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Antimicrobial Activities of Bergamot, Lavender and Lemon Essential Oils for Homemade Perfume

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Abstract: Homemade perfume is commonly prepared from essential oil mixed in a tube, but it does not ensure human health safety. This project studied the antimicrobial activities of essential oils used to create the perfume created by the Kirby-Bauer protocol. Three essential oils used in the test are the *Citrus Limon* (lemon), *Citrus Bergamia* (bergamot), and *Lavandula* (lavender). *Staphylococcus aureus* (*S. Aureus*) and *Escherichia coli* (*E. Coli*) were introduced as gram-positive and gram-negative bacteria growth on the Mannitol-Salt agar and Nutrient agar, respectively, with penicillin and ampicillin as positive controls and distilled water as a negative control. The antibiotics that acted as the positive control shows that penicillin has better performance against *S. Aureus* than ampicillin against *E. Coli*. In comparing lemon and bergamot essential oils, the study found that lavender essential oil had strong antibacterial activity in both bacteria plates. However, the perfume showed no inhibitory effect in both antimicrobial activities as no inhibition zone was present after the test. In conclusion, this homemade perfume is suggested for use on humans constantly for a short period as it has an inferior performance against bacteria and which could affect human health.

Keywords: Antimicrobial activities, *E. Coli*, Essential oils, Kirby-Bauer, Perfume, *S. Aureus*

1. Introduction

Essential oils are natural, volatile, complex, oily, or lipid-like in origin and waft a strong scent as a plant molecule. They are soluble in lipids, alcohol, organic solvents, and other hydrophobic compounds and are generally liquid at room temperature [1]. Essential oils make up a small percentage of the most weight of plant material, often around 1% or less. Therefore, the antimicrobial components from essential oil are often used to describe the characteristics of essential oils.

Secondary plant metabolites have traditionally been defined as all plant-produced chemicals that do not appear to be needed for plant growth and have no evidence for development purposes. However,

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secondary metabolites have been discovered to play roles in defense, signaling, and secondary metabolism due to increased interest and inquiry in recent years [2].

A formal investigation of essential oils in terms of biological activity includes antimicrobial activity, antibacterial activity, antifungal activity, antiviral activity, antiprotozoal activity, and anticancer activity [3]. Therefore, the essential oils were selected as therapeutic components that waft pleasant smells for smelling without endangering their health. In addition, lavender, bergamot, and lemon essential oils have substantial effects that reduce anxiety and depression [4-6]. Thus, considering its significant impact on people, these essential oils were chosen to continue the research by investigating the antimicrobial effects of lavender, bergamot, and lemon essential oils separately and after being combined into a homemade perfume [1]. Perfume that has antimicrobial activities might be harmful to the human skin.

Microorganisms such as bacteria, viruses, fungi, and protozoa cause many infectious diseases. The compounds with specific antimicrobial activity are the best weapon for treating these diseases. Plant-based medicines containing compounds with antimicrobial activity included essential oils attempting to treat such illnesses [7]. Antimicrobial essential oils differ significantly in terms of the variety of plants from which they can be obtained and the chemical composition of each oil. Nevertheless, antimicrobial activity is present in most essential oils, with some oils and components demonstrating a substantial activity level [8].

Many studies of essential oils' antibacterial activity have found that they are more effective against one of the two major bacterial divisions; Gram-positive or Gram-negative bacteria. Monoterpenes, sesquiterpenes, and nonterpenaceous components such as phenylpropanoids are the principal classes of chemicals identified in essential oils that have antimicrobial activity. Gram-positive and Gram-negative bacteria have exhibited susceptibility to essential oils and components in vitro. Although most essential oils include active antimicrobial components at various doses, there is still a considerable risk of microorganism contamination [8].

