

## Assessment of the antibacterial activity of lemongrass-extracted essential oil

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### Abstract

Lemongrass is one of the medicinal plants having significant applications to cure various diseases. This study aims at extracting oil from lemongrass plants and examining their antibacterial activities. The sample of lemongrass for this study was collected from Kailali District, Nepal. The leaves of lemongrass were collected, washed, cut into small pieces and dried in the sun. Then dried pieces of lemongrass were steam distilled for about four hours using the Clevenger apparatus, and oil was extracted. The extracted essential oil was further utilized to study its antibacterial activities in human pathogens, namely *Staphylococcus aureus* (*S. aureus*: gram-positive bacteria) by Agar diffusion and broth dilution method. It was found that the zone of inhibition (ZOI) for *S. aureus* in concentration of 100%, 75%, 50% and 25% was about 25 mm, 11 mm, 9 mm and 0 mm respectively. Furthermore, this study demonstrates the sensitivity of gram-positive bacteria to lemongrass essential oils. The lowest and highest ZOI were observed at 25% and 100% concentration respectively, against *S. aureus*. This study suggests that lemongrass essential oil possess antibacterial activity at high concentration. Along with medicinal uses of lemongrass essential oil, it also has promising applications in industrial especially for the purpose of food storage and food packaging.

### Keywords

Lemongrass, Essential oil, Extraction of essential oil, Anti-bacterial activity.

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## 1 Introduction

Lemongrass (*Cymbopogon citratus*) is a well-known medicinal plant in Nepal. It is composed of volatile aromatic chemicals, belongs to the Gramineae family, which grows primarily in tropical and subtropical region of the world [1]. They possess antibacterial, antifungal, anti-inflammatory, anticancer, antiseptic, and antioxidant properties. Lemongrass is widely utilized as a flavouring agent in the food business, as well as in the pharmaceutical, cosmetic, soap, and detergent sectors [2]. Essential oils can be extracted utilizing various plant parts, particularly the leaves and aerial parts. Basically, plants with medicinal properties are used to extract essential oils because of their antibacterial properties against bacteria, fungal, and viral pathogens. The presence of alkaloids, phenols, and other functional groups in essential oil (EO) contributes to their antibacterial activities. Essential oils derived from several medicinal plants are thought to be alternative natural antibacterial agents [3]. Moreover, due to the antibacterial and antifungal properties of lemongrass-extracted essential oil, it has an extensive potential to be employed in food preservatives. This sort of significant feature of essential oil can be useful and feasible for farmers in the rural locations [1]. Various ethnic groups have employed plant-derived natural products as traditional medicine. The essential oil isolated from *Origanum onites*, for example, has demonstrated considerable biological activity such as antibacterial, antifungal, and antioxidant characteristics, and it has also been shown to be useful against colon cancer [4]. Similarly, the leaves of *Cymbopogon citratus* have medicinal benefits and are a source of essential oils, as claimed by Manvitha and Bidya [5]. A study on *Cymbopogon citratus* revealed pharmacological activity such as anti-moebic, antibacterial, antidiarrheal, antifilarial, antifungal, and anti-inflammatory effects. It also highlighted that antibacterial activity of lemongrass can be utilized as a supplement treatment for fever, respiratory disorders, dental hygiene, and other conditions, and it also possesses anti-diabetic and anti-cancer properties [6]. Furthermore, citral compounds have also been utilized in the perfume business, cleaning wounds, and treating skin ailments. The essential oil extracted from *Cymbopogon citratus* does not have any disturbance in antibacterial activity, whether it is extracted in water as a medium or alcohol [7]. With respect to the type of organism, the gram-positive organisms were more sensitive to lemongrass oil as compared to gram-negative organisms [8]. Therefore, lemongrass essential oil can be effective against drug-resistant organisms.

