Antibacterial effect of eucalyptus (globulus Labill) and garlic (Allium sativum) extracts on oral Cariogenic bacteria

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ABSTRACT: The antibacterial activity of the plant extracts including garlic and eucalyptus extracts against cariogenic bacteria, Streptococcus mutans and Lactobacillus acidophilus were investigated. To evaluate the effect of different concentrations of alcoholic extracts of eucalyptus and garlic on S. mutans and Lactobacillus acidophilus, 72 sheep blood Muller Hinton agar were prepared. The sensitivity profile of the bacteria was determined according to the method of Bauer-Kirby. The sterile blank papers stained by seven different concentrations of the extracts were dried and put on the media inoculated with the test organisms. Four media from each group were considered as controls, two of which were added and the other two were added effective antibiotics, penicillin (10 IU) and erythromycin (15 µg/ml) disks. Inhibition zones were measured after 24 h of incubation at 37°C in the presence of 5% CO₂. Interpretation of resistance was compared with the media containing effective antibiotics. Both bacteria, S. mutans and Lactobacillus acidophilus, were resistant to eucalyptus extract but sensitive to garlic extract. The inhibition zone diameter increased as concentration of garlic extract increased. Furthermore, both bacteria were susceptible to penicillin and erythromycin. However, S. mutans was more sensitive to erythromycin while Lactobacillus acidophilus was more sensitive to penicillin. It seems that garlic extract has a potential antibacterial effect against cariogenic agents such as S. mutans and Lactobacillus acidophilus in vitro, while eucalyptus extract had no effect. However, we need more scientific evidence based on randomized clinical trials in order to investigate the antimicrobial activity of garlic against these bacteria in vivo and its effect on prevention or reduction of dental caries.

Keywords: Plant extracts, Cariogenic agents, Dental caries, Streptococcus mutans, Lactobacillus

INTRODUCTION

Dental caries and periodontal diseases are the most common chronic diseases in the world (Swift et al., 2002). The acidic oral environment is well tolerated by Streptococcus mutans and Lactobacillus acidophilus. These bacteria are strongly stimulated by sucrose and are known as the main organisms responsible for human tooth decay. S. mutans mainly initiate tooth decay while Lactobacillus acidophilus makes it progress to dental cavity-like lesion (Swift et al., 2002). Agents such as S. mutans and facultative anaerobic Gram-positive bacteria are usually found in the human oral cavity. These bacteria is the most common pathogens isolated from human dental plaque and play an important role in
dental caries by converting sucrose into lactic acid and predisposing teeth to decay (Ryan and Ray, 2004).

Garlic (Allium sativum) has antibacterial, antifungal and antiviral properties (Dayaghi et al., 2007; Bakri and Douglas, 2005). There is evidence that garlic extract may inhibit the growth of oral pathogens and may have therapeutic value for periodontitis (Bakri and Douglas, 2005). In addition, investigations have shown that garlic is effective in vitro against several opportunist infections in acquired immunodeficiency syndrome (AIDS), including candidiasis, herpes and Cryptococcus infections as well as cryptosporidium, cytomegalovirus, and pneumocystis carinii (Müller et al., 2005; Geraci et al., 2001).

Eucalyptus essence has antimicrobial activity against some bacteria such as Vibrio cholerae, Aspergillus flavus and Staphylococcus aureus (Mahboobi et al., 2007). Furthermore, garlic extract has antimicrobial effect on both Gram-positive and Gram-negative bacteria and can inhibit protease activity of Porphromonas gingivalis (Bakri and Douglas, 2005). Eucalyptus has two or three major components at high concentration (Burt, 2004; Idaomar et al., 2008). The main components are terpenes and terpenoids as well as aromatic and aliphatic. The monoterpenes are the most representative molecules, constituting 90% of EOs and comprising a great variety of structures (Burt, 2004). The leaves of Eucalyptus have been used for wounds healing, fungal infections, boils, and gastrointestinal disorders (Mahady et al., 2012; Ashour, 2008). Traditionally, eucalyptus spp. essential oil has been used to treat respiratory tract disorders and infections such as pharyngitis, bronchitis, and sinusitis. Recently, the interest in natural medicine has been increasing and scientific attention to this field is expanding. Studies showed antibacterial and antiviral activity of myrtaceous in eucalyptus oil (Mohammadypour et al., 2006; Sakaino et al., 2004). Accordingly, eucalyptus has a number of components which have antimicrobial activity.

