A Comparative Study of the Antibacterial Activity of Clove and Rosemary Essential Oils on Multidrug Resistant Bacteria

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Abstract

The essential oils of *Syzygium aromaticum* (clove bud) and *Rosmarinus officinalis* L. (rosemary) were obtained by hydro-distillation. The antimicrobial activity of clove bud oil and rosemary oil was investigated by agar well diffusion method against four multidrug resistant strains namely *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* as well as two standard strains, *Staphylococcus aureus* ATCC29213 and *Pseudomonas aeruginosa* ATCC27853. Both essential oils exhibited inhibitory effects towards all the test organisms, clove essential oil had antibacterial activity little higher than of rosemary oil, MICs ranged from 0.312% (v/v) to 1.25% (v/v) for all tested bacteria while MICs for rosemary oil ranged from 0.312% (v/v) to 5 % (v/v). Based on this finding, it may be suggested that these essential oils may be used as natural antibacterial agents to treat infections caused by multidrug resistant bacteria.

Keywords: Antibacterial, Essential oil clove, Rosemary, MIC

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1 Introduction

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. There was a medical belief since antibiotics discovery and their uses chemotherapeutic agents that this would lead to the infectious diseases eradication. However, the major factor for the dissemination and emergence of multi-drug resistant bacterial strains was the overuse of antibiotics. The worldwide emergence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Acinetobacter baumannii* has become a majortherapeutic problem. Multi-drug resistant strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* are widely distributed in hospitals and are increasingly being isolated from community acquired infections. Thus, as a result of the rapid global spread of emergence of resistant bacteria and the need to find new antibacterial agents. However even new generations of antibiotics have low effectiveness. For this reason, Natural products provide unlimited opportunities for new drug. The search for new antibacterial compounds an alternative way for solving this problem so herbal products are increasingly turning the researchers attention to develop better drugs against Multidrug Resistant Bacteria (MDR).

Cloves (*Syzygium aromaticum*) are belonging to the family Myrtaceae which are dried unopened floral buds of an evergreen tree. Clove is used as flavoring agent and as spice. It has been known as aromatic, stimulant and carminative, used for dyspepsia and gastric irritations. Antimicrobial and antioxidant properties have been known to clove buds and their essential oils.

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest...
antioxidant activity. Rosemary essential oil is also used as an antibacterial, antifungal and anticancer agent.

The aim of the present study was to investigate the antibacterial effects of the oil extracts of Syzygium aromaticum and Rosmarinus officinalis against multi-drug resistant strains isolated from clinical infection.

2 Materials and Methods

2.1 Collection and identification of plant materials

The leaves of rosemary plant and the flower buds of clove used in this study were purchased from the local market of Baghdad, Iraq. The plants were identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, and University of AlMustansiriah.

2.2 Extraction of volatile oil by steam distillation

Steam distillation method was used in order to obtain the oil extracts of the dried flower buds of cloves and the dried rosemary by using the Clavenger apparatus as mentioned by Harbone. Briefly, 120g of grinded dried two plants were placed in a round bottom flask and the added water full approximately three-quarter of the flask. The distillation apparatus was then connected to the flask and by heating the water boiled, steam carrying volatile condensed by condenser and over anhydrous sodium sulphate the oil was drained and dried.

Determination the density of the oil was carried according to the weight: volume ratio (w/v).

2.3 Bacterial test isolates

Four multidrug resistant bacteria clinical isolates and two standard strains were selected for this study. The multidrug resistant bacteria comprised Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis were isolated from different clinical samples (stool, wound, ear, throat and sputum) and the standard strains were Staphylococcus aureus ATCC 29213 and Pseudomonas aeruginosa ATCC27853. The bacteria were cultured on nutrient agar medium. The agar plates were incubated at 37°C for 24 h.

2.4 Antibacterial assay

Determination the antibacterial activities of each plants oils was carried out by agar well diffusion method. Briefly, one hundred microlitres (100μL) of inoculums (10^6 CFU/mL; 0.5 Mac-Farland) of each test bacterium was spread on to a sterile Muller-Hinton Agar plate (Hi Media). The plates were allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 50μL volume of each dilution of the oil with DMSO (10% to 0.312% v/v) introduced in triplicate wells into Muller-Hinton Agar plate. Sterile DMSO served as the negative control. The plates were allowed to stand for at least 1h for diffusion to take place and then incubated at (37 °C for 24h). The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 6mm.

2.5 Determination of minimum inhibitory concentration (MIC)

The MIC was defined as the lowest concentration that completely inhibited the growth for 24 h. The MIC for the oils of each plant was determined by the agar well diffusion method. A two-fold serial dilution of the clove oil and rosemary oil was prepared in sterile DMSO to achieve a decreasing concentration range of 10% to 0.039% (v/v). A 50 μL volume of each dilution was added aseptically into the wells in Mueller Hinton agar plates that had been inoculated with standardized inoculums (10^6 CFU/mL) of the test bacteria. The agar plates were incubated at 37°C for 24 h. All experiments were performed in triplicate. The lowest concentration of clove and rosemary oils showing a clear zone of inhibition was considered as the MIC.

3 Results

The essential oils obtained were yellow in color with a density of 1.04 mg/ml for Syzygium aromaticum flower buds and 1.07 mg/ml and Rosmarinus officinalis L.

