The antibacterial activity of aqueous extract of peppermint and Bay leaf against *Staphylococcus aureus*

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ABSTRACT

**Background:** Microbial resistance to antibiotics especially among *Staphylococcal* strains is a major threat to public health. Therefore plant extracts are alternatives now to treat resistant organisms. **Materials and Method:** The effect of different concentration of two aqueous extracts (peppermint and bay leaf) against *Staphylococcus aureus* was evaluated using the agar diffusion method and determination of minimum bactericidal concentration (MBC) value of peppermint and bay leaf were also done in this study. **Results:** The results of this study demonstrated that peppermint extract exhibit more activity than bay leaf on *Staphylococcus aureus* but both of them were found to have inhibitory effect against *Staphylococcus aureus*. The minimum bactericidal concentration was shown that 25% was the MBC for peppermint and 35% was the MBC for bay leaf. **Conclusion:** This in vitro study suggests that peppermint and bay leaf may be used as antibacterial agent against *Staphylococcus aureus*. **Key words:** Antibacterial activity, peppermint, bay leaf, *Staphylococcus aureus*. (J Bagh Coll Dentistry 2011;23(146-150)).

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (1). The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. *Staphylococcus aureus* is recognized as one of the major cause of nosocomial infections and food poisoning (2). Microbial resistance to antibiotic especially among *Staphylococcus* strains is a major threat to public health. Therefore it is extremely important to find new antimicrobial agents that are effective for the treatment of infectious diseases caused by *Staphylococcus aureus* (3).

Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (4). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine and constitute a major source of natural organic compound (1).

Plants extracts shown to possess antibacterial, antifungal, and antioxidant properties like peppermint and bay leaf, theses extracts have been traditionally used by people for various proposes in different part of the world (5,6).

Peppermint (*Mentha piperita*) is the most popular herbal remedies all over the world that belong to the family *Lamiaceae* (7). In vitro, peppermint has significant antimicrobial and antiviral activities, strong antioxidant, antiulcer and antitumor actions, antispasmodic, anti-inflammatory, antiseptic and some antiiallergenic potential (8). Bay leaf (*Laurus nobilis*) belongs to the family *Lauraceae* has shown antimicrobial and antifungal characteristics, hypoglycaemic and antiulcerogenic properties. Bay leaf has been used to treat epilepsy, neuralgia and Parkinsonism (9,10).

The present study was undertaken in order to evaluate the in-vitro antibacterial activity of peppermint and bay leaf aqueous extract against *Staphylococcus aureus*.

MATERIALS AND METHODS

Evaluation of antibacterial effects of two aqueous extracts (peppermint and bay leaf) against *Staphylococcus aureus* was done in this study.

The two aqueous extracts were prepared according to Cowans (11) that 100g of dried powder plants were infused in 1000 ml of boiled distilled water, and left for one hour at room temperature. Agitation of the infusion with stirrer had been done alternatively. Then the infusions were filtered by filter paper and residue discarded. The two extracts left to dry in glass petridishes at the incubator (37 °c) the resulted powder kept in tightly closed glass container in refrigerator until used to prepare different concentrations.

Five diagnosed isolates of *Staphylococcus aureus* obtained from Baghdad teaching laboratories were used in the present study. An 18
hours culture of *Staphylococcus aureus* at 37 °C in 10 ml Mueller Hinton broth was used. The cultures were adjusted to approximately \(10^5\) CFU/ml with sterile saline solution.

Agar diffusion technique was applied to study the antibacterial effect of the previously mentioned extracts. Final concentration of 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60% and 70% of the aqueous extract of peppermint and bay leaf in Mueller agar were obtained for each extract separately.

Mueller Hinton agar plates were swabbed with a suspension of *Staphylococcus aureus* prepared as mentioned earlier, using sterile cotton swab. Plugs were removed from each agar plate producing holes.

To each hole 100 μl from different concentration of each extract was added and allowed to diffuse at room temperature for 20 min, the plates were then incubated aerobically overnight 37 °C.

Determination of the minimum bactericidal concentration, the lowest concentration of the antimicrobial agent causing negative growth (fewer than three colonies) was done by serial dilution of Mueller Hinton broth and lineation of Mueller Hinton agar \(^{12,13}\).

RESULTS

Each extract was tested against the five isolates of *Staphylococcus aureus*. The antibacterial activity of the extracts was recorded as the mean diameter of the resulting inhibition zones of growth measured in (mm). The antibacterial activity of peppermint and bay leaf aqueous extract is in figure (1).

The results revealed that peppermint exhibited antibacterial activity against *Staphylococcus aureus*. As the mean of the diameter of inhibition zones were 7mm, 8.5mm, 10mm, 11mm, 12.5mm, 13mm, 15mm 16mm for the concentrations of 10%, 15%, 20%, 30%, 40%, 50%, 60% and 70% respectively.

![Figure 1: The relationship between the concentrations of peppermint and the bay leaf in (%) with the diameter of inhibition zones against *Staphylococcus aureus* in (mm).](image)

Figure 1 also showed that bay leaf extract exhibited antibacterial activity against *Staphylococcus aureus* and the mean of the diameter of inhibition zone were 8mm, 10mm, 11mm, 12mm, 13.5mm, 14.5mm for the concentrations of 20%, 30%, 40%, 50%, 60% and 70% respectively while the concentrations of 5%, 10%, 15% did not give any inhibition zone.