Essential oils are a part of the plant's immune system with highly concentrated volatile chemicals extracted from different parts of plants, each with its medicinal and energising properties [9]. These volatile liquids are complex chemical compounds with incredibly efficient and potent effects. First, it is made from the plant's essence, rich in natural tastes and active substances, and released by the cells of specific plant parts. Next, the secretory organs are distilled or pressed to obtain valuable liquids [1]. Finally, steam or hydro-distillation, Soxhlet extraction, solvent extraction, or continuous extraction technologies extract essential oils from aromatics and plants. They are typically colourless and volatile and soluble in organic solvents. The essential oil can be found in all plant parts, including buds, leaves, fruits, bark, root, stems, twigs, and flowers [10].

Perfume is a liquid-based mixture that emits pleasant odours from biological or synthetic elements. Perfume extraction techniques ensure that natural bioactive compounds are preserved and do not degrade during the extraction process. The employment of perfumes has a significant impact on the applicants' mood and self-appeal [11]. The most volatile species would theoretically be smelled first as a first impression. The tonality will gradually shift to middle and base notes that linger in the air for hours [12]. As a result of the evaporation and diffusion processes, a blending of vapour fragrances evolves in the air above the liquid. The human nose simultaneously perceives those single odorants at different intensities [13].

2. Materials and Methods

2.1 Materials

The material used for the first step of this project which is the preparation of microbial culture, is Mannitol-Salt agar powder, Nutrient agar powder, *E. Coli*, and *S. Aureus*. The materials for the preparation of microbial culture are obtained from the Lab of Biology and Function 1 (MBF1), Universiti Tun Hussein Onn Malaysia (UTHM). Next, natrium chloride is used to make saline, and

0.5 McFarland standard estimates the bacterial density. Finally, the pure bergamot, lavender, lemon essential oils from Young Living is obtained from MBF1 UTHM lab, and pure aloe vera carrier oil purchased from the online store is used to produce the perfume. Besides, distilled water, penicillin, and ampicillin were also used in the Kirby-Bauer test.

2.2 Methods

2.2.1 Perfume Solution

In this study, the lavender essential oil was set as the base note, while the middle and top notes were bergamot and lemon essential oils, respectively. The ratios of the mixture of the essential oils were 2:5:3, where 20% was from the base note fragrance, 50% from the middle note, and 30% were from the top note. Firstly, 2mL lavender essential oil, 5mL bergamot essential oil, and 3mL lemon essential oils were pipetted into the 30mL perfume bottle. The essential oil mixture was then combined with 9mL aloe vera as a carrier oil, and the bottle was shaken for roughly 1 minute before being ready to use as a perfume. Finally, the carrier oil was added to dilute the concentration of the essential oils. Therefore, the perfume was safe to use on human skin.

2.2.2 McFarland Standards: Turbidity of Bacterial Suspension

One loop of bacteria (*E. Coli* and *S. Aureus*) was cultivated on an agar plate and inoculated into the universal bottle, which contained saline. The bacterial suspensions turbidity in saline was visually compared to the McFarland standards used to estimate the bacterial density. If the turbidity of the saline was higher and cloudier than the 0.5 McFarland standard, then more saline was added to the universal bottles. Difference to the process, if the 0.5 McFarland standard was cloudier than the saline, then one loop of microbe was added into the saline and vortexed again. These processes were repeated until the turbidity of the bacterial suspension in saline was in the same turbidity with the 0.5 McFarland standard.

2.2.3 Antimicrobial Activity of Essential Oils

First, agar plates were divided into three sections for the control discs. Each section was labelled as a positive control (antibiotics), negative control (distilled water), and the essential oil utilized for the plate. In separate plates, 0.5 µL of antibiotics, distilled water, and essential oils were pipetted to each disc, which was then let to partially dry before being placed on the agar surface. First, pipette 100 µL of bacteria suspension (saline) solution made previously in the McFarland standards into the sterile agar plates to inoculate the agar plates. The plates were then left for about 3 to 5 minutes with the lids slightly ajar at room temperature before proceeding to the following procedure. The final step in this approach was to immerse the control discs in agar and incubate them. The control disc was taken and dropped into the agar plates in pieces using sterile forceps. Before and after inserting the disc into the agar plate, the forceps were sterilized with a Bunsen burner. To prevent agar contamination, each time a disc was placed in their section, the lid had to be closed and opened again to place another disc. After inserting the disc into the agar plates, the parafilm was used to seal the plates completely and were placed in the incubator at 37°C for 24 hours before the results could be measured.