Several studies have been conducted in the field of extraction of essential oil from different plant

species, Wong et al. [9] discussed the extraction of essential oil from *Cinnamomum zeylanicum* by steam distillation and Soxhlet extraction method. Cinnamon essential oil has cinnamaldehyde as a major component. The amount of cinnamaldehyde in essential oil is high (about 90%) from steam distillation and low (about 62-73%) from Soxhlet extraction. The antibacterial tests for both essential oil showed the formation of clear zones all around petri dish which confirms the antimicrobial activity. High microbial properties were shown by cinnamon essential oil against the gram-positive bacteria *Bacillus subtilis* and gram negative bacterium *Escherichia coli*. Pandey et al. [10] discussed the extraction of essential oil from the leaves of medicinal plant *S. officinalis* and evaluated its antimicrobial activity. They concluded that *S. officinalis* is a natural medicinal plant that can be used to cure many diseases, like inflammation. Hanamantagouda et al. [11] extracted essential oils from the dried leaves of *Lavandula pinnata*. The *Lavandula pinnata*-extracted essential oil was found as an herbal medicine, and it shows antimicrobial activity against both bacteria and fungi. Reyes-Jurado et al. [12] highlighted the antimicrobial activity of essential oils against a wide range of organisms. They also found that the yield and antimicrobial activity of essential oils mainly depend on environmental conditions of the season in which plant grow and on extraction methods employed.

This study includes the extraction of essential oil from the dried leaves of lemongrass using Clevenger apparatus and calculating the yield of oil in percentage. With the help of the zone of inhibition, we made five well systems, where four well systems contain four different concentrations of essential oil while the other one contains a common antibiotic, Cloxacillin, against *Staphylococcus aureus* (gram-positive bacteria). In addition, variable concentrations of essential oil and control have provided an antibacterial property against *Staphylococcus aureus*, gram-positive bacteria. Due to its effective antibacterial activity, lemongrass-extracted essential oil is considered a naturally occurring antibacterial agent.

## 2 Materials and Methods

### 2.1 Materials

The leaves of *Cymbopogon nardus* (lemongrass) collected from Botanical garden, Clevenger apparatus, distilled water, microorganism (*S. aureus*) collected from Seti Provincial Hospital laboratory, Dhangadhi, Nepal. The media MHA (Mueller-Hinton Agar), petri dish, test tube, a condenser, sterilized cotton, heating mantle, Cloxacillin, Zone of inhibition (ZOI), measuring scale, Round bottom

flask, Incubator, etc.

## 2.2 Methodology

### Extraction of Essential oil

In this study, lemongrass leaves were collected from Dewariya Botanical Garden (Jokhor Lake), Dhangadhi, Nepal. These leaves were washed properly with tap water to remove dust, mud, and foreign substances. Further, these leaves were cut into small pieces and left dry in sunlight for about 30 minute until water was removed from the leaves. In addition, the extraction was carried out on Clevenger apparatus with two sets in order to check the yield of the essential oil. The first set contains 200 gm of dried leaves in a round bottom flask with 1000 ml of distilled water, and the second set contains 80 gm of dried leaves in a round bottom flask having 500 ml of distilled water. The duration of distillation for both extraction was 4 hours and 15 minutes. Both sets provided a successful result; the 200 gm and 80 gm leaves yielded 4.5 ml and 1.9 ml respectively. Finally, after the distillation process the essential oil collected in a hard glass tube [8]. The experimental setup for the extraction of lemongrass essential is shown in the Figure 1.

### Culture of organism

Staphylococcus aureus was selected as the microorganism and collected from the Seti Provincial Hospital, Dhangadhi, Kailali. To initiate the bacte-

ria culture, *S. aureus* was mixed with nutrient broth in a test tube. McFarland turbidity standard number 0.5 was employed as a reference to determine the concentration. The test tube containing the bacterial culture was left untouched for 4-5 hours, allowing the culture to settle. After settling period, a sterilized cotton swab was utilized to consistently distribute the bacteria on the surface of prepared media in the petri dish. The petri dish, now containing the bacteria-infused media, was then placed in an incubator set to a temperature of . The incubation period lasted for 24 hours, allowing the bacteria to grow and develop under optimal conditions [13–15].

### Determination of Anti-bacterial property by Zone of Inhibition (ZOI)

To assess the antibacterial activity, five wells were formed in a petri dish containing *S. aureus* bacteria. The four wells were designated for *Cymbopogon nardus* oil at different concentrations, including 100%, 75%, 50%, and 25% and remaining one well was designated Cloxacillin (antibiotic medicine) as a positive control. The petri dishes were then placed in an incubator set at and incubated for 24 hours, ensuring no contamination occurred during this period. After incubation, the diameter of the zones of inhibition around each well was measured using a scale in millimetres. This measurement represents the extent of bacterial growth inhibition and is referred to as the “zone of inhibition” [13–15].



