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ORIGINAL RESEARCH

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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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

effects, plant remedies are recognized as the important alternatives by the scientists.[4]

The peppermint (botanical name: *Mentha piperita*), member of Lamiaceae, a large mint family, is a perennial herb which grows fast and can reach up to 1.5 m height under favorable conditions. The extremely variable species *M. piperita* is distributed around 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.[5]

Considering all these 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.

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 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 in to 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.[6]

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.[7] 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

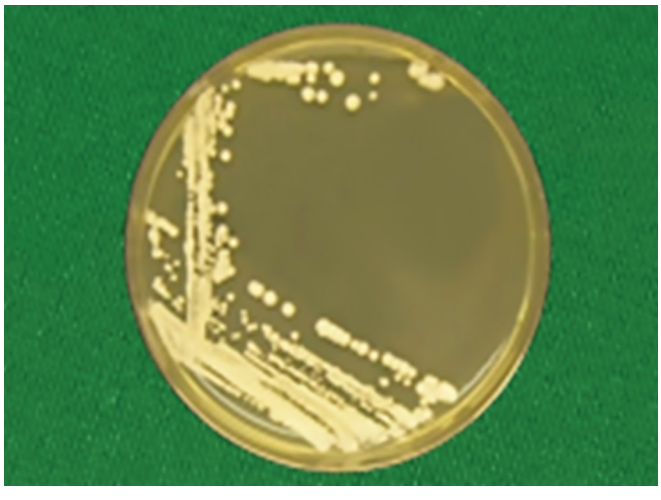

Fig. 1: Zone of inhibition of *C. albicans*

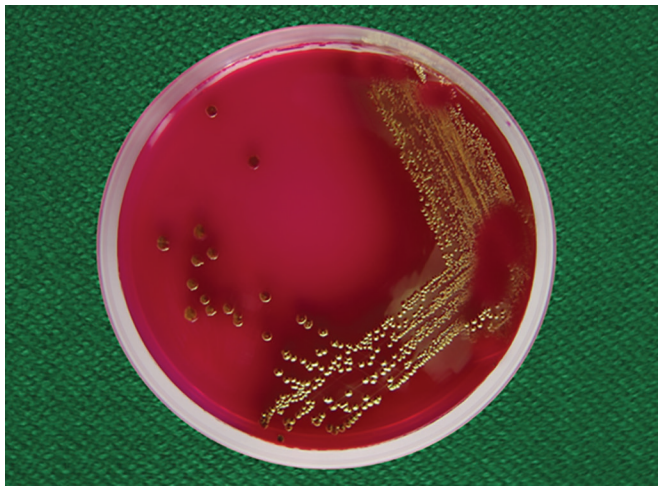

Fig. 2: Zone of inhibition of *S. mutans*

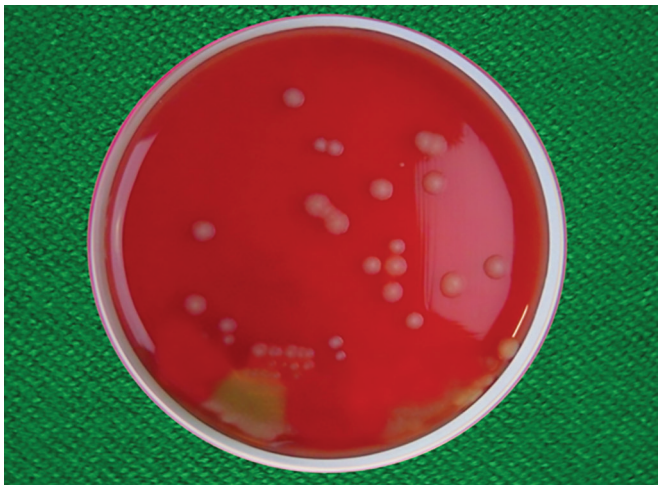

Fig. 3: Zone of inhibition of *A. actinomycetemcomitans*

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.

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 alternative for the treatment of voracious infectious diseases.[8]

Table 1: Mean zone of inhibition of *M. piperita* extracts against oral pathogens at 24 hours

	Mean of zone of inhibition (in millimeters)	
Microorganism	*M. piperita extracts*	*0.2% chlorhexidine*
S. mutans	20.16 ± 0.36	32.64 ± 1.34
A. actinomycetemcomitans	18.34 ± 1.09	28.45 ± 0.22
C. albicans	15.83 ± 1.37	27.66 ± 1.85

Table 2: Mean zone of inhibition of *M. piperita* extracts against oral pathogens at 48 hours

	Mean of zone of inhibition (in millimeters)	
Microorganism	*M. piperita extracts*	*0.2% chlorhexidine*
S. mutans	34.18 ± 1.46	40.11 ± 0.98
A. actinomycetemcomitans	30.48 ± 1.82	37.76 ± 1.78
C. albicans	28.75 ± 2.57	33.62 ± 1.54

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

Extract	*Microorganism*	*Mean ± SD*	*Standard error*	*f-value*	*p-value*
M. piperita extracts	*S. mutans*	20.16 ± 0.36	0.1742	64.520	0.08
	A. actinomycetemcomitans	18.34 ± 1.09	0.2260		
	C. albicans	15.83 ± 1.37	0.0890		

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

Extract	*Microorganism*	*Mean ± SD*	*Standard error*	*f-value*	*p-value*
M. piperita extracts	*S. mutans*	34.18 ± 1.46	0.0054	58.164	0.001
	A. actinomycetemcomitans	30.48 ± 1.82	0.6879		
	C. albicans	28.75 ± 2.57	0.1752		

Table 5: Comparison of mean zone of inhibition with positive control after 24 hours

Extract	*Microorganism*	*Mean ± SD*	*Standard error*	*f-value*	*p-value*
0.2% chlorhexidine	*S. mutans*	32.64 ± 1.34	0.0211	73.683	0.04
	A. actinomycetemcomitans	28.45 ± 0.22	0.1532		
	C. albicans	27.66 ± 1.85	0.1332		

Table 6: Comparison of mean zone of inhibition with positive control after 48 hours

Extract	*Microorganism*	*Mean ± SD*	*Standard error*	*f-value*	*p-value*
0.2% chlorhexidine	*S. mutans*	40.11 ± 0.98	0.1773	69.546	0.001
	A. actinomycetemcomitans	37.76 ± 1.78	0.0034		
	C. albicans	33.62 ± 1.54	0.2908		

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.[9] It has 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.[10]

The cold water leaf extract used in this study was similar to the Zamin et al[11] 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[4] 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[12] and Mathur et al[13] 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[14] 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 are 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.[15] *Mentha piperita*'s organic leaf extracts showed its wide range of broad-spectrum antibacterial activity as stated by Bupesh et al.[16] 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[17] 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 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.

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