Antifungal Activity of Lemon Grass (*Cymbopogon citratus*) Leaves Extract against *Malassezia Furfur* Formulated as Ointment

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Abstract*— Medicinal plants are endowed with an innate ability to synthesize therapeutic chemicals. Lemongrass (Cymbopogon citratus) is a perennial grass that is extensively distributed throughout the tropics and is widely utilized for its pleasant flavor and medicinal benefits. The discovery of new medications with significant therapeutic potential has resulted from the extraction and characterization of secondary plant metabolites from medicinal plants. Thus, the purpose of the study was to formulate and evaluate the antifungal activity of *C. citratus* against *Malassezia furfur*. This study utilized true experimental research using quantitative and qualitative approaches. The results gathered were tabulated and subjected to One-way analysis of Variance (ANOVA). The extract of *C. citratus* was tested for phytochemical analysis which showed that it is positive of the following constituents: phenols, tannins and terpenoids. The lemongrass extract also has undergone the Disk Diffusion Method to evaluate its antifungal activity using three different concentrations 1%, 5%, and 10% against positive control, Clotrimazole. The phytochemical analysis of lemongrass leaves revealed the presence of terpenoids, tannins, and phenols, of which terpenoids are the active component responsible for inhibiting *M. furfur*. Based on the findings, it showed that 10% concentration of lemongrass ointment is more effective when compared to the formulated 5% and 1% concentrations in inhibiting the growth of Malassezia furfur. Also, the positive control, Clotrimazole, exhibited the highest inhibition against *M. furfur*. In conclusion, the 10% concentration has exhibited antifungal activity but not as effective as the positive control, Clotrimazole.

Keywords*— Cymbopogon citratus, Malassezia furfur, antifungal ointment

I. Introduction

Lemongrass has been shown to have biological antimicrobial properties. *Malassezia furfur* is a fungus that belongs to a monophyletic genus that is lipid-dependent, commensal yeasts accounting for more than 80% of the total fungal population of human skin (Vest & Krauland, 2020). Other *Malassezia* species, such as *Malassezia furfur*, *Malassezia restricta*, *Malassezia globosa*, and *Malassezia sympodialis*, have been associated with dermatological diseases in humans, such as pityriasis versicolor, dandruff/seborrheic dermatitis, and atopic dermatitis. Malassezia is found in mycelial and yeast forms, the latter of which is more prevalent on the skin. Pityriasis versicolor is a non-contagious skin infection caused by *M. furfur*, a fungus that is normally found in the cutaneous microbiota (Mcbain et. al., 2016). Pityriasis versicolor may be treated effectively with topical and/or systemic agents but its recurrence may occur despite effective treatment. Topical medications are considered the first-line therapy (Leung et al., 2022).

Fungi and their infections are rarely responsible for major illnesses such as skin disease. Their capacity to escape the host immune system and their pathogenicity have expanded the range of diseases they cause. Each year, more than 1.5 million individuals die from fungal infections, which impact over a billion people. Despite the knowledge that the majority of deaths from fungal infections are avoidable, public health authorities continue to ignore the issue. With the rise in fungal infections and antifungal resistance, researchers are seeking to develop new and more effective antifungal medicines (Pekmezovic et. al., 2016; Sharma et al., 2017). Various plant essential oils have recently been demonstrated to successfully suppress phytopathogenic fungus growth in vitro and in vivo. New effective antifungal medicines, particularly for local fungal infections that do not require systemic antifungal therapy, such as skin disorders, are still needed. Furthermore, the utilization of natural antifungal medications may help to avoid the development of antifungal drug resistance (Barac et. al., 2017).