Figure 1: Clevenger Apparatus setup for the extraction of lemongrass oil.

## 3 Results and Discussion

To complete the entire experimentation, two extractions were performed, one with 200 gm and another with 80 gm of sun-dried leaves of lemongrass. The

duration of distillation process was 4 hours and 15 minutes for both extractions. The yields obtained for 200 gm and 80 gm of lemongrass leaves were 4.5 ml (2.25%) and 1.9 ml (2.37%), respectively. The yields of these two extractions are shown in Figure

2(a).

To access the antibacterial activity of lemongrass oil, *S. aureus* (gram-positive bacteria) was selected. The result revealed that *Cymbopogon nardus* exhibited significant antibacterial activity in comparison to a positive control i.e. Cloxacillin (COX). This suggests that the essential oil of *Cymbopogon nardus* possesses antibacterial properties that can effectively inhibit the growth of gram-positive bacteria. Hence, it can be used as a natural alternative against bacterial infections, further supporting its traditional use as a medicinal plant with antibacterial properties. The picture of the zone of inhibition in the five-welled system with four wells containing different concentration of EO and one well containing a positive control (Cloxacillin) is shown in Figure 2(b). The zone of inhibition of five-welled system is tabulated in Table 1.

A study conducted by Timung et al. [16] found that the essential oil extracted from lemongrass leaves has a yield of 2.38%. We also obtained yield of lemongrass EO as 2.25% and 2.37% which is in agreement with Timung et al. [16]. We used extracted lemongrass essential oil for testing its antibacterial activities against *S. aureus* (gram-positive bacteria). With regard to gram-positive bacterium, *S. aureus*, the ZOI demonstrated by the essential oils at four different levels of concentration including 100%, 75%, 50%, and 25% was 25 mm, 11

mm, 9 mm, and 0 mm, respectively. This shows as concentration increases, ZOI also increases which is in agreement with Naik et al. [8]. This implies that the gram-positive bacterium *S. aureus* can be successfully inhibited from growing by the essential oil of *Cymbopogon nardus* due to its antibacterial capabilities. The results of this investigation demonstrate that the antibacterial activity of essential oil increases progressively as concentration of oil increased. Our results clearly shows the antibacterial activity of lemongrass essential oil against *S. aureus* microorganism. Along with *S. aureus*, lemongrass EO found to possess antibacterial activity against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae* however it is found neutral against *Pseudomonas aeruginosa* microorganism [8].

In addition, the penicillin class of antibiotics includes cloxacillin as a subtype. It is frequently employed to treat bacterial infections brought on by specific bacterial species, such as *S. aureus*. It might also be ineffective in combating germs that have become resistant to penicillin-based antibiotics [17]. In addition, the penicillin class of antibiotics includes cloxacillin, which had a ZOI of 24 mm against *S. aureus*. This implies that gram-positive bacterium *S. aureus* can be successfully inhibited from growing by the essential oil of *Cymbopogon nardus* due to its antibacterial capabilities.

Table 1: Zone of inhibition (ZOI) in mm of a five-welled system, four for EO and one for COX

Micro-organism (Bacteria)	Concentration of lemongrass oil (%v/v)				
	100%	75%	50%	25%	Cloxacillin (COX)
<i>S. aureus</i> (gram positive bacteria)	25	11	9	0	24

