Comparative Study between Percolation and Ultrasonication for the Extraction of Hibiscus and Jasmine Flowers Utilizing Antibacterial Bioassay

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ABSTRACT
Different extraction techniques can produce extracts of divergent active compounds from plants. Percolation with soxhlet and ultrasonication were used with ethanol solvent to extract hibiscus and jasmine flowers. These extracts were tested for antibacterial effect on Escherichia coli and Staphylococcus aureus with susceptibility test. The study was conducted by extracting the flowers with soxhlet and ultrasonic apparatus using ethanol as solvent. The extracts were separated from the solvent by evaporating the solvent with rotary evaporator. Discs of 6 mm were prepared from filter paper and were impregnated with the extracts. Sub-cultured test organisms, E. coli and S. aureus were swabbed on Mueller Hinton (MH) agars for susceptibility testing. The prepared discs were placed on the swabbed MH agar plates. Commercially prepared jasmine oil and hibiscus tea extracts’ discs were used as standards. Gentamicin discs were used as positive control and ethanol impregnated discs were used as negative control. Observations were recorded after 24 hours of incubation at 37 °C. Soxhlet and ultrasonic extracts of hibiscus flower showed antibacterial effect on E. coli and S. aureus. The hibiscus standard did not produce any effect on both E. coli and S. aureus. Soxhlet and ultrasonic extracts of jasmine flower showed no effect E. coli. Soxhlet and ultrasonic extracts of jasmine flower showed antibacterial effect on S. aureus. The jasmine standard showed antibacterial effect on both E. coli and S. aureus. Based on the results, it can be concluded that different extraction techniques administered on plants will yield different extracts. These extracts will display varying effects on bacteria like E. coli and S. aureus.

Key words: Percolation, Ultrasonication, Hibiscus and Jasmine Flowers, Antibacterial Bioassay, Escherichia coli and Staphylococcus aureus.

INTRODUCTION
Extraction is used in medical field to separate medically active portion of a plant by using selective solvent in standard extraction procedures. Soxhlet apparatus is designed to extract compound that has limited solubility in a solvent and the impurities are insoluble in that solvent. Ultrasonic extraction uses ultrasonic waves to extract active compounds of a plant. The ultrasonic field enables generation, locally of microcavitations in the liquid surrounding the plant material. Escherichia coli and Staphylococcus aureus are naturally occurring commensals in the human body. Both bacteria can cause a variety of diseases that are difficult to treat due to the increased drug resistance. There is a need to search for new drugs to overcome this and Malaysian flowers like hibiscus and jasmine has shown antibacterial effect on E. coli and S. aureus. Previous studies have shown that hibiscus possesses many biological activities, such as antipyretic, analgesic and anti-inflammatory activities. It has also been reported that the plant’s flower possesses antispermaticogenic, androgenic, antitumor and anticonvulsant properties in addition, the leaves and flowers have been found to be aid in the healing of ulcers. Parts of jasmine plant such as leaf, stem, bark and roots are also very useful and important in pharmaceutical industries. All the parts of this plant contain manitol. The plant is extensively used in manufacturing incenses, room fresheners and soaps. Juices from the leaves of jasmine plants are applied to treat ulcers, remove corns, effecting in expelling worms, regulating menstrual flow, to clean kidney waste, inflamed and blood shot eyes. Therefore this study was conducted to determine if extracts of hibiscus and jasmine from different extraction techniques have antibacterial effects on E. coli and S. aureus.

MATERIALS AND METHOD
Plant Material: The plant materials of hibiscus and jasmine were collected from a nursery located in Klang, Selangor. Plant Extraction: The plant flowers were collected, washed, the petals were removed from its stem and stamen and shadow dried. The flowers were then pulverised to coarse powder with an electric blender. The coarse

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Powders were used for extraction with ethanol using soxhlet and ultrasonic apparatus. The extracts were evaporated by rotary evaporator, labelled and stored for further use.

Optical Density Measurement of Extracts: All collected extracts’ optical density were measured using a...
spectrophotometer to determine their respective concentrations.[20]

Test Microorganisms: *Escherichia coli* and *Staphylococcus aureus* were obtained from Faculty of Health and Life Science of Management and Science University (MSU), Malaysia. The cultures of the bacteria were maintained in their appropriate agar plate at 4°C throughout the study and used as stock cultures.[19]

Culture Preparation for Susceptibility Test: The test microorganisms were sub cultured from their agar plates into nutrient broth. A single bacterial colony was removed from the agar and incubated in nutrient broth for 24 hours at 37°C.[20]

Discs Preparation: Filter paper was used to prepare 6 mm paper discs that were incubated with extracts and were air dried.[20] The positive control used was gentamicin discs. The negative control was ethanol incubated discs. Commercially prepared jasmine oil and hibiscus tea extracts were also incubated in paper discs and used as a comparison.