Lemongrass (*Cymbopogon citratus*) is a perennial grass that is extensively distributed throughout the tropics, South and Central America, and is widely utilized for its pleasant flavor and medicinal benefits (Oladeji et. al., 2019). Lemongrass plants are primarily cultivated for their essential oils (Mukarram et. al., 2021). Lemongrass essential oil is mostly composed of the chemical compound citral, but it also contains numerous other compounds such as neral, geraniol, -myrcene, limonene, linalool, proximadiol, eranyl acetate, borneol, terpinolene, estragole, methyl heptanone, methyleugenol, citronellal, pinene, careen-2, farnesol, alpha-terpineol, d β-caryophyllene, (+)-cymbodiacetal and linalyl acetate (Ademuyiwa et. al., 2015). According to various research, the antibacterial properties of lemongrass essential oil are entirely dependent on the composition. Lemongrass contains a lot of citral and its cis-isomer (neral), beta myrecene and other aldehydic chemicals, esquiterpene monoterpenes, both oxygenated and non-oxygenated, and phenolic molecules. It possesses antifungal effects as a result of its high citral content, flavonoids, and tannins, which may account for its antibacterial activities against gram negative and gram-positive bacteria and fungus (Nyamath and Karthikeyan, 2018; Praveen and Sharma 2021). *C. citratus* has anti-amoebic, antibacterial, anti-diarrheal, anti-filarial, antifungal, and anti-inflammatory effects. Lemongrass is also used in the pharmaceutical, cosmetics, soap, and detergent sectors (Oladeji et. al., 2019). According to Nyamath and Karthikeyan (2018), the various pharmacological effects of lemongrass have also been connected to secondary active metabolites of a range of components. The most often extracted and recognized components from the leaves are aldehydes, alkaloids, saponin, terpenes, alcohols, ketone, and flavonoids, which have a variety of medical properties. Due to lemongrass essential oil's ability to inhibit the growth of all bacteria, it may be beneficial as an antifungal and anti-inflammatory agent in the prevention and treatment of acute inflammatory skin disorders (Ali etal., 2017).

Medicinal plants are endowed with an innate ability to synthesize therapeutic chemicals. The discovery of new medications with significant therapeutic potential has resulted from the extraction and characterization of bioactive compounds from medicinal plants (Mirghani et. al., 2012). Because of developed resistance to a wide range of medications, the use of essential oils against fungal infection has recently attracted much attention. The discovery of antifungal properties of plants are essential for a better understanding of the human pathogenic fungi biology. Despite advances in antifungal therapies over the last 30 years, antifungal resistance remains a major concern in clinical practice (Vandeputte et. al., 2012). In many nations, lemongrass (*C. citratus*) is frequently utilized in traditional medicine, antibacterial and antifungal capabilities are among the qualities attributed to it (Revathi et. al., 2016). The study of Boukhatem et. al., 2014 suggested that lemongrass essential oil has a potential for the formulation of new dosage form for treating fungal infections and skin inflammation. This study focuses on the fungicidal efficacy of Lemongrass (*C. citratus*) against *Malassezia furfur* formulated as ointment using Disc Diffusion Methods.

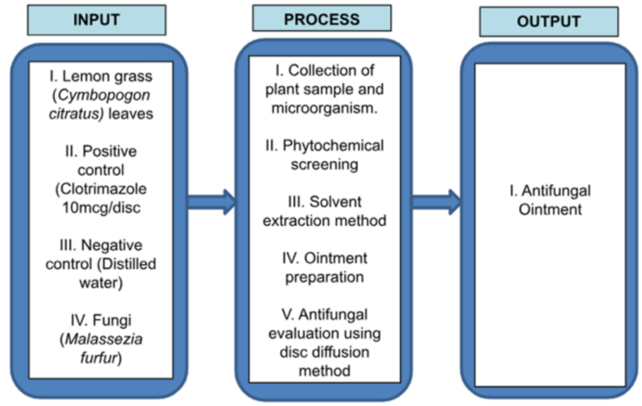

Fig. 1. Methodological framework of the study

II. METHODS

This study was designed to evaluate the antifungal activity of C. citratus against Malassezia furfur using different concentrations and to compare the formulated ointment's efficacy with that of Clotrimazole ointment. The study utilized true experimental research design using quantitative and qualitative approaches in gathering needed information. The extract was evaluated quantitatively, and the acquired data was described qualitatively.