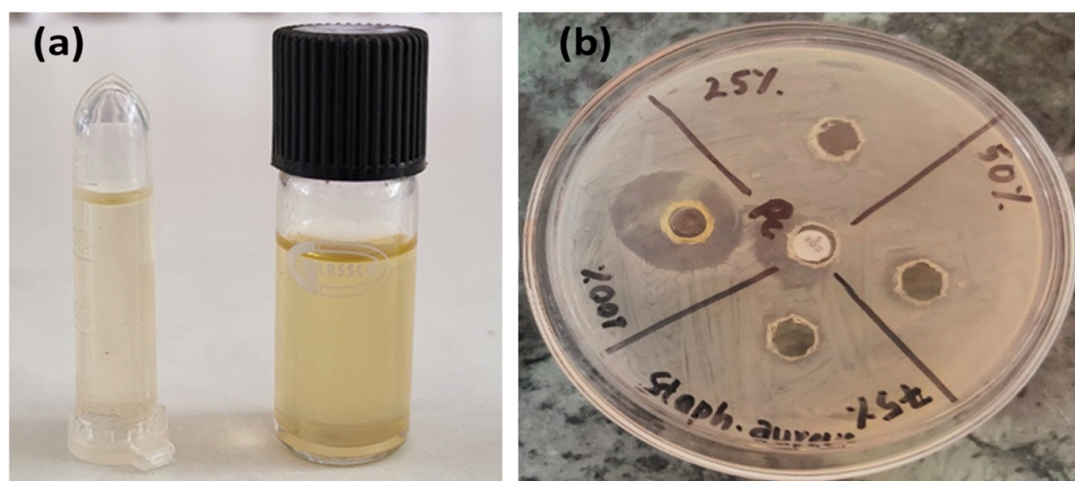


Figure 2: (a) Essential oil extracted from *Cymbopogon nardus* leaves (b) ZOI shown by the essential oil of *Cymbopogon nardus* in four welled plates with four different concentrations (100%, 75%, 50% and 25%) and Cloxacillin (an antibiotic medicine) in one welled plate on *Staphylococcus aureus*.

Furthermore, EO at concentration of 75%, 50%, and 25% had fewer antibacterial benefits than cloxacillin antibiotics, although EO at concentrations of 100% offered marginally more antibacterial benefits than cloxacillin antibiotics. However, several studies had already been conducted by several researchers showing that essential oils extracted from lemongrass have significant antibacterial activities. Timung et al. [17] reported that citronella oil possesses significant antibacterial activity, so essential oil can be utilized for various bacterial infections. In addition, Naik et al. [8] reported that gram-positive organisms are more sensitive to lemongrass oil as compared to gram-negative organisms. Moreover, U et al. [18] reported that extracts of lemongrass leaves and roots possessed intermediate antimicrobial activity against *S. aureus*, and Azizi et al. [19] mentioned that EO possessed antimicrobial and antiviral activities. Similarly, the lemongrass-extracted essential oil can be used as a treatment for fungal infection and skin inflammation, as reported by Boukhatem et al. [20]. Lemongrass EO can be helpful in curing infections caused by gram positive and gram negative organisms [8]. Pathogenic and Nonpathogenic diseases can be treated by the use of EO, like garlic extracted essential oil can be used to cure patients with coronary heart diseases [21]. Lemongrass EO has potential use in the medicine and surgical devices because of its antibacterial and antifungal property. It can also be used to modify the materials in medicinal devices so as to make such surfaces resistant to biofilm formation. It is rich in bioactives and is highly sensitive with human cells, especially has pronounced anticancer activity. This shows lemongrass EO has a potential for its use in the treatment of cancer [22]. Along with the significant applications of lemongrass EO in medicine, it has the prominent use in the food industry due to noticeable potential against various microorganisms. Mainly, it has been used in strengthening food packaging and food storage systems [23]. Tzortzakos and Economakis [24] also highlighted that lemongrass EO can be used as an alternative to synthetic fungicides, and it can also find an application in storage and packaging. It has a capacity to reduce the disease growth mainly by limiting the spread of pathogen and lowering the spore production.

#### 4 Conclusion

Medicinal plants are very important to humans because they have many benefits such as being antibacterial, antifungal, anti-inflammatory etc. The lemongrass extracted oil is used as a treatment for various infectious and respiratory diseases. A species of lemongrass, *Cymbopogon nardus*, also possesses economic importance in the production of

soap and detergent, perfume, beverages, food, and cosmetics. In this study, the leaves of lemongrass were subjected to hydro distillation using Clevenger apparatus for a period of over 4 hours, which provided a 2.37% yield of EO. Furthermore, the oil was then analysed to evaluate its antibacterial activity using ZOI. The ZOI for four different concentrations, such as 100%, 75%, 50%, and 25% of EO and antibiotic (COX), was approximately 25 mm, 11 mm, 9 mm, and 0 mm, and 24 mm respectively. This study clearly shows that EO from *Cymbopogon nardus* exhibits significant antibacterial activity against *S. aureus* (gram-positive bacteria). The sensitivity of EO found to be enhanced at higher concentration. This finding supports the traditional use of *Cymbopogon nardus* as a valuable plant for its antibacterial properties and suggests its potential for further exploration as a natural alternative for treating bacterial infections. Along with the antibacterial activity lemongrass EO has a potential use in industries especially in food packaging and food storage.

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