Susceptibility Test: The nutrient broth inoculated with the test organisms were swabbed on prepared Mueller Hinton agar with sterile cotton swabs. Then the discs with extracts impregnated were placed directly on the surface of the swabbed Mueller Hinton agar plates and were incubated at 37°C for 24 hours.[20] The test was done in triplicates. The

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**Table 2: Optical density of hibiscus samples**

<table>
<thead>
<tr>
<th>Hibiscus Sample</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Sample</td>
<td>0.000</td>
</tr>
<tr>
<td>Soxhlet Extract</td>
<td>0.913</td>
</tr>
<tr>
<td>Ultrasonic Extract</td>
<td>0.371</td>
</tr>
</tbody>
</table>

**Table 3: Effects of jasmine extracts**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Gentamicin (+ve)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>18.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>18.0</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Hibiscus Extracts: Hibiscus flowers extracted using soxhlet and ultrasonic extractions both have antibacterial effect on *Escherichia coli* and *Staphylococcus aureus* (See Figure 1 and Figure 2). Soxhlet extract of hibiscus flower has produced 6 mm of zone of inhibition and ultrasonic extract of hibiscus flower has produced 7 mm on *E. coli*. Whereas on *S. aureus*, soxhlet extract of hibiscus has produced 8 mm of zone of inhibition and ultrasonic extract of hibiscus flower has produced 11 mm (See Table 1). Standard sample of hibiscus did not exhibit any effect on *E. coli* and *S. aureus*. These observations show that both extracts of hibiscus flowers from soxhlet and ultrasonic has extracted the active compounds that can inhibit the growth of *E. coli* and *S. aureus*.

Optical density of the hibiscus samples were measured at 800 nm to determine the samples concentration. Based on the measurements (See Table 2), Soxhlet extract was more concentrated compared to the ultrasonic extract of hibiscus flower. However ultrasonic extract exhibited better antibacterial effect on *E. coli* and *S. aureus* compared to soxhlet extract (See Figure 3).

Soxhlet extract utilizes heat to produce extract, thus only heat stable compound were extracted and heat sensitive compounds were deactivated. Whereas in ultrasonic extraction, all types pf extracts were extracted because it did not utilize heat and ultrasonic extraction technique can extract three times better than normal extraction techniques. Therefore, it can be concluded that ultrasonic extraction technique has extracted more active compounds from hibiscus flower responsible for the inhibition of *E. coli* and *S. aureus* compared to the soxhlet extraction technique.

Jasmine Extract: Jasmine flowers extracted using soxhlet and ultrasonic extractions both have antibacterial effect on *Escherichia coli* and *Staphylococcus aureus* (See Figure 4 and Figure 5). Soxhlet extract of jasmine flower did not produce any effect but ultrasonic extract of jasmine flower has produced 7 mm on *E. coli*. Whereas on *S. aureus*, soxhlet extract of jasmine has produced 8 mm of zone of inhibition and ultrasonic extract of hibiscus flower has produced 9 mm (See Table 3). Standard sample of jasmine exhibited antibacterial effect on *E. coli* and *S. aureus*. These observations show that both extracts of jasmine flowers from soxhlet and ultrasonic has extracted the active compounds that can inhibit the growth of *S. aureus* but not *E. coli*.

Optical density of the jasmine samples were measured at 650 nm to determine the samples concentration. Based on the measurements (See Table 4), ultrasonic extract was more concentrated compared to soxhlet extract. Hence ultrasonic extract exhibited better antibacterial effect on *S. aureus* compared to soxhlet extract (See Figure 6).

Since soxhlet extract and ultrasonic extract did not show any effect on *E. coli*, it suggests that both extraction techniques did not succeed in extracting the active compound responsible to inhibit *E. coli*. Whereas for *S. aureus*, both soxhlet and ultrasonic extraction techniques have extracted the active compound responsible for the inhibition of the bacteria. Moreover, ultrasonic extraction technique has been able to extract more active compounds compared to soxhlet extraction technique, hence the more concentrated sample and better antibacterial effect on *S. aureus*. Therefore, it can be concluded that ultrasonic extraction technique has extracted more active compounds from jasmine flower responsible for the inhibition of *S. aureus* compared to the soxhlet extraction technique, but both extraction techniques failed to produce active compounds for the inhibition of *E. coli*.

CONCLUSION

In this study, it can be concluded that ultrasonic extraction of hibiscus flowers can produce better resulting extracts compared to soxhlet extraction to inhibit the *Escherichia*
coli and Staphylococcus aureus. Besides, jasmine flower extracted with ultrasonic extraction technique showed better effect compared to jasmine soxhlet extraction technique on inhibition of S. aureus. Therefore in conclusive, this study conveys that different extraction techniques can generate different active elements from hibiscus and jasmine flowers that may or may not exhibit antibacterial effects on common pathogenic bacteria such as E. coli and S. aureus.

REFERENCES