A. *Collection and Preparation of Plant Sample*

The leaves of *C. citratus* were collected from the Provinces of Kalinga and Apayao and transported to the Pharmacy Laboratory of the University of Saint Louis, Tuguegarao, for preparation. Due to its ample number, plant samples were collected in May. The researchers brought the plant sample to the Department of Agriculture, Cagayan, for authentication. After drying the plant sample at room temperature for a maximum of eight days, it was then stored in a sealed plastic bag at ambient room temperature, protected from sunlight.

B. *Collection and Preparation of Test Microorganism*

The researchers acquired the specimen of the fungus *M. furfur* through skin scraping, and it was cultured at the Department of Science and Technology, Cagayan Valley.

C. *Solvent Extraction Method*

The plant materials were soaked for 30 minutes in distilled water before the extraction technique to increase the oil's collection efficiency. A 225g of the dried lemongrass was placed in a 1 lit clean flask with a flat bottom. The flask was filled with 750ml of N- hexane solvent. The flask and contents were left to stand for 36 hours to extract the lemongrass oil fully. The extract was then decanted into a new 1-liter beaker. A 300 ml of ethanol was added to the mixture to extract the essential oil since essential oil is soluble in ethanol. After transferring the mixture to a 500ml separating funnel, it is separated using a method called liquid/liquid separation. The contents of the separating funnel, which split into two layers, were permitted to re-establish equilibrium (depending on their different density). The bottom ethanol extract was collected and the higher hexane layer into two separate 250ml beakers and set in a 78°C water bath. This process eliminates the ethanol, leaving just the pure essential oil (Suryawanshi et al., 2016).

D. Ointment Formulation

For the ointment preparation, white wax was first mixed with petrolatum in an evaporating dish and melted using a water bath to make the white ointment base. After that, the extracted oil of lemongrass (1% w/w, 5% w/w, and 10% w/w concentration) was incorporated into the ointment base. The ointment was transferred into a suitable container and cooled to room temperature (El-Gied et al., 2015)

TABLE I. FORMULATION OF THE C. CITRATUS (LEMONGRASS) OINTMENT

Ingredients	1% C. *citratus* Ointment	5% *C. citratus* Ointment	10% *C. citratus* Ointment
White wax	1.25 g	1.15 g	1.1 g
Petrolatum	23.5 g	22.6 g	21.4g
Lemongrass oil	0.25	1.25 g	2.5 g

E. Evaluation of the Ointment Formulation

a) Physical evaluation (El-Gied et al., 2015). The formulations were evaluated for their color, odor and texture.

b) Spreadability (El-Gied et al., 2015). A specific amount of sample was imparted between the two glass slides to compress them to a consistent thickness. Spreadability was calculated using the formula:

$$S = M*L/T$$

Where:
M = wt. tied to the upper slide,
L = length of glass slides,
T = time taken to separate the slides.

F. Antifungal evaluation using the disc diffusion method

For the disc diffusion test, the Sabouraud Dextrose Agar medium was prepared. After sterilization, it was subjected to sterile Petri plates and allowed to harden. For inoculum preparation, a one-day-old fresh *M. furfur* culture was used, suspending the culture in 0.9 percent NaCl solution. A sterile cotton swab was used to swab *M. furfur* culture onto the surface of sterile Sabourauds dextrose agar plates.

Filter paper discs were sterilized and soaked in an undiluted (100 percent) concentration of oils. Oil saturated discs of 1%, 5%, and 10% concentration were aseptically placed over different Sabourauds dextrose agar plates seeded with the respective test microorganism using ethanol dipped and flamed forceps. As a positive control, clotrimazole (10 mcg/disc) was placed aseptically over the seeded Sabourauds dextrose agar plates to compare the antifungal activity of lemongrass. The plates were incubated for 24 hours at 37°C. Following the incubation, the zone of inhibition was measured using a ruler in mm units. The back of the petri dish was then viewed with the naked eye to measure each zone. Using reflected light, the plate was placed a few inches above a black, non-reflective surface. To determine antifungal activity, Whatman No. 1 filter paper discs with a diameter of 6.0 mm were sterilized by dry heat at 140°C for one hour in an oven.

