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Preformulation of Lemongrass Oil (*Cymbopogon citrates* (DC) Stapf) and Turmeric Extract (*Curcuma xanthorrhiza* Roxb.) on the Growth of *Escherichia coli* Bacteria

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Abstract

Background: Lemongrass and turmeric are medicinal plants known for their antibacterial properties due to the presence of compounds such as alkaloids and flavonoids in lemongrass oil, and saponins and flavonoids in turmeric. Typically, lemongrass oil is used for massage oil, and turmeric is commonly used as a spice and traditional medicine. However, there is limited scientific information on the combination of both. **Objective**: This study aims to evaluate the antibacterial potential of a formulation combining lemongrass oil and turmeric extract against *Escherichia coli* bacteria, both in extract form. **Method:** The research steps include the preparation of the plant materials, extraction, granulation process, and antibacterial testing. Extraction is performed using boiling with distilled water as the solvent. The antibacterial activity testing method employed is the good diffusion method. **Results:** The formulation of lemongrass oil (*Cymbopogon citratus* (DC) Stapf) and turmeric (Curcuma xanthorrhiza Roxb.) has demonstrated antibacterial activity against *Escherichia coli* in both extract and granule forms across five different formulations. **Conclusion:** The highest inhibition diameter was observed with the combination of lemongrass oil and turmeric extract at 19.05 mm, and lemongrass oil and turmeric granules at 18.71 mm, in Formula 3 (0.25 lemongrass oil: 0.75 turmeric).

Keywords: Antibacterial, *Escherichia coli, Cymbopogon citrates* (DC) Stapf, Curcuma xanthorrhiza Roxb.

INTRODUCTION

The benefits of lemongrass, particularly from its stems and leaves, are widely recognized. It is frequently used as a cooking spice, in perfumes, as an ingredient in traditional herbal medicine (Jamu), and for producing essential oils. Lemongrass stems can serve as a diuretic, a sweat inducer, an expectorant for treating coughs, a mouthwash, a body warmer, a remedy for digestive disorders, stomach aches, colds, a fever reducer, and an antiemetic, among other uses. Lemongrass contains compounds that give it a distinctive aroma and a slightly spicy taste [1].

Temulawak (*Curcuma xanthorrhiza* Roxb.) is a medicinal plant belonging to the *Zingiberaceae* family. It is extensively cultivated and used as a raw material for traditional medicine. Temulawak is well-known not only in Indonesia but also globally. In 2012, the production of Temulawak in Indonesia reached 44,122 tons [2], [3].

Temulawak is widely utilized as a raw material in the pharmaceutical industry. Research has identified several compounds in temulawak, including curcuminoids [1]. In traditional medicine, Temulawak is commonly used to treat digestive disorders, jaundice, and leucorrhea, to boost the immune system, and to maintain overall health [4]. Additionally, Temulawak has anti-inflammatory, antioxidant, antimicrobial, antitumor, hepatoprotective, and antihyperlipidemic properties [5].

Previous studies have indicated that Temulawak has antibacterial potential. Given the medicinal properties of lemongrass and Temulawak in treating digestive disorders, this study investigates the antibacterial activity of lemongrass oil and Temulawak against bacteria commonly associated with traditional medicine. One such bacterium is *Escherichia coli* ATCC, which is a normal gut bacterium but can become

pathogenic under certain conditions, often causing diarrhoea and serving as an indicator of faecal contamination in water [6].

The combination of herbal medicines involves using two or more remedies simultaneously to enhance their individual effects. Traditional medicines are beneficial and safe when used correctly, considering the appropriate dosage, timing, method of use, and selection of ingredients that align with their indications and pharmacological effects (complementary effects) to determine treatment effectiveness.

