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Antibacterial Evaluation of *Etlingera elatior* Ultrasonicated Extracts

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ABSTRACT: This study investigated the antibacterial potential of *Etlingera elatior*, a medicinal plant abundant in Southeast Asia. The research focused on the antibacterial effects of hexane, ethyl acetate, methanol and aqueous extracts of the *Etlingera elatior* flower against various bacteria, including *Staphylococcus aureus, Escherichia coli, Shewanella oneidensis,* and *Bacillus* UMK DG-1. The extraction process involved ultrasonication, and disk diffusion tests were used to assess antibacterial activity. The methanolic extract of the *Etlingera elatior* flower exhibited significant antibacterial activity, where it exhibited the highest inhibition zones against *S. aureus* and *E. coli*. The aqueous extract displayed inhibition against *S. aureus* and *E. coli* as well, indicating its potential antibacterial properties. In addition, antibacterial activity evaluation on the growth of *Bacillus* UMK DG-1 and *E.coli* presented the most successful inhibition in the hexanoic extraction meanwhile, in ethyl acetate extraction, *E.coli* showed the most outstanding inhibition. Due to its easy availability and cost-effectiveness, *Etlingera elatior* holds promise as a natural source of antibacterial compounds for pharmaceutical use where ultrasonication extraction is one of the methods to obtain these properties.

Keywords: antibacterial; etlingera elatior; ultrasonic extraction; UAE

1. Introduction

The significance of herbs and natural products for therapeutic purposes is well recognized, with extensive research conducted on bioactive compounds, especially those from plant sources. Phytochemicals in medicinal plants provide health benefits beyond nutrients. Traditional medicine heavily relies on plant extracts, especially in developing countries. In Malaysia, herbs and spices are consumed like vegetables, particularly among Malays.

Etlingera elatior is a member of the ginger family known as Zingiberacea and is native to Indonesia and Malaysia [1]. *Etlingera elatior*, known as "bunga kantan" in Malaysia, belongs to the ginger family and has various uses including culinary and traditional medicinal applications. It is widely cultivated and recognized for its therapeutic properties. The plant's various varieties are used as spices, cut flowers, and ornaments. *Etlingera elatior* is native to Malaysia and Southeast Asia, known for its antibacterial and antioxidant properties. Previous studies have highlighted its antibacterial effects against common pathogens. An earlier investigation of E. elatior flower extracts found that they had pharmacological activities and that natural products could be used to generate new treatments for a variety of ailments. Numerous investigations have documented the antibacterial activity of *E. elatior* flower extracts. Lachumy [2] discovered that, methanol extract of *E. elatior* flower possesses antibacterial activity against common human infections like *Staphylococcus aureus, Escherichia coli, Bacillus thuringiensis, Bacillus subtilis, Microccocus spp., Salmonella spp., Proteus mirabilis, Aspergillus niger*, and *Candida albicans*.

Ghasemzadeh [3] discovered that, both aqueous and methanol extracts of the *E. elatior* flower possessed antibacterial efficacy against a variety of bacteria, including *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Listeria monocytogenes, Escherichia coli*, and *Salmonella Typhimurium.* An extraction method known as solvent extraction was employed in each and every one of these studies. Extraction is the first step to separate the desired natural product from the raw material. Extraction methods include solvent extraction, distillation methods, pressing and polishing according to the principle of extraction [4]. This study aimed to investigate the antibacterial activity of methanol, aqueous, ethyl acetate and hexane extracts of *Etlingera elatior* flowers using ultrasonication assisted extraction method.

2. Materials and Method

2.1 *Etlingera elatior* Sample preparation

The *Etlingera elatior* flowers were cleaned and dried for two weeks in sunlight, followed by a 4-hour drying process at 140°C in an oven. The dried flowers were ground into fine powder and stored in airtight containers. For extraction, 4 g of *Etlingera elatior* was mixed with 60 ml of methanol, aqueous, hexane and ethyl acetate solvents,

and ultrasonication was carried out for 45 minutes, repeating the process three times at 60% amplitudes. The extracts were then evaporated using a rotary evaporator at 40-60°C and stored at -80°C.

