Effectiveness of *Mentha piperita* Leaf Extracts against Oral Pathogens: An *in vitro* Study

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**ABSTRACT**

**Aim:** The study aims to assess the *Mentha piperita* leaf extract’s effectiveness against oral pathogens.

**Materials and methods:** The leaf extract of *M. piperita* was prepared using cold water method. The three microbial strains, i.e., *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, and *Candida albicans* were used as microbiological materials. Chlorhexidine 0.2% was used as positive control. The digital caliper was used to measure the zone of inhibition to know the antimicrobial activity at 24 and 48 hours. To compare the activity within and between the different microbial strains, one-way analysis of variance (ANOVA) was used. To analyze the data, Statistical Package for the Social Sciences (SPSS) software version of 21.0 was used. The p-value <0.05 was considered as statistically significant.

**Results:** Maximum inhibition zone was seen in both *M. piperita* extracts and 0.2% chlorhexidine with *S. mutans* at 24 and 48 hours, followed by *A. actinomycetemcomitans*, and *C. albicans* respectively. The statistical analysis ANOVA reveals the statistically significant association of *M. piperita* extracts with *p*-value <0.001. The comparison with 0.2% chlorhexidine at 24 hours showed a *p*-value of <0.04 and at 48 hours, it showed a *p*-value <0.001, which was statistically significant.

**Conclusion:** The present study concluded that *M. piperita* showed antimicrobial activity against the oral microorganisms which are causing major less or more severe oral diseases and it can be administered as an alternative medicine for the conventional treatment.

**Clinical significance:** The study results serve as a guide in selecting and providing information about the efficacy of *M. piperita* extracts to the dental professionals. The discovery of a potential herbal medication would be a great development in the field of antimicrobial therapies.

**Keywords:** Antimicrobial activity, *Mentha piperita*, Oral pathogen, Zone of inhibition.

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**Conflict of interest:** None

**INTRODUCTION**

The major issue worldwide in health care field is the oral disease. The issues include tooth loss, periodontal diseases, orofacial disorders, and dental caries. These are some of the diseases among the important oral health issues. Some of them may cause health concerns which are significant.1

Evidences which are associated with oral health and chronic conditions are considered, such as the association of poor oral health and aggressive periodontal diseases with that of the systemic diseases, such as lung disease, osteoporosis, strokes, rheumatoid arthritis, diabetes, heart attack, and other cardiovascular diseases.2 In addition to it, periodontal disease may also cause complication during pregnancy, such as preterm low birth weight. In adult patients, 20% of the tooth loss is mainly due to poor periodontal health, resulting in significant morbidity and may lead to premature death.3

The ancient custom followed around the world is the use of oral care agents made up of herbal products. As an alternative to the expensive antibiotics and their side
The effectiveness of Mentha piperita leaf extracts against oral pathogens is investigated. Mentha piperita, a member of the Lamiaceae family, is a perennial herb that grows quickly and can reach up to 1.5 m in height under favorable conditions. The variable species M. piperita is distributed around the Mediterranean region, eastward into Asia, and in Europe. In India, minor sore throat and irritation of the throat or minor mouth are treated with peppermint leaves. It is used in the treatment of minor sprains and aches and used as a nasal decongestant. In addition, it has antiseptic, antiparasitic, carminative, and stimulant properties.

To assess the effectiveness of leaf extracts of M. piperita against three oral microorganisms, such as C. albicans, A. actinomycetemcomitans, and S. mutans, an in vitro study was conducted.

**Materials and Methods**

The present in vitro study was conducted at the Department of Periodontology, Educare Institute of Dental Sciences, Kerala, India. The present in vitro study was conducted at the Department of Periodontology, Educare Institute of Dental Sciences, Kerala, India.

**Collection of Leaf**

Matured, disease-free, healthy leaves of M. piperita were collected from the local garden directly. The leaves were washed under tap water and are cleaned in the research laboratory of the Department of Microbiology. The leaves were chopped into small pieces, air dried for 7 days, at room temperature. The dried leaves were finely powered using a blender machine.

**Cold Water Extract Method**

The crude preparation was done using a conical flask; 15 gm of powder was mixed in 100 mL of distilled water (cold water extract) and was left overnight inside the shaker at 35°C. The preparation was centrifuged at 2500 rpm for 10 minutes. The centrifuged product was transferred into a preweighed beaker. The supernatant plant extract was concentrated by evaporating the solvent at 60°C. The weighed crude extract was dissolved in dimethyl sulfoxide of known volume. The sterilized final concentration was filtered through Millipore filters (0.45 µm). The extracts in aqueous form were stored at 4°C in sample bottles prior to use.