Combining various components of essential oils with mild to moderate strength can produce synergistic or mutually reinforcing effects. This study will formulate a mixture of lemongrass oil (*Cymbopogon citratus* (DC) Stapf) and Temulawak extract (*Curcuma xanthorrhiza* Roxb.) to test its effects on the growth of *Escherichia coli* ATCC. The long-term goal of this research is to provide a scientific basis and preliminary assessment for utilizing a combination of lemongrass and temulawak as an herbal medicine.

RESEARCH METHODS

Preformulation Evaluation

Preformulation testing was conducted to determine if the granules produced are suitable for encapsulation. This testing includes flow time, angle of repose, and tap index [7].

Determination of Inhibition Zone Diameter

Antibacterial activity testing is conducted for each extract concentration obtained using the agar well plate diffusion assay method, with sterile metal supports [8].

The base layer of the medium is prepared by pouring 10 ml of Mueller Hinton Agar (MHA) into a sterile petri dish and allowing it to solidify. Once solidified, 0.1 ml of bacterial inoculum suspension is spread on the surface of this layer, followed by 25 ml of Mueller Hinton Agar (MHA) as a second layer, which is then homogenized. Sterile metal supports are immediately placed and arranged on the surface of the medium in such a way that the observation areas do not overlap. The metal supports are then slowly removed using sterile tweezers from the solidified agar surface, forming wells (holes) that will each receive the test extract solutions at various concentrations. The test extract solutions at various concentrations and a blank mixture of dimethyl sulfoxide: ethanol (4 ml: 3 ml) are each introduced into the available wells at 0.1 ml per well. The petri dishes are immediately covered and left for 30 minutes, then incubated in an incubator at 35±2°C for 24 hours. Observations are made by measuring the clear zones around the wells using a calliper to determine the diameter of the inhibition zone in millimetres (mm). Data is collected from three replicates.

RESULTS AND DISCUSSION

The flow time test was conducted by introducing a quantity of granules into a flow funnel until it was 2/3 full. The granules were then allowed to flow by opening the bottom cover of the funnel while simultaneously starting a stopwatch. The flow time recorded was 4.72 seconds. This result indicates that the average flow time meets the specified requirement, which states that the flow time for granules should be less than 10 seconds [9], [10].

The angle of repose test for the granules was performed by introducing the granules into a flow funnel and allowing them to flow out completely. The diameter and height of the resulting granule pile were then measured, yielding a diameter of 9.33 cm and a height of 2.16 cm. Consequently, the angle of repose obtained was 24.45°, which falls within the acceptable range of $20° < 0 < 40°$. A smaller angle of repose indicates better flow properties of the powder [11].

Antibacterial activity was determined using the agar well diffusion method. The principle of this method is the measurement of the diameter of the inhibition zone formed. The good diffusion method was chosen because it can produce a larger inhibition zone diameter, and osmolarity occurs more uniformly and homogenously compared to the disc diffusion method. In the good diffusion method, each well is filled with a concentration of the extract, leading to higher and more potent extract concentrations that inhibit bacterial growth more effectively. The data is shown in **Table 1** [12].

Table 1. Inhibition Zone Diameter Combination of Lemongrass Oil and Tumeric

Formula	Inhibition Zone Diameter	
	Lemongrass Oil: Tumeric Extract	Lemongrass Oil: Tumeric Extract Granule
Formula 1	18.41	15.4
Formula 2	11.38	8.40
Formula 3	19.05	18.71
Formula 4	18.73	13.73
Formula 5	11.71	14.73
Chloramphenicol	28.73	13.06
Untreated	0.00	0.00

Notes: Formula 1: Lemongrass oil:Tumeric Extract (1:0); Formula 2: Lemongrass oil:Tumeric Extract (0:1); Formula 3: Lemongrass oil:Tumeric Extract (0,25:0,75); Formula 4: Lemongrass oil:Tumeric Extract (0,5:0,5); Formula 5: Lemongrass oil:Tumeric Extract (0,75:0,25)

The results from the above table and graph indicate that the combination of lemongrass oil and turmeric, in both extract and granule forms, exhibited inhibitory zones. The combination of lemongrass oil and turmeric extract showed the largest inhibition zone in Formula 3, measuring 19.05 mm, and the smallest in Formula 2, measuring 11.38 mm. Similarly, the combination of lemongrass oil and turmeric granules in Formula 3 produced the largest inhibition zone of 18.71 mm, while Formula 2 showed the smallest inhibition zone of 8.40 mm.

