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Extraction and antibacterial activity of pure and diluted eucalyptus oil against *Corynebacterium diphtheriae* and *Streptococcus* species

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Abstract

This study involved the extraction of pure eucalyptus oil using a Clevenger apparatus and its subsequent biological activity assessment. Oral swabs were collected from two individuals infected with SARS-CoV-20 and cultured on agar plates to isolate bacterial strains. Growth of *Corynebacterium diphtheriae* and *Streptococcus* species was observed. The sensitivity of these bacteria to pure eucalyptus oil and its dilutions in dimethyl sulfoxide (DMSO) was tested. The results demonstrated that pure eucalyptus oil exhibited high antibacterial activity (+++), while the 50% diluted oil showed moderate activity (++). However, further dilutions at 35% and 25% were found to be ineffective (-).

Keywords: Dimethyl sulfoxide (DMSO), SARS-CoV-20, antibacterial activity, eucalyptus oil

Introduction

The Eucalyptus, also known by various names such as Kalibtos, Qalimtus, or Qalam Tuz in Iraq, comprises a genus with a diverse history and applications. Initially, about 25 species of Eucalyptus were native to South America, but excessive exploitation during the 16th and 17th centuries nearly led to their extinction. Later, botanists identified approximately 700 similar species in Australia, many of which have found significant use in medicine ^[1].

Eucalyptus is a genus belonging to the family Rubiaceae, encompassing around 25 species originating from the Andean rainforests of South America. A limited number of species have been discovered in Central America, Jamaica, French Polynesia, Sulawesi, Saint Helena in the South Atlantic, and São Tomé and Príncipe near the tropical African coast. Some of these species are known for their herbal medicinal properties, particularly as a source of quinine and other compounds. The genus name was introduced by Linnaeus in 1742, inspired by the Countess of Chinchón, who was treated with its medicinal bark in 1638. Historical accounts from the Quechua people, dating back to 1560–1570, also describe the therapeutic benefits of the bark. Today, Eucalyptus is considered a national tree in Ecuador and Peru ^[2, 17].

Eucalyptus oil offers a variety of health benefits, including alleviating coughs ^[4, 3], disinfecting wounds ^[5], improving respiratory conditions ^[6], reducing joint pain ^[7], repelling insects, boosting the immune system ^[8], relieving eczema ^[9], treating headaches ^[10], improving oral health ^[11], and aiding in managing symptoms of Attention Deficit Hyperactivity Disorder (ADHD) ^[12]. It has been traditionally used for its expectorant, anti-inflammatory, analgesic, antimicrobial, and insecticidal properties.

This study explores the unique botanical, ecological, and pharmacological characteristics of Eucalyptus, with a focus on its significant role in traditional medicine and its application in modern industries.

Materials and Methods

The study utilized a Clevenger apparatus comprising a round-bottom flask, condenser, extraction tube, and fractionation column. Additional equipment included a heating mantle, submersible pump, container, stand and clamp, and rubber tube. The materials used were eucalyptus leaves, water, ice cubes, swabs for sample collection, media culture plates, and

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Eppendorf tubes. This setup was essential for extracting pure eucalyptus oil and conducting the biological activity analysis.

Procedure

The process of extracting essential oil was carried out using the water distillation method with a Clevenger apparatus, as shown in Fig. 2, ensuring the preservation of the active components present in the oil. Fresh eucalyptus leaves were carefully harvested from public gardens and subjected to thorough washing with clean water to eliminate dust, soil, and other impurities. This preparatory step was crucial to obtaining a pure, uncontaminated yield of essential oil. The washed leaves were then placed into a 500 mL round-bottom flask, and a sufficient amount of water was added to completely immerse the leaves. This setup helped to ensure that the steam generated during the distillation process effectively interacted with the plant material for optimal oil extraction.

The round-bottom flask was placed on a heating mantle, and the temperature was maintained at 50 °C to provide controlled heating without agitation. As the water began to boil, steam carried the volatile components of the essential oil through the apparatus. The vapors passed into the condenser, which is a critical cooling component of the system. To enhance the efficiency of the condensation

process, the condenser was kept at a very low temperature by circulating cold water mixed with ice cubes, facilitated by a submersible pump. This setup ensured that the volatile compounds condensed effectively into a liquid form, which was then directed into the collection chamber of the Clevenger apparatus.