**Strains used**

Three microbial strains, such as S. mutans, A. actinomycetemcomitans, and C. albicans, which mainly cause more or less oral infections of severe intensity were collected from Sudharma Metropolis, Thrissur.

**Disk Diffusion Method**

To determine the bacterial growth inhibition by plant extract, the disk diffusion method is used. In this method, the disks were aseptically placed over the bacterial culture of agar-nutrient plates incubated for 24 hours at 37°C. A digital Vernier caliper was used to measure the zones of inhibition around the disk after inoculation.

**Evaluation of Antimicrobial Activity**

The disk diffusion method will determine the efficacy of M. piperita leaf extract to inhibit the formation of new bacterial and fungal colonies by forming an inhibitory zone. Candida albicans was suspended in sterile saline of 2 mL, and each bacterium was suspended under peptone water of 2 mL. The turbidity of the suspension was set to 0.5 McFarland standard using turbidimeter. A lawn was created on Sabouraud dextrose agar (C. albicans) (Fig. 1) and Mueller–Hinton blood agar medium (S. mutans and A. actinomycetemcomitans) (Figs 2 and 3) plates using the sterile cotton swab dipped in the suspension. Using...
sterile forceps, the sterile disks were impregnated with
M. piperita leaf extract of 80 µL and were applied on the
agar surface. The agar plates were incubated at 37°C. And
0.2% chlorhexidine used as positive control. The zone of
inhibition was measured at 24 to 48 hours in millimeters,
using a digital caliper.

Statistical Analysis
The analysis of the results was done by calculating
mean and standard deviation (SD) using the SPSS
software version 21.0. To compare within and between
microbial strains, one-way ANOVA was used. The data
with a significance level of p < 0.05 are statistically
significant.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>M. piperita extracts</th>
<th>0.2% chlorhexidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans</td>
<td>20.16 ± 0.36</td>
<td>32.64 ± 1.34</td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td>18.34 ± 1.09</td>
<td>28.45 ± 0.22</td>
</tr>
<tr>
<td>C. albicans</td>
<td>15.83 ± 1.37</td>
<td>27.66 ± 1.85</td>
</tr>
</tbody>
</table>

Table 3: Comparison of mean zone of inhibition with M. piperita extracts after 24 hours

<table>
<thead>
<tr>
<th>Extract</th>
<th>Microorganism</th>
<th>Mean ± SD</th>
<th>Standard error</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. piperita extracts</td>
<td>S. mutans</td>
<td>20.16 ± 0.36</td>
<td>0.1742</td>
<td>64.520</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>A. actinomycetemcomitans</td>
<td>18.34 ± 1.09</td>
<td>0.2260</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>15.83 ± 1.37</td>
<td>0.0890</td>
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<td></td>
</tr>
</tbody>
</table>

Table 4: Comparison of mean zone of inhibition with M. piperita extracts after 48 hours

<table>
<thead>
<tr>
<th>Extract</th>
<th>Microorganism</th>
<th>Mean ± SD</th>
<th>Standard error</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. piperita extracts</td>
<td>S. mutans</td>
<td>34.18 ± 1.46</td>
<td>0.0054</td>
<td>58.164</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>A. actinomycetemcomitans</td>
<td>30.48 ± 1.82</td>
<td>0.6879</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>28.75 ± 2.57</td>
<td>0.1752</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS
The mean zone of inhibition of M. piperita extracts at
24 hours against oral pathogens is shown in Table 1. The
maximum zone of inhibition with both M. piperita extracts
and 0.2% chlorhexidine was seen with S. mutans (20.16 ±
0.36 and 32.64 ± 1.34), followed by A. actinomycetemcomitans
(18.34 ± 1.09 and 28.45 ± 0.22) and C. albicans (15.83 ±
1.37 and 27.66 ± 1.85).

The mean zone of inhibition of M. piperita extracts at
48 hours against oral pathogens is shown in Table 2. The
maximum zone of inhibition with both M. piperita extracts
and 0.2% chlorhexidine was seen with S. mutans (34.18 ±
1.46 and 40.11 ± 0.98), followed by A. actinomycetemcomitans
(30.48 ± 1.82 and 37.76 ± 1.78) and C. albicans (28.75 ±
2.57 and 33.62 ± 1.54).

The comparison of the mean zone of inhibition at
24 and 48 hours with M. piperita extracts is shown in
Tables 3 and 4. The analysis of covariance showed a
highly statistical significant association at 48 hours with
p-value <0.001.