2.2 Ultrasonication Assisted Extraction of Etlingera elatior

Four grams of *Etlingera elatior* was weighed and mixed with 160 ml extraction solvents (hexane, ethyl acetate, methanol and water). The mixture underwent ultrasonication assisted extraction for 45 minutes. The extraction process was repeated three times with 60% amplitudes. The extract was evaporated with a rotary evaporator at 40-60°C, depending on the solvents boiling temperature. Once dried, the crude extract was stored at -20°C until further use.

2.3 Antibacterial Activity

The efficiency of the *Etlingera elatior* extract against bacteria was evaluated with antibacterial activity test. The *Etlingera elatior* extract was analyzed using the Kirby-Bauer method with Gram-positive bacteria (S. aureus and *Bacillus UMK DG-1* [5]) and Gram-negative bacteria (E. coli and Shewanella). The Kirby-Bauer disc diffusion method is a commonly used method for assessing the resistance of bacterial isolates [6]. The zone inhibition is measured, as it will show the antibacterial activity where the greater the inhibitory zone, the more potent the antibacterial action. These bacteria were obtained from stock culture maintained at the Microbiology Lab, Universiti Malaysia Kelantan. *Etlingera elatior* extracts were dissolved in a 10% DMSO solution to achieve a final concentration of 25 mg/ml and filtered. Inoculum was prepared by mixing bacterial suspension (turbidity equals to 0.5 MacFarland standard) with sterile distilled water and swabbed onto the agar plates using cotton swabs. About 10ml of the extracts were applied to sterile paper discs (6mm diameter) and placed on nutrient agar plates. A blank paper disc served as a negative control and ampicillin was used as the positive control. After incubation (24 hours at 30°C for *Bacillus* UMK DG-1 and *Shewanella oneidensis*, and 37°C for *E. coli* and *Staphylococcus aureus*), the growth inhibition zones were measured using a ruler. The antibacterial activity procedure was adapted from [7] where the concentration of *Etlingera elatior* extract used was 25 mg/ml. Every test was performed in triplicates (n=3) and presented as mean ±SD. All data was analyzed by GraphPad Prism8 Software.

3. Results and Discussion

3.1 Antibacterial analysis of *Etlingera elatior* extracts

Four different microorganisms - *S. aureus, E. coli, Bacillus* UMK DG-1, and *Shewanella oneidensis* were used to assess the antibacterial activity of various *Etlingera elatior* extracts as shown in Table 1. All four bacteria displayed inhibition in response to the methanol extract, with inhibition zones ranging from 6.67 to 10 mm. This suggests that the methanolic extract had potential antibacterial activity against all tested bacteria. In agar disc-diffusion tests, *Etlingera elatior* flowers demonstrated significant antibacterial activity against these pathogenic bacteria. Notably, the inhibition zone diameter was 7.0 mm for S. aureus, 6.67 mm for E. coli, 9.0 mm for *Bacillus* UMK DG-1, and 10.0 mm for *Shewanella oneidensis*, indicating strong inhibitory effects. In comparison, the positive control (ampicillin) exhibited inhibition zones ranging from 19.0 ± 1.0 to 41.67 ± 10.4 mm against the same bacteria (not stated in the table). However, all bacteria did not show any growth inhibition in the negative control. The results in Table 1 illustrate that the methanolic extract of *E. elatior* effectively inhibits the growth of *E. coli*, resulting in an average inhibition zone of 6.67 mm. Higher extract concentrations could potentially lead to larger inhibition zones. In the case of the methanolic extract, *Shewanella oneidensis* exhibits inhibition with an average diameter of 10.0 ± 2.65 mm. The table shows that the aqueous extract of *Etlingera elatior* only inhibited the growth of *S. aureus* and *E. coli*, not *Bacillus* UMK DG-1 or *Shewanella oneidensis*. The inhibition zones for *S. aureus* and *E. coli* were relatively large, ranging from 14.67 mm to 20.67 mm. The negative control had no effect on bacterial growth, while the positive control was effective against all four bacteria. In contrast, aqueous extracts showed antibacterial effects against some microorganisms, with *S. aureus* and *E. coli* being the most affected.

Table 1: Measured density and moisture content of the manufactured wood composites.