In the Clevenger tube, the condensed mixture of water and oil accumulated, and the process of reflux ensured that the water returned to the flask, creating a continuous cycle of distillation. The distillation process was allowed to proceed for 5 to 6 hours to ensure the complete extraction of the essential oil from the fresh eucalyptus leaves. Over time, the mixture in the Clevenger tube naturally separated into two distinct layers based on their density: the upper layer comprised the essential oil, while the lower layer consisted of water.

Once the extraction was complete, the valve at the bottom of the Clevenger tube was carefully opened to drain off the water, leaving only the oil in the collection chamber. The essential oil was then transferred into a clean, airtight container to prevent contamination and preserve its purity and quality. This meticulous method of extraction, utilizing the Clevenger apparatus, ensured the recovery of a high-quality essential oil with all its active chemical components intact.



Fig 1: Clevenger apparatus

Results and Discussion

Effect of extraction time

The effect of extraction time on eucalyptus oil extraction was studied until equilibrium was reached at 100 °C, using a water to eucalyptus leaves ratio of 6:1 (ml: g) and a mixing speed of 200 rpm. The data, presented in Figure 2, indicate that the amount of eucalyptus oil increased with time. Initially, the extraction rate was fast, but it slowed down gradually as the free oil on the surface of the eucalyptus leaf particles was extracted quickly. This resulted in a rapid

increase in the extraction rate when the material was exposed to fresh solvent. At the beginning of the extraction process, the oil concentration in the solvent was low, which facilitated the rapid diffusion of oil from the solid material to the liquid phase due to mass transfer. However, as extraction continued, the oil concentration in the solvent increased, leading to a decrease in the diffusion rate. These observations are consistent with previous studies, including the findings of Cassel and Vargas ^[14].

