and cost-effective solutions for managing fungal infections. In regions where antifungal resistance poses a significant challenge to public health, integrating such natural treatments into healthcare practices could enhance treatment options and improve patient outcomes. Overall, the study paves the way for further investigations into the pharmacological properties of these herbs, as well as their potential roles in integrative medicine and sustainable healthcare practice.

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