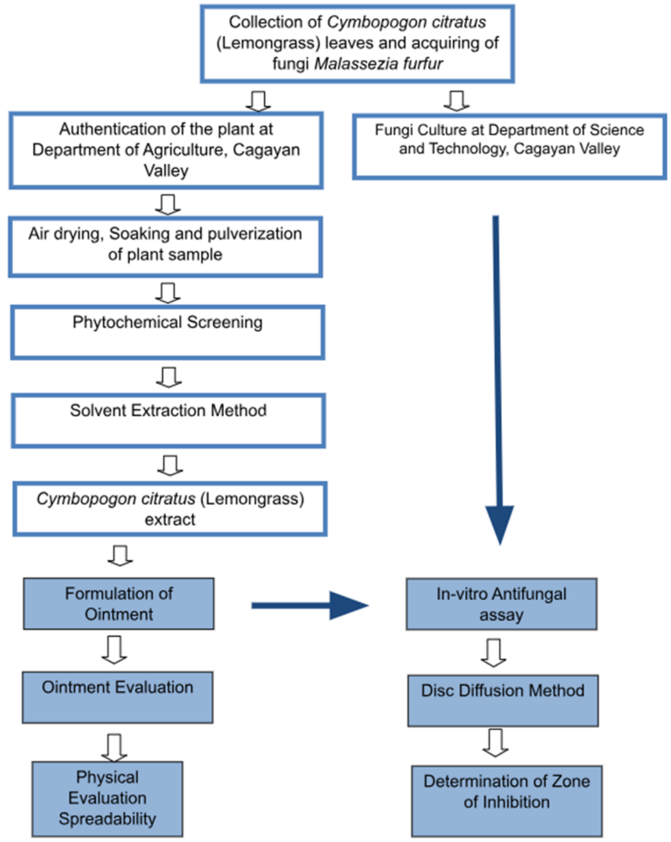

Fig. 2. Methodological framework of the study

G. Waste Disposal Management

The researchers subjected the needed apparatuses to an autoclave for sterilization to ensure microorganisms will not interfere when conducting the study.

H. Data Analysis

The results gathered were tabulated and subjected to statistical treatment, the one-way analysis of variance (ANOVA). ANOVA was performed to compare the different concentrations of lemongrass and the positive control, and the results were measured as mean +/- SD. A p-value less than 0.05 was considered statistically significant, and $p<0.0001$ was considered highly significant

TABLE II. QUALITATIVE OF THE ZONE OF INHIBITION FOR ANTIFUNGAL EVALUATION USING DISC DIFFUSION METHOD

Zone of Inhibition	Qualitative Interpretation
< 10 mm	Inactive
10-13 mm	Partially Active
14-19	Active
>19 mm	Very Active

III. RESULTS AND DISCUSSION

TABLE III. PHYTOCHEMICAL CONSTITUENTS OF LEMONGRASS (*C. CITRATUS*) LEAF ETHANOLIC EXTRACT

Phytochemical Constituents	Description
Alkaloids	Negative
Anthocyanins	Negative
Flavonoids	Negative
Phenols	Positive
Quinones	Negative
Saponins	Negative
Steroids	Negative
Tannins	Positive
Terpenoids	Positive

The data shows that phenols, tannins, and terpenoids are present in the extract of *C. citratus*. Citral are common terpenoids occurring in this plant. It has an antifungal effect which may be responsible for inhibiting *M. furfur*. This is consistent with the findings of previous studies (Babatunde et al., 2019).