2.3 Dilution formula

The dilution formula used in the preparation of nutrient agar, Mannitol-Salt agar, and antibiotics as positive control is written as:

$$M_1 V_1 = M_2 V_2 \qquad \textbf{Eq. 1}$$

where M_1 is initial molarity, V_1 is initial volume, M_2 is final molarity and V_2 is the final volume.

3. Results and Discussion

The present study has elucidated the antimicrobial activity of essential oils and perfume. However, the inhibition growth value less than 7 mm diameter is considered no antimicrobial activity in *E. Coli* and *S. Aureus* bacteria. **Figure 1** shows the inhibition zone of *Citrus Bergamia*, *Citrus Limon*, *Lavandula* essential oils, and perfume in agar plate disk diffusion after 24 hours of incubation for 37°C temperature.

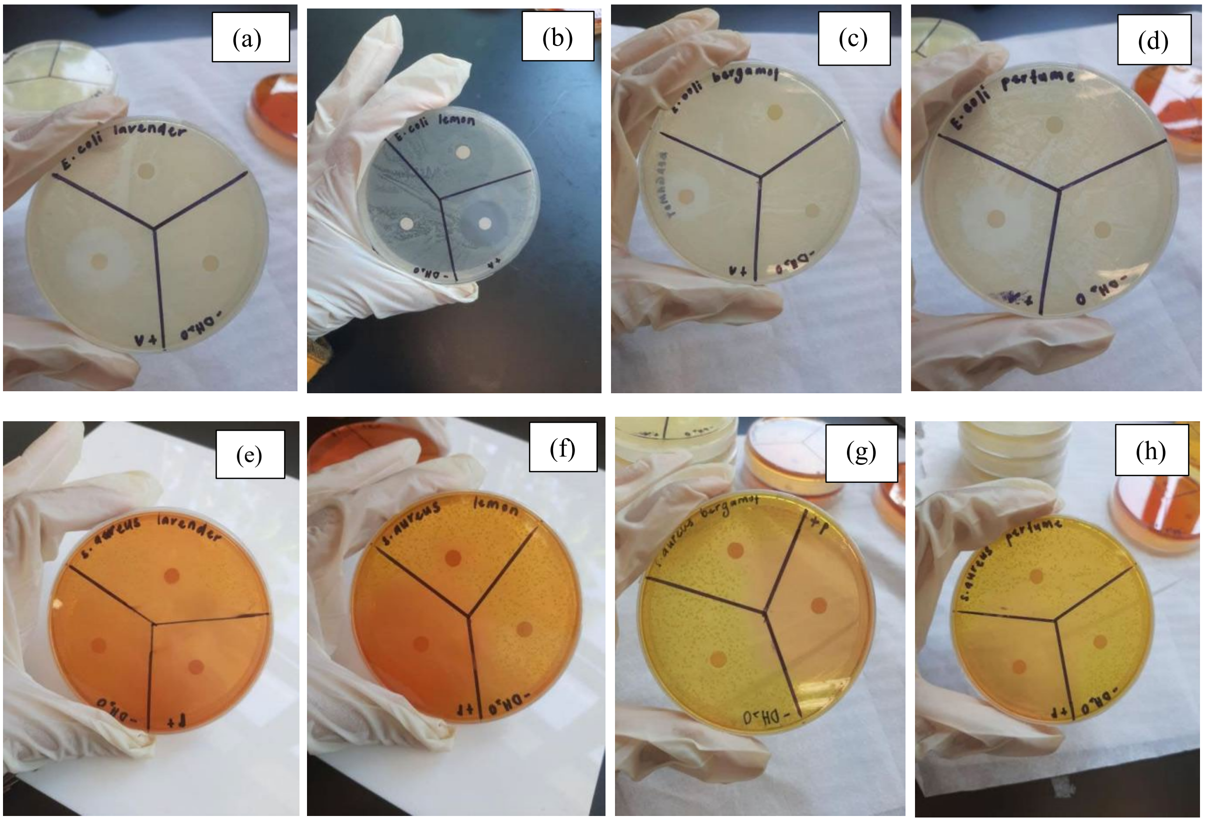

Figure 1: Zone of Inhibition (mm)^2 in *Staphylococcus aureus* for (a) *Lavandula,* (b) *Citrus Limon,* (c) *Citrus Bergamia* and (d) Perfume for *E. Coli* and (e) *Lavandula,* (f) *Citrus Limon,* (g) *Citrus Bergamia* and (h) Perfume for *S. Aureus.*

Figure 2 shows the susceptibility rates of *Citrus Bergamia*, *Citrus Limon*, *Lavandula*, and *perfume* against gram-negative; *E. Coli*. The samples indicated the antimicrobial values against inhibition growth of *E. Coli*. As shown, *Lavandula* essential oil possessed the most excellent antimicrobial activity value with 11mm diameter, continue down with *Citrus Limon* at 8mm and *Citrus Bergamia* with only 6mm. This is because *Lavandula* contain the highest