Bacteria	Hexanoic extract	Ethyl acetate extract	Methanolic extract	Aqueous extract
Shewanella oneidensis	6.67±2.9	NIL	10.0 ± 2.65	NIL
E.coli	8.0±2.0	9.7±2.3	6.67 ± 1.15	14.67 ± 4.5
Staphylococcus aureus	NIL	6	7.0 ± 1.0	20.67 ± 8.1
Bacillus UMK DG-1	8.0±7.2	6.3±0.6	9.0 ± 1.73	NIL

*Mean ± standard deviation; NIL= No inhibition zone

The hexanoic extract could only inhibit three bacteria which are *Shewanella oneidensis, E.coli*, and *Bacillus* UMK DG-1. However, the extract could not inhibit *S.aureus*, which concludes that the extract could not go against the bacteria. The hexanoic extract has the highest antibacterial activity against *Bacillus* UMK DG-1 and E.coli with zone inhibition of 8 mm and *Shewanella* with 6.67 mm of zone inhibition. The positive control for four bacteria showed the ampicillin could inhibit all four of them with zone inhibition ranging from 17 mm to 45 mm. The ethyl acetate extract could inhibit only three bacteria: *S.aureus*, *E.coli*, and *Bacillus* UMK DG-1. However, the extract could not inhibit *Shewanella oneidensis*, which concludes that the extract did not exhibit antibacterial activity against this bacterium. The hexanoic extract has the highest antibacterial activity against *E.coli* with a 9.7 mm diameter of zone inhibition, followed by *Bacillus* UMK DG-1 with zone inhibition of 6.3 mm and *S.aureus* with 6 mm of zone inhibition.

According to Wahyu [8], the differences in torch ginger flower source, extraction method, and solvent used can affect the concentration of secondary metabolites in the plants, consequently influencing their antibacterial activity. Differences in the diameter of inhibition zones at each concentration may have resulted from variations in the amounts of bioactive compounds present at each concentration. Factors such as the organism's level of sensitivity, the culture medium, the incubation circumstances, and the antibacterial compound's rate of diffusion all had an impact on the inhibition zone's size [9]. Antibacterial agents can affect bacteria differently depending on their sensitivity. Typically, compared to Gram-negative bacteria, Gram-positive bacteria are more sensitive to antibacterial agents. The cell wall of Gram-negative bacteria has an exterior barrier that prevents some antibiotic substances from penetrating or damaging it [10]. The most effective antibacterial activity was demonstrated by the methanol extracts of the examined plants against both Gram-positive and Gram-negative bacteria.

Ultrasonicator was employed in this research because it is a fast and efficient extraction technology that utilises ultrasonic to induce rapid movement of solvents, resulting in a faster mass transfer rate and acceleration of extraction [11]. Compared to other modern extraction procedures, the ultrasonicator is more economical, environmentally friendly, and practical because its reduced time consumption, improved oil quality, and lower cost. By creating cavitational bubbles, an ultrasonicator uses high frequency (20 kHz) pulses to create local hotspots with high shear stress and temperature on a macroscopic scale [11]. Simple, less time-consuming, and solvent-intensive than other extraction processes, the ultrasonicator can be simply used with other extraction techniques. This approach, which can be performed at room temperature, can prevent the oxidation and breakdown of natural compounds of interest. The three main variables determining the efficiency of ultrasonicator extraction are extraction time, solvent composition, and input power. The ultrasonicator can extract bioactive components in a short amount of time, at a low temperature, while requiring less energy and solvent [12].

4. Conclusions

The antibacterial study of *E. elatior* flower ultrasonicated-assisted extracts revealed that each of the solvents; methanol, aqueous, hexane and ethyl acetate extracts exhibited antibacterial activity. The methanolic extract effectively inhibited the growth of Gram-positive and Gram-negative bacteria at a concentration of 25 mg/ml. The aqueous extract showed stronger activity against *S. aureus* and *E. coli*, but not against *Bacillus* UMK DG-1 and *Shewanella oneidensis*. Antibacterial activity evaluation on the growth of *Bacillus* UMK DG-1 and *E.coli* revealed the inhibition in hexanoic extraction with 8 mm; meanwhile, in ethyl acetate extraction, *E.coli* showed inhibition with 9.7 mm. This suggests potential therapeutic applications of *E. elatior* flower extracts in the pharmaceutical industry against range of important pathogens. Furthermore, the ultrasonication-assisted extracts proved to exhibit the antibacterial activity which indicated that this method should be employed for plant extraction studies because it is economical, uses less solvents and less time-consuming.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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