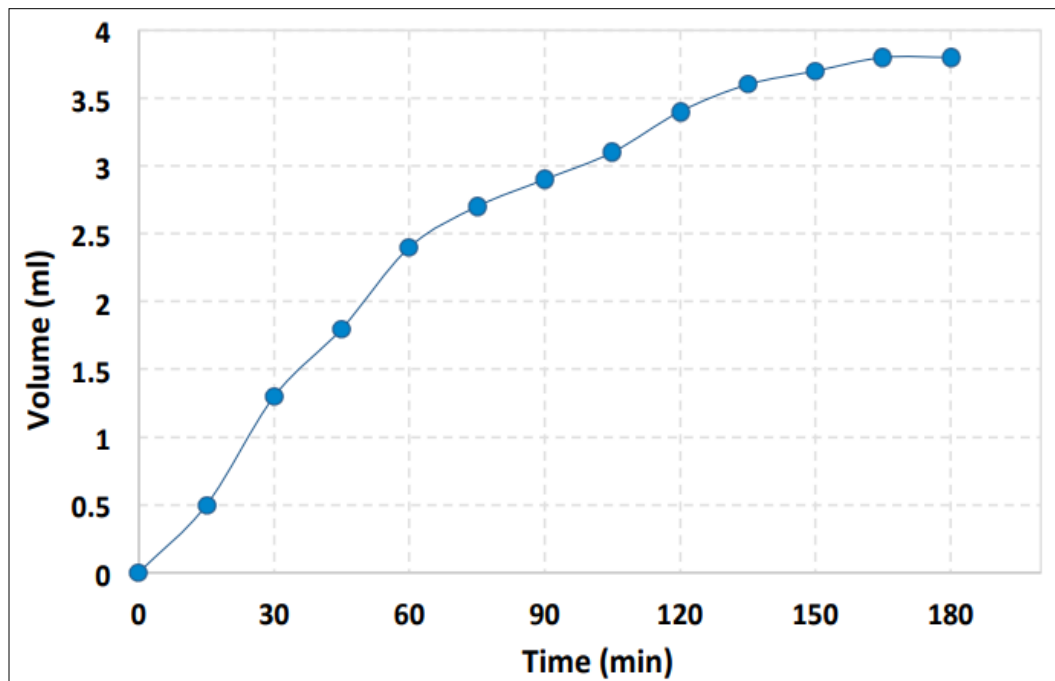


Fig 2: The effect of extraction time on amount of eucalyptus oil

Effect of extraction temperature

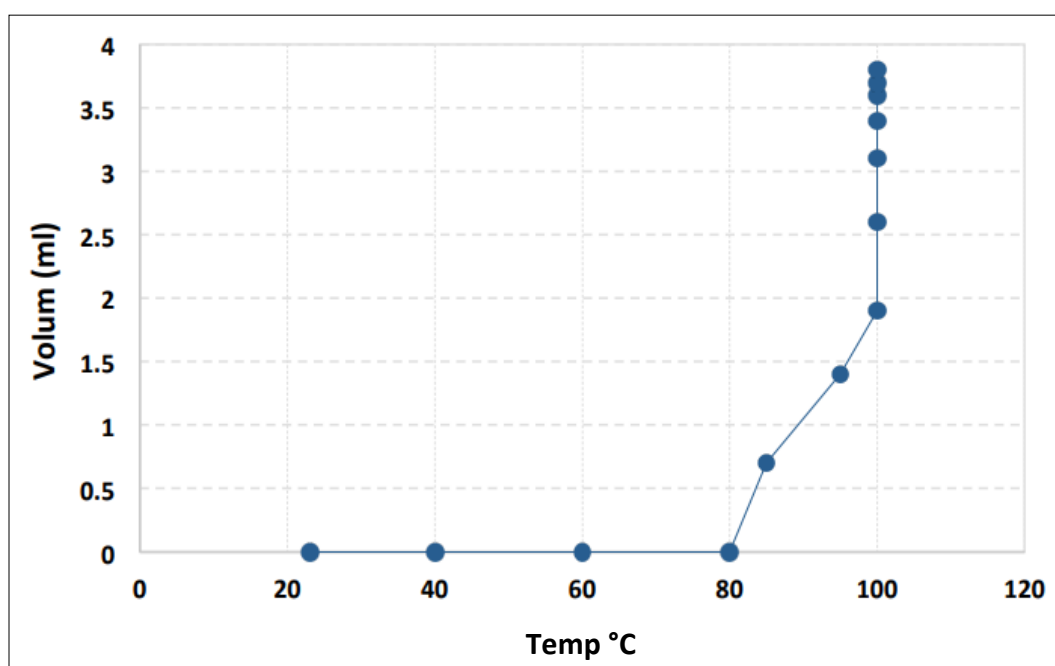


Fig 3: The effect of extraction temperature on amount of eucalyptus oil

The effect of extraction temperature was evaluated at various temperatures, maintaining a water to eucalyptus leaves ratio of 6:1 (ml: g), a particle size of 0.5 cm for the eucalyptus leaves, and a mixing speed of 200 rpm. As shown in Figure 3, the amount of eucalyptus oil increased with higher extraction temperatures due to the enhanced vaporization of eucalyptus oil at elevated temperatures. Additionally, higher temperatures led to an increase in the oil's diffusion coefficient, resulting in a faster diffusion rate of eucalyptus oil ^[15].

Effect of agitation speed

In order to evaluate the influence of agitation velocity on the extraction of eucalyptus oil via the water distillation

technique, this investigation was executed at various agitation velocities ranging from 0 to 200 rpm, while sustaining a consistent extraction temperature of 100 °C. The ratio of water to eucalyptus leaves was established at 6:1 (ml: g), with a particle dimension of 0.5 cm for the eucalyptus leaves. Figure 4 depicts the relationship between agitation speed and the temporal progression of eucalyptus oil extraction. It was noted that the yield of eucalyptus oil was augmented with increasing agitation velocities. Furthermore, an elevation in agitation speed improved the diffusion coefficient of the oil, as it facilitates eddy diffusion, thereby enhancing the transfer of material from the particle surface to the bulk solution, which consequently elevates the mass transfer rate ^[16].