The inhibition zone resulting from the extract was larger than that from the granule form, indicating reduced antibacterial activity in the granule form. This reduction could be due to the numerous processing steps and the presence of additional materials that might diminish the chemical compounds in the sample.

The results indicate that the highest inhibition zone for the combination of lemongrass oil and turmeric, in both extract and granule forms, was observed in Formula 3, where the amount of turmeric was greater than the amount of lemongrass oil. This is because the active compounds in turmeric are more potent at killing microbes when combined with a smaller amount of lemongrass oil. When the quantities of lemongrass oil and turmeric were equal, the inhibition zone was not as large. Increased dilution of a test substance results in a reduction of active compounds with antibacterial potential. The inhibition zone for the combination was larger than that for the individual components, likely due to a higher concentration of main secondary metabolites in the combination sample compared to the individual samples [3], [13].

The data show that in formulas 1 to 4, the inhibition zones produced by the granule form of turmeric were smaller than those produced by the turmeric extract. However, in Formula 5, the inhibition zone increased when the sample was in granule form.

Observation of the inhibition zones or clear areas around the wells indicated the presence of active compounds in lemongrass oil and turmeric extract that inhibit bacterial growth. Lemongrass oil contains alkaloids, terpenoids, and flavonoids. Alkaloids, which have many benefits in the health and cosmetic fields, are potent antibacterial agents because they can damage the cell wall by inhibiting cell wall synthesis, leading to cell lysis and death. Flavonoids form complexes with extracellular proteins, damaging the bacterial cell membrane and causing the release of intracellular compounds [14], [15].

Compounds in turmeric, such as saponins and flavonoids, also exhibit antibacterial activity by disrupting the stability of bacterial cell membranes, causing cell lysis [3]. An effective inhibition zone is defined as having a diameter of approximately 14-16 mm. An inhibition zone diameter of ≤ 5 mm indicates weak antibacterial activity, 5-10 mm indicates moderate activity, 10-20 mm indicates strong activity, and > 20 mm indicates very strong antibacterial activity [16].

The bacterial growth inhibition zone is indicated by the clear area around the wells where lemongrass oil and turmeric are applied. The tool used to measure the inhibition zone diameter is a calliper (mm), measuring the diameter twice at right angles. The data obtained show that the formulation of lemongrass oil and turmeric can inhibit the growth of *Escherichia coli* bacteria [12].

The antibacterial equation is as follows: Y = 15.97A + 13.28B + 9.87AB
Where:
- Y = Response
- A = Lemongrass Oil Component
- B = Turmeric Component
- AB = Interaction between the two components

The coefficients obtained are A (15.97), B (13.28), and AB (9.87). Based on these coefficients, both the combination formula of lemongrass oil and turmeric and the individual formulas enhance antibacterial activity, as indicated by the positive coefficients. The coefficient for lemongrass oil is higher than that for turmeric and their combination, suggesting that the single lemongrass oil formula has a more dominant effect on enhancing antibacterial activity according to the SLD method [11].

The contour plot of the formula shows that the combination of lemongrass oil and turmeric extract has a synergistic effect on antibacterial testing. The combination of equal amounts of lemongrass oil and turmeric extract results in the most potent (optimal) antibacterial activity. Thus, in the contour plot, the optimal antibacterial activity effect is observed in the combination form, while the single form shows a lower effect than the combination of the two samples.

CONCLUSION

The formulation of lemongrass oil and turmeric extract, as well as the antibacterial activity test of the lemongrass oil and turmeric granule formulation against *Escherichia coli* bacteria, demonstrated the presence of inhibition zones.

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