Based on the results of the data gathered, the phytochemical screening of the lemongrass (*C. citratus*) revealed that terpenoids, tannins and phenols are present. Previous studies (Nyamath & Karthikeyan, 2018; Praveen & Sharma 2021) showed that lemongrass possesses antifungal effect as a result of its high citral content, tannins and flavonoids which may account also to its antibacterial activities against gram negative and gram-positive fungi. The diverse pharmacological actions of lemongrass have also been associated with secondary active metabolites of a spectrum of components. The most typically extracted and identified components from the leaves include aldehydes, alkaloids, saponin, terpenes, alcohols, ketone, and flavonoids, which have a range of medicinal effects. Due to its capacity to inhibit the development of all bacteria, lemongrass essential oil may be useful as an antifungal and anti-inflammatory agent in the prevention and treatment of acute inflammatory skin conditions (Ali et al., 2017) Additionally, Studies says that lemongrass oil is known for its monoterpene compounds, of which citral is the most abundant, exhibits an antifungal activity (Boukhatem et. al.,2014; Maldonado et. al., 2020).

TABLE IV. PHYSICAL CHARACTERISTICS OF THE FORMULATED LEMONGRASS (*C. CITRATUS*) OINTMENT

Treatments	Color	Odor	Texture	Spreadability (g.cm/s)
T2. 1% *C. citratus ointment*	Cream	Pleasant odor	Smooth	246.53
T3. 5% *C. citratus ointment*	Beige	Pleasant odor	Smooth	261.94
T4. 10% *C. citratus ointment*	Brown	Pleasant odor	Smooth	246.53

The table shows the physical characteristics of the different ointments prepared. Moreover, the three ointment formulations showed three different colors: cream, beige, and brown depending on the different concentrations of active ingredient which was used in this study. It also shows that the three formulations have a pleasant odor and smooth texture.

Based on the results of Table 6 and 7, formulated ointments showed three different colors: cream, beige, and brown depending on the concentrations of the active ingredient used with a pleasant odor and smooth texture. The spreadability values revealed that the *C. citratus* ointment 2 had the highest spreadability. Previous studies also revealed that formulated creams and ointments, 1%, 5%, and 10% concentration exhibited three different colors: light brown, brown and dark brown, respectively, with a smooth and homogenous appearance. The formulated formulations passed the short-term test, indicating the product's physical characteristics and spreadability (Aldawsari et al., 2015; El-Gied et. al., 2015).

TABLE V. ANTIFUNGAL ACTIVITY OF THE DIFFERENT TREATMENTS IN TERMS OF ZONE OF INHIBITION

Treatments	Zone of Inhibition				Qualitative Interpretation
	R1	***R2***	***R3***	***Mean***	
T1. Negative Control	6	6	6	6	Inactive
T2. 1% *C. citratus ointment*	7	8	6	7	Inactive
T3. 5% *C. citratus ointment*	10	9	7	9	Inactive
T4. 10% *C. citratus ointment*	15	14	13	14	Active
T5. Positive Control	33	35	32	33	Very Active

This table shows that among the experimental treatments (TII to TIV) only the 10% *C. citratus* ointment has an active inhibitory activity and is therefore able to inhibit the growth of the *M. furfur* fungus.

TABLE VI. TEST OF DIFFERENCE IN ANTIFUNGAL ACTIVITY AMONG ALL TREATMENTS

F-value	p-value	Decision
289.700	0.000*	Reject Ho

* SIGNIFICANT AT 0.01 LEVEL

The data shows that the negative control, positive control, and the three different concentrations of Lemongrass ointment at p-value of 0.000 has a significant difference. Since there is a significant difference among the five treatments, multiple comparisons were made using the least significant difference to determine which treatment has a significant difference.

TABLE VII. MULTIPLE COMPARISON ANALYSIS OF THE DIFFERENCE IN ANTIFUNGAL ACTIVITY AMONG ALL TREATMENTS

	T2	T3	T4	T5	T1
T2					
T3	.108				
T4	0.000*	0.000*			
T5	0.000*	0.000*	0.000*		
T1	.314	0.018*	0.000*	0.000*	

* SIGNIFICANT AT 0.01 LEVEL

The data show that the negative control (distilled water) has the least antifungal activity against the fungus *M. furfur*. All treatments (5%, 10%, and positive control (Clotrimazole) have significantly higher antifungal activity compared to the negative control. The 5% concentration has no difference in antifungal activity in comparison to 1% concentration. The 10% concentration has higher antifungal activity compared to the 1% and 5% concentrations of lemongrass ointment. Additionally, the positive control (Clotrimazole) has the highest antifungal activity in contrast with the three concentrations and negative control (distilled water).