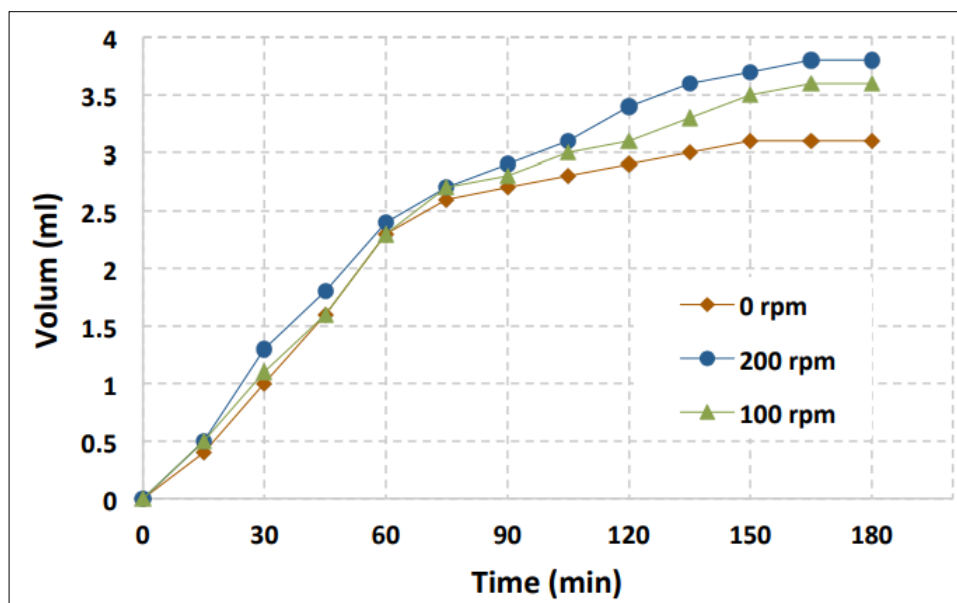


Fig 4: Effect of agitation speed on amount of eucalyptus oil

Effect of Leaves Particle Size

The effect of particle size was investigated using two different sizes (0.5 cm and 1 cm) under the conditions of a water to eucalyptus leaves ratio of 6:1 (ml/g), an agitation speed of 200 rpm, and an extraction temperature of 100 °C, until equilibrium was reached. As shown in Figure 6, a smaller amount of eucalyptus oil was extracted from the

larger eucalyptus leaves particles compared to the smaller particles. This is due to the larger particles having a smaller interfacial area between the leaves and the liquid phase. Consequently, less eucalyptus oil is transferred from the interior of the larger leaf particles to the surrounding solution, in contrast to the smaller particles.

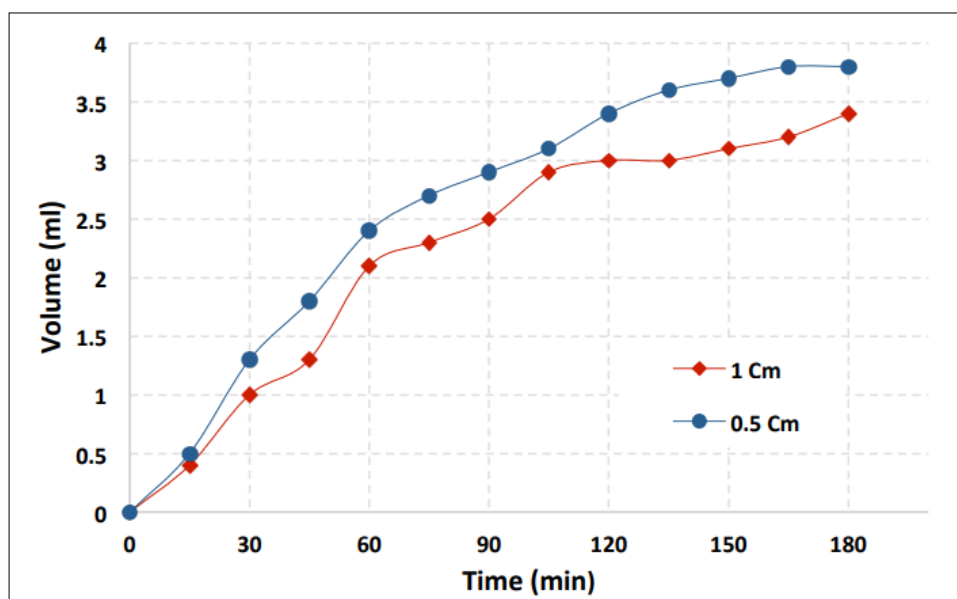


Fig 6: Effect of leaves particle Size on amount of eucalyptus oil extraction

Evaluation of the biological activity of the extracted oil

The biological activity of the essential oil extracted from eucalyptus leaves was evaluated in a detailed study. Sterile swabs were used to collect bacterial samples from the oral cavities of two individuals (designated A and S) who were diagnosed with SARS-CoV-20. These samples were cultured on Blood Agar, a nutrient-rich medium, to promote bacterial growth. Observations indicated the presence of two specific types of bacteria: *Corynebacterium diphtheriae*, which causes diphtheria, and *Streptococcus pyogenes*, a hemolytic streptococcus responsible for pharyngitis and tonsillitis.