Despite numerous studies which investigated antibacterial effect of garlic extract, there are limited evidence to assess the effect of antibacterial effect of eucalyptus extract. Furthermore, there is limited evidence showing the antibacterial effect of eucalyptus and garlic against cariogenic bacteria. Thus, the aim of this study was evaluation of antibacterial activity of eucalyptus and garlic extracts against S. mutans and Lactobacilli which are two major cariogenic bacteria.

MATERIALS AND METHODS

In this experimental study, which was conducted in July 2011, extracts from eucalyptus (globulus Labill) and garlic (Allium sativum) were provided. In order to prepare eucalyptus extract, first, 10 gr of dried eucalyptus leaf was ground (Ahmad and Beg, 2001). Then, added 50 ml of ethanol 96° (96%). The mixture was stored at lab temperature (22°C) for 24 hours and mixed it every two hours. Then, the supernatant was centrifuged at round 3000 g for 10 min, passed the obtained alcoholic extract through 0.45 micrometers filter, put in dark containers, and kept at refrigerator temperature (4°C) (Najar et al., 2005).

In order to prepare garlic extract, 10 gr of garlic was ground and added it to 80 ml of ethanol 96° (96%). The mixture was incubated at 22°C for 24 hours. Then, the lipid mixture was centrifuged at round 3000 g for 10 min, passed the obtained alcoholic extract through 0.45 micrometers filter, put in dark containers, and kept at refrigerator temperature (4°C) (Najar et al., 2005).

To evaluate the effect of different concentrations (non diluted, 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64) of alcoholic extracts of eucalyptus and garlic on S. mutans PTCC 1683 and Lactobacillus acidophilus PTCC 1643 (obtained from Microbiology Department of Hamadan University of Medical Sciences), 72 Muller Hinton agar (MHA) (MERCK CO) with 5% sheep blood (four groups of 18 each) were prepared.

Antimicrobial susceptibility was tested by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clsci., 2007). The sterile blank papers (BBL Co) were stained by seven different concentrations of the extracts were dried at 37°C for half an hour and placed on the surface of 72 sheep blood MHA inoculated with the test organisms (equal to McFarland standard No 0.5), of which, 36 media for S. mutans and 36 for Lactobacillus acidophilus. Four media from each group were considered as controls, two of which were added nothing and the other two were added effective antibiotics, penicillin G (10 IU) and erythromycin (15 µg/ml) disks. Inhibition zones were measured after 24 h of incubation at 37°C in the presence of 5% CO2. Interpretation of resistance was compared with the media containing effective antibiotics.

Pearson correlation coefficient used to assess the correlation between the diameter of non-growth zone and concentration of garlic extract using statistical software Stata 10 (StataCorp, College Station, TX, USA).

RESULTS

S. mutans was sensitive to garlic extract. The diameter of non-growth zone increased as the concentration of garlic extract increased (Table 1). There was a strong positive correlation between the diameter of inhibition zone and the
Table 1. The effect of garlic and eucalyptus extracts on *streptococcus mutans* and *Lactobacillus* in Muller Hinton agar.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Diameter of <em>streptococcus mutans</em> inhibition zone (mm)</th>
<th>Diameter of <em>Lactobacillus</em> inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Garlic extract</td>
<td>Eucalyptus extract</td>
</tr>
<tr>
<td>Non diluted</td>
<td>64</td>
<td>56</td>
</tr>
<tr>
<td>1/2</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>1/4</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>1/8</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>1/16</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>1/32</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>1/64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean [95% CI]</td>
<td>25.9 [15.5, 36.3]</td>
<td>0</td>
</tr>
<tr>
<td>Pierson correlation coefficient</td>
<td>0.9215</td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

concentration of garlic extract (r=0.9215). However, *S. mutans* was not sensitive to different concentrations of eucalyptus extract and the non-growth zone was seen in none of the media containing eucalyptus extract.

*Lactobacillus acidophilus* was sensitive to different concentrations of garlic extract on blood agar medium. The diameter of inhibition zone increased as the concentration of garlic extract increased Table 1 and Figure 1.. The correlation between the concentration of garlic extract and inhibition zone was strongly positive (r=0.9549). However, *Lactobacillus acidophilus* was not sensitive to different concentrations of eucalyptus extract leading to lack of inhibition zone.

Both *S. mutans* and *Lactobacillus acidophilus* were sensitive to the antibiotics penicillin and erythromycin. However, *S. mutans* was more sensitive to erythromycin than to penicillin while *Lactobacillus acidophilus* was more sensitivity to penicillin than to erythromycin (Table 1).

**DISCUSSION**

We indicated that alcoholic garlic extract has antibacterial effect against *S. mutans* and *Lactobacillus acidophilus* at different concentrations. Nonetheless, eucalyptus extract has no antibacterial activity against these bacteria even at high concentrations.