The results of the present study using t-test demonstrated that there are significant differences for peppermint and bay leaf for most concentrations used in this study and this was demonstrated in table 1.

Table 2 showed the minimum bactericidal concentration (MBC) of peppermint and bay leaf aqueous extract. The results of this study demonstrated that the minimum bactericidal concentration (MBC) of peppermint aqueous extract for *Staphylococcus aureus* was 25% the majority of *Staphylococcus aureus* isolates were
sensitive at this concentration it seems to be toxic to these bacteria.

For bay leaf aqueous extract the concentration of 35% was able to make all isolates or strains of Staphylococcus aureus sensitive at this concentration and it was considered the MBC.

Table 1: Students t-test between peppermint and bay leaf aqueous extract for each test concentration in relation to the sensitivity of Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Percent concentration</th>
<th>Mean (mm)</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10%</td>
<td>7</td>
<td>S</td>
</tr>
<tr>
<td>15%</td>
<td>8.5</td>
<td>S</td>
</tr>
<tr>
<td>20%</td>
<td>10</td>
<td>S</td>
</tr>
<tr>
<td>30%</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>40%</td>
<td>12.5</td>
<td>S</td>
</tr>
<tr>
<td>50%</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>60%</td>
<td>15</td>
<td>S</td>
</tr>
<tr>
<td>70%</td>
<td>16</td>
<td>S</td>
</tr>
</tbody>
</table>

S: Significant difference at level P<0.05.
Mean: mean of diameter of inhibition zones

Table 2: Minimum bactericidal concentrations of peppermint and bay leaf aqueous extract for Staphylococcus aureus.

<table>
<thead>
<tr>
<th>type of extract</th>
<th>frequency</th>
<th>No. of isolates within the MBC of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>Peppermint extract</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Bay leaf extract</td>
<td>5</td>
<td>0</td>
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</table>

DISCUSSIONS

The antimicrobial effect of medicinal plants is well documented and the results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agent even against some antibiotic-resistant strains of bacteria (14).

In this current study using the agar diffusion technique it was observed that extract of peppermint produce antibacterial activity against Staphylococcus aureus; these results confirmed the observation of earlier studies of Botani et. al. (15). Gupta et. al also (16) reported that peppermint was effective against Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, the mode of antibacterial action of this extract is related to it’s ability to inactivate microbial and cell envelope proteins (14).

Deans and Baratta (17) shows that the compounds from peppermint possess potent antimicrobial activity and suggesting that the peppermint extracts should contains the effective active constituents responsible for eliminating the bacterial pathogens. Menthol is the active compound in peppermint in addition to many potent compounds such as menthone, menthol acetate, menthofuran, and limnone and the earlier studies suggested that the antibacterial activity of peppermint was probably due to this compound found in peppermint and Menthol is virucidal against influenza, herpes and other viruses (18).

In another hand Chamsai et. al. (19) reported that the effect of peppermint on Staphylococcus
Staphylococcus aureus appears to have good preventive treatment with regard to pathogen when using it as hand sanitizer on hand and shoe higher antimicrobial activity compared to water washes. Tassan et al. (20) also demonstrated that a low concentration of essential oil of peppermint (<0.1%), the addition of glucose in the growth medium prevented the formation of staphylococcal enterotoxin B.

The results of our study revealed that bay leaf extract also exhibited antibacterial activity against Staphylococcus aureus. This was in agreement with the results of Hammer et al. (21) who found that bay leaf extract inhibit the growth of Staphylococcus aureus this was also reported by Özkan et al. (22) Dadalioglu and Evrendilek (23)

The effect of bay leaf may be explained by the action of its main components like 1, 8- cineole, eugenol, sabinene and 4-terpineol (9). While Celikel and Kavas (24) found Staphylococcus aureus showed low susceptibility to bay leaf extract. This may be due to the differences in bacterial strains of and differences in the preparation production of extract, in this study we used crude aqueous of bay leaf.

Sensitivity of Staphylococcus aureus to different concentrations of peppermint and bay leaf extracts were tested separately. As mentioned earlier both of them have antibacterial activity against Staphylococcus aureus and the inhibition effect of each extracts increases with increasing concentration (24).

Findings of the present study are similar to those reported by Liu and Nakano (25) who found that bay leaf showed potent and maximum activity against Staphylococcus aureus.

There are a few informations on the mechanism of inhibition of bacteria by plants extracts, some potential modes of action suggested that interference with the phospholipid bilayer of the membrane may cause increased permeability and loss of cellular constituents or impairment of a variety of enzyme system and may affect the production of cellular energy and synthesis and structural components (26).

From our results we noticed that peppermint extract had more effects than bay leaf extract for most concentrations used on Staphylococcus aureus. While another study found that bay leaf exhibited greater activity than peppermint on Staphylococcus aureus. This may be due to the differences in mode of action of each extract and the differences of molecular weight of the active ingredient in each extract and the main factors that determine antibacterial activity are type, composition of the extract used, pH and temperature of the environment (25). In conclusion peppermint and bay leaf were found to have important antimicrobial activity against Staphylococcus aureus.

REFERENCES


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