The comparison of the mean zone of inhibition at
24 and 48 hours with positive control is shown in Tables 5
and 6. The analysis of covariance showed a statistically
significant association at 24 and 48 hours with p-value
less than 0.04 and 0.001 respectively.

DISCUSSION
Increase in the antibiotic resistance and its side effects has
led the researchers to suggest the plant extracts as an alter-
native for the treatment of voracious infectious diseases.8

Fig. 3: Zone of inhibition of A. actinomycetemcomitans
Mentha piperita is a good antibacterial, antiseptic, and antiviral agent. It is clean, light, and has a refreshing aroma; it is a good insect repellant. It has a strengthening and stimulating effect used in the treatment of shock, neuralgia, and as a relief agent in general debility, migraines, and headaches. Its antispasmodic and antiseptic effect helps to reduce sinusitis, throat infections, flu, asthma, cold, bronchitis, mucus, and in relieving coughs. It is used as inhalants, applicants, or bathing agents. It has a cleansing and cooling effect to soothe itchy skin and relieve inflammation. The peppermint property gives the mouth fresh feel, adds taste to the formula and also increases salivation which helps in dry mouth condition resulting in halitosis.

The cold water leaf extract used in this study was similar to the Zamin et al study, which suggested that cold water M. piperita leaf extract has broad-spectrum antimicrobial activity, though the degree of vulnerability may differ within different microorganisms. This antimicrobial activity is found to prove the presence of secondary metabolites either in combination with various chemical compositions or individual component of a plant.

Aggregatibacter actinomycetemcomitans showed the zone of inhibition more than C. albicans and less than that of S. mutans at 24 and 48 hours, which is similar to that of Karicheri and Antony study, which showed that A. actinomycetemcomitans demonstrated antibacterial property out of 68 strains using disk diffusion method; 53 (77.9%) were sensitive against M. piperita and 52 (76.5%) were sensitive against M. arvensis oil.

Chlorhexidine 0.2% was used as a positive control in the present study. It is similar to Balagopal and Arjunkumar and Mathur et al studies, which mention about the chlorhexidine formulations which are considered as gold standard anti-gingivitis and antiplaque mouth rinses due to their extended broad-spectrum activity toward microorganisms and plaque-inhibitory potential.

Candida albicans showed the minimum zone of inhibition in the current study, similar to that of Doddanna et al study, which stated that few extracts of plant, such as onion bulb and leaves, curry leaves, tea leaves, and aloe vera, which have medicinal values, are screened to evaluate their antimicrobial activity against C. albicans. Candida albicans were strongly repressed by the alcoholic curry leaves followed by the aqueous tea leaves. The alcoholic mint leaves, alcoholic aloe vera, alcoholic onion bulb, alcoholic tea leaves, and alcoholic onion leaves were known to inhibit the C. albicans’ growth in increasing order, but are not as strong as above-mentioned extracts.

A good antibiofilm activity was exhibited by M. piperita against gram-positive pathogens, L. monocytogenes as per Sandasi et al. Mentha piperita’s organic leaf extracts showed its wide range of broad-spectrum antibacterial activity as stated by Bupesh et al. These activities are attributed to the presence of potential compounds including menthone, menthofuran, menthyl acetate, and menthol. Menthol alone has been proved to inhibit the organisms, such as bacteria, viruses, and fungi which contribute to the overall antimicrobial activity of the plant extract of M. piperita.

The compounds of M. piperita that were investigated by Baratta et al proved to have antimicrobial activity and they suggested that the leaf extract of M. piperita contains the active component which is effectively responsible for eradicating the pathogens.

The data proved that the herbal leaf extract exhibits its variation in the effectiveness of their antimicrobial property against microorganisms which are tested. Considering the limitation of the in vitro studies, it is necessary to mention that these results may alter in in vivo analysis because the environment tested will differ from that of the oral cavity. Therefore, in vitro studies are necessary to support further clinical investigations. The result from this study can provide the information to the dental professionals regarding the efficacy of the M. piperita leaf extract and acts as a supporting document for further studies on M. piperita.

The low effectiveness, high cost, and toxicity of the recent antimicrobial agents available in the market make...
it inefficient. The discovery of potent plant medication will be a great development in the line of antimicrobial therapies. This shows a need to develop new antimicrobial agents which can satisfy the current demand.

**CONCLUSION**

The present study concluded that *M. piperita* has been proved to have antimicrobial activity against oral microorganisms and can be used as an alternative medicine and as an adjunct to the conventional therapy, which would help the countries which are developing and having financial constraints and with limited oral health care facility for the concerned population.

**REFERENCES**