There is considerable interest in the using of new classes of antimicrobials for the control of infection, because of bacterial resistance to antibiotics. Garlic has been used as a medication since ancient times (Bakri and Douglas, 2005). Garlic extract has inhibitory effect against methicillin-resistant *S. aureus* (Cutler and Wilson, 2004) and *C. albicans* (Bakri and Douglas, 2005), and multi drug resistant tuberculosis (Geraci et., 2001). It is very effective against a range of oral Gram-negative species while being less active against oral Gram-positive species. However, allicin may also have therapeutic use for periodontitis and possibly other oral infections (Bakri and Douglas, 2005).

Antibacterial effect of garlic extract against bacteria other than *S. mutans* and *L. acidophilus* has been reported in previous studies. Li et al. reported in 2007 that garlic extract is effective against not only *S. mutans* and *L. acidophilus* but also against *Actinomyces, Wolinella, Peptococcus, Prevotella,* and *Fusobacterium* (Zhu et al., 2007). Groppo et al indicated in 2002 that both garlic and chlorhexidine have antimicrobial activity against *S. mutans*, but have no effect on other oral microorganisms (Sartoratto et al., 2002). In addition, Groppo et al reported in 2007 that 2.5% garlic mouthwash solution have in vivo antimicrobial activity and is capable to inhibit the activity of *S. mutans* and other oral microorganisms (Sartoratto et al., 2007).

The antimicrobial properties of eucalyptus essential oils have been known for many years and have been used against a wide variety of bacteria and fungi, including oral pathogens [22,23]. Eucalyptus was used as potential natural agents for food preservation as pharmaceutical products because of antimicrobial activities and chemical composition (Rota et al., 2011). The activity of eucalyptus globulus essential oil was studied for 120 isolates of *S. pyogenes*, 20 isolates of *S. pneumoniae*, 40 isolates of *S. agalactiae*, 20 isolates of *S. aureus*, 40 isolates of *H. influenzae*, 30 isolates of *H.*
Figure 1. Algorithm for adding different concentrations of garlic and eucalyptus extracts to the media containing *streptococcus mutans* and *Lactobacillus*
**parainfluenzae**, 10 isolates of *K. pneumoniae*, 10 isolates of *S. maltophilia* and two viruses, a strain of adenovirus and a strain of mumps virus. *H. influenzae*, *H. parainfluenzae*, and *S. maltophilia* were the most susceptible, followed by *S. pneumonia*, and *S. agalactiae*. *K. pneumoniae* did not show susceptibility to eucalyptus oil (Quaglio et al., 2008). Eucalyptus oil also has anti herpes virus activity (Reichling et al., 2001).

Essential oils significantly retard biofilm formation and caries progression so it could be used as anti-caries treatments (Claffey, 2003). Essential oils antimicrobial properties have been described for many years (Astaneh et al., 2009). Mouthwashes of essential oil is effective against oral microorganisms, which has safe components for daily oral health (Claffey, 2003). It is recommended that twice daily brushing with essential oil-containing toothpastes may have clinically significant incremental benefit [28]. Clinical studies have demonstrated that essential oil mouthwash have excellent safety and tolerability profiles with no side effects such as extrinsic tooth stain , no aberration of intra-oral soft-tissue, no changes in taste perception and no increase in calculus formation (Astaneh et al., 2009). Eessential oils can affect biofilm formation and decrease bacterial adhesion with potential ability to act as novel agents in preventing caries and periodontal disease (Astaneh et al., 2009).

We showed that eucalyptus extract had no antibacterial effect on cariogenic bacteria such as *S. mutans* and *L. acidophilus*. So far, antimicrobial activity of eucalyptus against cariogenic bacteria has not been investigated. Nonetheless, the results of previous studies indicated that eucalyptus extract could prevent tooth decay through inhibition of biofilm formation of plaques. For example, Nagata et al (Kataoka et al., 2008) conducted a randomized double-blinded clinical trial in 2008 in order to assess the effect of eucalyptus extract against periodontal diseases and caries. They reported that using chewing gum containing eucalyptus extract could improve gingival index, decrease bleeding during probing, and reduce periodontal diseases compared with the control group.

**CONCLUSION**

It seems that garlic extract has a potential antibacterial effect on cariogenic bacteria such as *S. mutans* and *Lactobacillus acidophilus in vitro* while eucalyptus extract had no effect. However, we need more scientific evidence based on randomized clinical trials in order to investigate the antimicrobial activity of garlic against these bacteria *in vivo*.

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**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no conflicts of interest.

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