To further assess the sensitivity of these bacteria to the extracted oil, additional samples were prepared and cultured on Nutrient Agar. Sensitivity testing was performed using serial dilutions of the essential oil, prepared in various concentrations as outlined in the Table 1. The dilutions were made using dimethyl sulfoxide (DMSO), a solvent chosen for its inert nature, as it does not interfere with bacterial viability or oil activity.

The test involved applying different concentrations of the oil to the cultured bacteria on Nutrient Agar and observing their growth inhibition patterns. Results revealed that the eucalyptus oil exhibited a significant antibacterial effect,

with varying degrees of sensitivity noted across the tested bacterial strains. The findings highlight the potential of eucalyptus essential oil as an effective antibacterial agent, particularly against *Corynebacterium diphtheriae* and *Streptococcus pyogenes*. These results open avenues for further exploration into the therapeutic applications of eucalyptus oil in combating oral and respiratory infections.

Table 1: Serial dilutions of eucalyptus essential oil prepared using DMSO for sensitivity testing

Oil dilution in solvent (DMSO)	Strain A response	Strain S response
Pure oil (100%)	+++	+++
50% dilution	++	++
35% dilution	-	-
25% dilution	-	-

Table 1 highlights the strong antibacterial activity of eucalyptus leaf essential oil against *Corynebacterium diphtheriae*. The data clearly show a significant inhibitory effect when the oil is used in its undiluted form, with both bacterial strains (A and S) displaying marked sensitivity, as indicated by the strong bacterial inhibition (+++). However, as the oil concentration is reduced through dilution with DMSO, its antimicrobial effectiveness decreases proportionally.

At a 50% dilution, the oil still exhibits moderate antibacterial activity (++), capable of inhibiting bacterial growth, although it is less effective compared to the undiluted oil. Further dilutions to 35% and 25% result in a complete loss of antibacterial activity (-), with neither strain showing any sensitivity to these lower concentrations.

This pattern emphasizes the critical role of concentration in determining the efficacy of eucalyptus essential oil as an antimicrobial agent. The results suggest that the oil's active compounds are most potent at higher concentrations, with a noticeable reduction in efficacy as the concentration decreases. These findings underscore the potential therapeutic value of undiluted or lightly diluted eucalyptus oil in treating bacterial infections, especially those caused by *Corynebacterium diphtheriae*. The results also highlight

the importance of carefully considering the oil concentration in both clinical and experimental contexts to fully exploit its antimicrobial properties.



Fig 7: Bacterial culturing method on blood agar medium



Fig 8: Growth of *Corynebacterium diphtheriae* and streptococci on blood agar



Fig 9: Eppendorf tubes containing eucalyptus oil at different dilution concentrations



Fig 10: Antibacterial activity of bacterial strain (A) on nutrient agar against pure and diluted eucalyptus oil

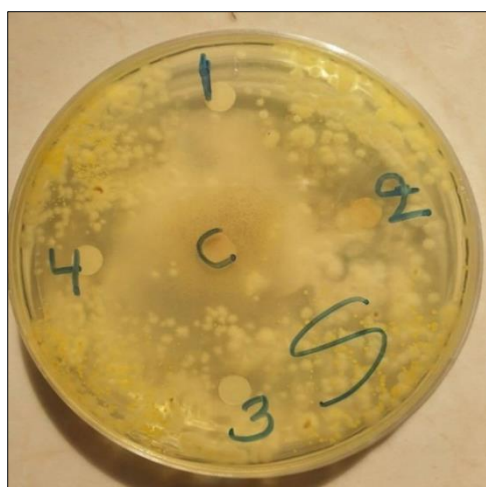


Fig 11: Antibacterial activity of bacterial strain (S) on nutrient agar against pure and diluted eucalyptus oil

Conclusion

Based on the results obtained from the extraction of eucalyptus oil and the subsequent study of its biological properties, it was found that the extracted oil is pure and concentrated, characterized by its pungent, hot, and refreshing taste, as well as its distinctive aroma. This unique fragrance is attributed to the presence of the compound 1,8-cineole, also known as eucalyptol. Furthermore, when examining the biological properties of the oil, it was observed that it inhibits the activity of *Corynebacterium diphtheriae*, a bacterium known for causing infections such as pharyngitis, tonsillitis, and congestion. This suggests that eucalyptus oil has the potential to be utilized in treating various ailments caused by this bacterium, offering insights into the relationship between traditional remedies and modern pharmaceutical treatments.

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