In the present investigation, we have tested the essential oil extracts of the two plants for their antimicrobial activity against four multidrug resistant strains. Two ATCC strains, one of Gram-positive bacterium (Staphylococcus aureus ATCC 29213) and one of Gram-negative bacterium (Pseudomonas aeruginosa ATCC 27853) were also used as control sensitive strains.

The two plants essential oils showed antibacterial activity against all microorganisms tested, as exhibited by an agar diffusion assay. Acinetobacter baumannii was found to be the most sensitive to rosemary oil at the higher concentration (10% v/v) with an inhibition zone diameter of 35 mm, followed by Pseudomonas aeruginosa (34 mm). Staphylococcus aureus and Enterococcus faecalis were found to be less sensitive to the test oil with an inhibition zone diameter of 30mm and 32 mm, respectively. However the lower concentrations of rosemary oil under (5% v/v) were found to be not effective in all tested multidrug resistant bacteria except Acinetobacter baumannii as shown in table 1.

The growth of the tested bacteria was also inhibited by clove oil. The widest inhibition zone diameter was produced against Acinetobacter baumannii (28mm) at the higher concentration (10% v/v) followed by Enterococcus faecalis and Pseudomonas aeruginosa produced an inhibition zone diameter of (25mm) and (17mm) respectively (table 2). Acinetobacter baumannii and Enterococcus faecalis were found not
sensitive to the concentration less than (1.25% v/v) and *Staphylococcus aureus* was not sensitive to the concentration less than (0.625% v/v).

As it can be seen, both essential oils exhibited inhibitory effects towards all the test organisms. Clove essential oil exhibited a little higher antimicrobial activity than that of rosemary essential oil, which was similar to the results of sensitivity test (Tables 1 and 2). MICs for clove essential oil ranged from 0.312% (v/v) to 1.25% (v/v) for all test microorganisms, while MICs for rosemary oil ranged from 0.312% (v/v) to 5% (v/v).

### Table 1: Antibacterial activities of rosemary essential oil

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Diameter of growth of inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>20±1.8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>34±2.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30±1.8</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>32±2.3</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>30±2.6</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 29213</td>
<td>25±3.4</td>
</tr>
</tbody>
</table>

(-) = no activity, Values, including diameter of the well (6 mm), are means of three replicates ± SD

### Table 2: Antibacterial activities of clove essential oil

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Diameter of growth of inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>28±2.3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>17±3.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20±2.8</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>25±2.6</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>25±1.4</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 29213</td>
<td>22±1.6</td>
</tr>
</tbody>
</table>

(-) = no activity, Values, including diameter of the well (6 mm), are means of three replicates ± SD

### 4 Discussion

The increasing occurrence, particularly in hospitals, of multidrug resistant bacteria has made therapy more difficult. The spread control strategies have been proposed and also searched for treatment MDR bacteria, the alternative treatment of these bacteria is by using natural compounds investigations.

The antibacterial activity were seen against MDR bacteria. However, the clove oil was more active against *Acinetobacter baumannii* and *Enterococcus faecalis* isolates at concentrations of 0.312% and above while rosemary oil was active against all bacteria tested at concentrations of 5% and above except *Acinetobacter baumannii* which was sensitive at concentrations of 0.312%.

Antibacterial properties of clove and rosemary have already been reported but in the present study we tested the antibacterial activity of clove and rosemary essential oils on multidrug resistance bacteria. The antibacterial activity of the essential oils of against clove and rosemary MDR bacteria has not been reported before.

MICs of clove and rosemary essential oils in this study were similar to the known literature with a little difference, which could be several reasons such as different growing environment of clove and
rosemary, different extracting methods of essential oils, and may be due to the use of MDR bacteria in the present study.

Different essential oils have different antimicrobial activity because of the components in them\(^{24}\). The antimicrobial activity of clove essential oils could be associated with Eugenol (2 methoxy-4 allylphenol)\(^{25}\), the main component of clove oil, which is already known to exhibit antibacterial and antifungal activity\(^{26}\). Clove antimicrobial activity also due to high tannin content (10-19\%)\(^{26}\). The main compounds present in rosemary essential oil were 1,8-cineole, α-pinene, camphor, etc., which have been evaluated for their antimicrobial effects\(^{31,32,33}\).

Based on these data, further chemical and pharmacological investigations are required for clove and rosemary essential oils. These in vitro results of the present study provide evidence that the two essential oils represent a potentially rich source for medicine and food preservatives against microorganisms.

5 Conclusion

On comparison of the antibacterial activities of clove oil and rosemary oil tested against the MDR bacterial strains, it was finally concluded that clove oil and rosemary oil emerged as the potent agent exhibiting high antibacterial activity in the treatment of the infections caused by multidrug-resistant bacteria and the clove oil is more potent than rosemary oil.

6 Competing interests

Plant extraction, antimicrobial evaluation, multidrug resistance

7 Author’s contributions

BHA carried out literature review, draft the manuscript, data collection and tabular form. BHA and SHF carried out the bacterial isolation and antibacterial evaluation. WJ participated in the plants extraction and data collection. All authors read and approved the final manuscript.

References


Abdullah et al. A Comparative Study of the Antibacterial Activity