amount of antimicrobial bioactive compound, beta-linalool, instead of *Citrus Limon* and *Citrus Bergamia* [14-16]. Furthermore, the perfume sample failed to inhibit *E. Coli* growth as it does not contain any antimicrobial value. It is thought to be due to antagonistic interactions between active hydrocarbon compounds in each essential oil and aloe vera carrier oil. [17]. Following that, the ampicillin as a positive control had the expected antimicrobial value, an average of 21 mm, indicating that the disc diffusion of each sample was a successful experiment.

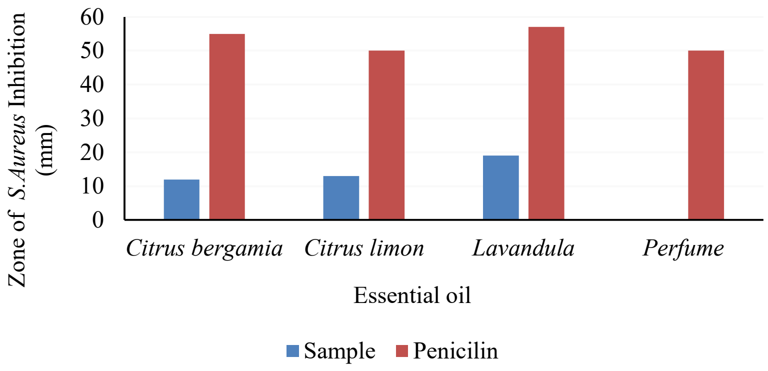

Figure 2: Antimicrobial activity of essential oils against *E. Coli.*

Figure 3 shows the susceptibility rates of *Citrus Bergamia, Citrus Limon, Lavandula,* and *perfume* against gram-positive; *S. Aureus*. These samples showed the antimicrobial values against inhibition growth of *S. Aureus.* Similar results in **Figure 2,** *Lavandula* essential oil, has the highest value of antimicrobial with 19mm diameter, followed by *Citrus Limon* with 13mm and *Citrus Bergamia* with 12mm. For *S. Aureus*, the antimicrobial values for all samples did not have a significant diameter comparison, with *Lavandula* containing the most antimicrobial activity and *Citrus Limon* and *Citrus Bergamia* incorporating a moderate amount of antimicrobial activity. Thus, these antimicrobial values cannot be compared to penicillin, the positive control, because it has much higher antibiotical activity with an average of 53 mm [2].