Based on the data given, table 5 shows that the null hypothesis is rejected inferring that there is a significant difference in the antifungal activity of *C. citratus* ointment when formulated in different concentrations and when compared to the positive control Clotrimazole ointment.

The growth of *M. furfur* was actively inhibited by the introduction of 10% concentration of lemongrass ointment. Clotrimazole was used as a reference drug for comparing antifungal activity of the ointment of *C. citratus*. Based on the statistical analysis conducted, it showed that 10% concentration of lemongrass ointment is more effective when compared to the formulated 5% and 1% concentrations in inhibiting the growth of *M. furfur*. Findings showed that the higher concentration, the higher the efficacy to inhibit the *M. furfur*. The study of El-Gied et. al. (2015) on Investigation of cream and ointment on antimicrobial activity of *Mangifera indica* which also utilized three different concentrations 1%, 5%, and 10%. Among the three concentrations of the ointment, the formula of ointment containing 10% of extract showed the highest antimicrobial effect. Additionally, study of Liu et.al., 2022 on the inhibitory activity of citral against *M. furfur* showed that citral has antifungal activity at high concentrations and can decrease the infection of M. furfur. In comparison with the three formulated ointments, statistical analysis also showed that the positive control, Clotrimazole, has the highest efficacy in inhibiting the *M. furfur*. However, statistical results also show that the negative control (plain distilled water) has exhibited the least inhibition of *M. furfur*, hence, the negative control has the least antifungal activity.

It was observed that 10% concentration of lemongrass prepared as an ointment was effective as an antifungal medication because of its capability to inhibit *M. furfur*. Similar studies also observed that 10% concentration lemongrass has growth-inhibiting effect of *M. furfur* and can be used as an alternative treatment. As a result, the ointment containing 10% *C. citratus* has the capacity to be used as an alternative medicine to treat *M. furfur* as supported by the study of (Abd Rashed et al., 2021; Bismarck et. al., 2020; Sahal et al., 2020).

IV. Conclusion

Based on the phytochemical screening result terpenoids, tannins and phenols are present in which terpenoids is the active constituent that is responsible for inhibiting *M. furfur*. Out of the three concentrations of lemongrass ointment, only the 10% concentration has an active inhibitory activity, meaning it can inhibit *M. furfur*. In conclusion, the 10 % concentration has exhibited higher antifungal activity compared with the 1% and 5% concentration but is not as effective as the positive control, Clotrimazole. In addition, the 10% concentration can be used as an alternative and is comparable to the commercially available ointments in terms of its antifungal effect and physical characteristics although it requires high concentration of active ingredient to produce same antifungal activity with that of commercially available ointments.

V. Recommendations

Based on the aforementioned findings and conclusions, the following recommendations and suggestions are deemed significant:

- The utilization of the ointment having a 10% concentration of lemon grass can be used since it is proven that it can inhibit the growth of *Malassezia furfur*.
- Future researchers should further investigate the antifungal properties of *Cymbopogon citratus* by isolating and identifying its active constituents.
- Future researchers may conduct the same study using different time frames in observing the effects of ointments. Also, future researchers may use different concentrations higher than the formulated concentrations used in the study.
- Future researchers may conduct a comparative study on different antifungal medications like Ketoconazole, Fluconazole, etc.
- Future researchers should further investigate the application of Lemongrass in treating different types of Tinea and other skin diseases.
- Other studies on the other uses of *Cymbopogon citratus*, such as medication for high blood pressure, digestive tract spasms, and achy joints, may be conducted in the future.

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