Overall, the antimicrobial activities of essential oils against two bacterial strains are summarized in **Figures 1** and **2.** The results represent the diameter of the inhibition zone including, 6 mm^2 of the paper disk. Based on **Figures 1** and **2,** *Lavandula* essential oil significantly shows antimicrobial activity against both *E. Coli* and *S. Aureus* bacteria. Different from *Citrus Limon,* which indicates moderate, consistent antimicrobial activity against the bacteria tested. Furthermore, *Citrus Bergamia* essential oil was weak against *E. Coli* and strong enough to inhibit the growth of *S. Aureus.* However, the perfume has no antimicrobial activity because the bacteria were resistant to the perfume solution. Adding aloe vera carrier oil into the perfume solution was done to reduce essential oil concentration and ensure safe skin contact. Unfortunately, the combination of essential oils and aloe vera carrier oil has antagonistic interactions, reducing antimicrobial activity against both bacteria tested [18].

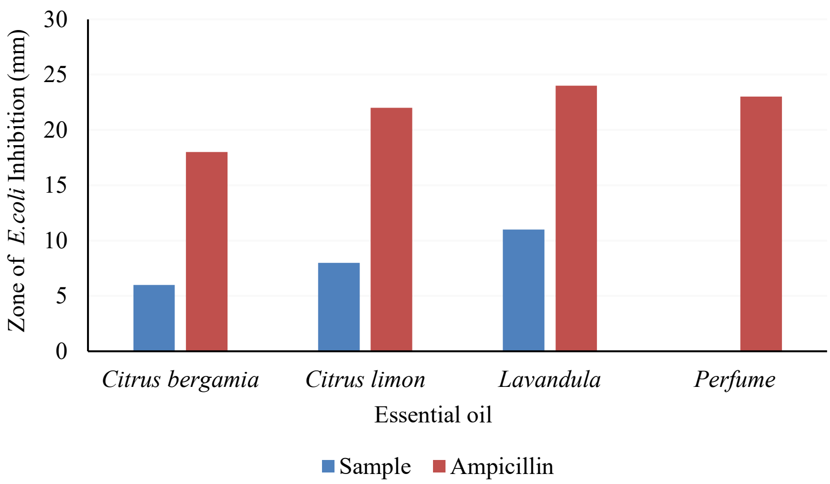

Figure 3: Antimicrobial activity of essential oils against *S. Aureus*.

Moreover, the comparison of antimicrobial values from **Figures 1** and **2** was much higher for gram-positive, *S. Aureus*. This is because gram-negative bacteria; *E. Coli,* form a barrier around the structure wall outer membrane to prevent the insertion of a hydrophobic compound, essential oils. The outer membrane barrier is lipopolysaccharides and lipoproteins compound as a defense mechanism for *E. Coli* protecting from an antimicrobial compound of essential oils [18]. This protective defense mechanism had lowered the antimicrobial value for essential oils in *E. Coli* instead of *S. Aureus* [8]. Lastly, the antibiotics positive control, ampicillin, and penicillin has no relation affecting the antimicrobial activity value of samples. The positive control purpose was to prove the sample indeed contains antimicrobial activity by the zone of inhibition value.

4. Conclusion

This study shows that bergamot essential oil, lemon essential oil, and lavender essential oil provide greater inhibition growth in *E. Coli and S. Aureus* than the drops of essential oils combined with aloe vera carrier oil is the perfume. In addition, individual single oils provided a more comprehensive antimicrobial benefit than observed by mixing these three essential oils with aloe vera carrier oil. In conclusion, the perfume is not suitable for daily use as it may irritate the skin due to the antimicrobial activities. However, more research may be needed to determine whether decreasing the amount of aloe vera carrier oil and increasing the amount of lavender essential oil improves the antimicrobial effects of the perfume.

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