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Original Article

Dentistry Section

Comparison of the Anti-microbial Efficacy of Clove, Cinnamon, Turmeric, Nutmeg, and Peppermint Essential Oil against Oral Pathogens: An In-vitro Study

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ABSTRACT

Introduction: Antibiotics are routinely used in dental practice to treat microbial diseases. However, the spread of drug resistant pathogens has drawn researchers' interest in finding new anti-microbial agents against oral microbial diseases.

Aim: To compare the anti-microbial activity of clove oil, cinnamon oil, turmeric oil, nutmeg oil, and peppermint oil against oral pathogens.

Materials and Methods: It was an in-vitro study. The organisms used in this study were *Streptococcus mutans, Candida albicans and Enterococcus faecalis.* Agar Well Diffusion Method was used to assess the anti-microbial efficacy of the five essential oils against oral pathogens. Five discs/concentration/micro-organism were used in the study. The zone of inhibition was measured after incubation. Kruskal Wallis Test was used to compare the difference in the anti-microbial activity of the oils.

Results: The mean zone of inhibition of clove oil against *Streptococcus mutans, Candida albicans,* and *Enterococcus faecalis* was highest at 100 µL (29.8 mm, 44.75 mm, 40.33 mm, respectively), cinnamon oil was highest at 100 µL (39.8 mm, 40.33 mm, 40 mm, respectively), turmeric oil was highest at 100 µL (41.8 mm, 40.50 mm, 40 mm, respectively), peppermint oil was highest at 100 µL (41.8 mm, 27.16 mm, 17 mm, respectively). Nutmeg oil against was highest at 100 µL (24 mm, 24.83 mm, 9.3 mm, respectively).

Conclusion: Clove oil, cinnamon oil, turmeric oil, nutmeg oil, and peppermint oil had anti-microbial activity against oral pathogens. Based on the findings, the most effective oil against *Streptococcus mutans* was turmeric oil and peppermint oil. The most effective oil against *Candida albicans and Enterococcus faecalis* was clove oil.

Keywords: Anti-bacterial, Anti-fungal, Oral microflora, Plant extract oil

INTRODUCTION

One of the most serious threats to the successful treatment of oral microbial diseases is the spread of drug-resistant pathogens. Hence, it has become necessary to find out new anti-microbial agents. Essential oils and other extracts of plants have drawn researchers' interest as sources of natural products [1]. Several small terpenoids and phenol compounds are present in essential oils which are responsible for their anti-microbial activity [2]. These compounds are mostly considered safe [3]. Essential oils such as clove oil, cinnamon oil, turmeric oil, nutmeg oil, and peppermint oil have been used traditionally.

Clove oil (derived from *Syzygium aromaticum*) has biological activities, such as anti-bacterial, anti-fungal, insecticidal, and anti-oxidant properties [4]. The strong biological and anti-microbial activities of clove essential oil are due to the high levels of eugenol in it. Cloves are used to alleviate the pain of toothaches, as disinfectant root canals in temporary fillings, and as an oral anaesthetic [5].

Cinnamon oil (derived from *Cinnamomum zeylanicum*) has many health benefits like-it is loaded with anti-oxidants, has anti-inflammatory properties, anti-microbial properties, lowers blood sugar level, protective against cancer, and may reduce the risk of heart disease [6]. Turmeric oil (derived from *Curcuma longa*), a rhizomatous herbaceous perennial plant also has many health benefits like anti-microbial and anti-cancer properties [7].

Peppermint oil (derived from *Mentha Piperita*) is also known to possess several anti-microbial, anti-septic, anti-spasmodic, and anti-bacterial properties [8]. Nutmeg oil (derived from *Myristica fragrans*) is known to have hypolipidemic and hypocholesterolemic effects, anti-microbial, anti-depressant, aphrodisiac, memory-enhancing, anti-oxidant, and hepatoprotective properties [9]. Since all these essential oils have shown anti-microbial properties, they were included in the study to assess their efficacy against oral pathogens.

Streptococcus mutans is known to be a significant contributor to dental caries [10]. *Candida albicans* is responsible for oral thrush [10,11]. *Enterococcus faecalis* is commonly detected in the root canals of teeth with post-treatment apical periodontitis or refractory/advanced marginal periodontitis [12]. It is highly resistant to anti-microbial agents since it carries virulence factors related to adhesion and biofilm formation [13].

These microorganisms were used in the study since they are commonly detected in the oral cavity and are a major source of concern. Since these pathogens have become drug resistant, the authors need to switch to alternative anti-microbial agents and using herbal products is quite a safe option [13-15].

Many studies have been conducted to assess the anti-microbial activity of clove, cinnamon, turmeric, nutmeg, and peppermint oil against oral pathogens [16-20], but no study has been done till date to compare these oils and find out which one is most effective. Hence, the aim of this study was to compare the anti-microbial activity of clove oil, cinnamon oil, turmeric oil, nutmeg oil, and peppermint oil against oral pathogens.

MATERIALS AND METHODS

It was an in-vitro study conducted in the Department of Microbiology, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India. The study was initiated in August 2022 and was conducted over a period of two weeks. Ethical clearance was obtained from the Institutional Ethical committee (SRB/SDMD03/18/PHD/22).

Procedure

Sample collection: Clove buds (*Syzygium aromaticum*), cinnamon sticks (*Cinnamomum zeylanicum*), turmeric roots (*Curcuma longa*), ground nutmeg (*Myristica fragrans*), and leaves of peppermint plant (*Mentha Piperita*) were purchased from the market. These samples were authenticated by Dr S Rajeshkumar, Professor, Department of Pharmacology, Saveetha Institute of Medical And Technical Sciences, Chennai, Tamil Nadu, India, using the Macroscopic method. The samples were authenticated based on characteristics such as size, colour, shape, surface characteristics and were compared to a standard reference material [21].

Extraction of essential oils: Approximately, 100 g of clove, cinnamon, turmeric, nutmeg, and peppermint samples were weighed and transferred into 2-litre round flasks separately. Each sample was then mixed with 1.35 liters of distilled water. Clevenger apparatus was used to obtain essential oil of the study samples. The flask was attached to the Clevenger trap and then heated. Each of the samples was heated for eight hour using hydrodistillation process. After eight hour, the oils were collected in the Clevenger and were allowed to cool at 28°C room temperature. At the bottom of the oils, water collected was first drained to separate from the oils. To remove the remaining trace water, the oils were treated with anhydrous sodium sulphate ($Na_2 SO_4$). The obtained essential oils were kept in a vial and then stored in a refrigerator. The procedure followed was in accordance to the method described by Jusoh S et al., and Ahmad FB et al., [22,23].

Test organisms: Pure cultures of *Streptococcus mutans, Candida albicans,* and *Enterococcus faecalis* were obtained from the Department of Pharmacology, Saveetha Dental College, Chennai, Tamil Nadu, India.

Anti-microbial assay: Agar Well Diffusion Method was used to assess the anti-microbial efficacy.

Media preparation: A 100 ml of Mueller Hinton agar (for *Streptococcus mutans* and *Enterococcus faecalis*) and 20 mL of ROSE Bengal Agar (for *Candida albicans*) was prepared, sterilised, and poured onto the petri-plates. The plates were allowed for solidification.

Swabbing: After solidification, the respective plates (5 plates/concentration/micro-organism) were swabbed with the oral pathogens-*Streptococcus mutans, Candida albicans, and Enterococcus faecalis.*

Well formation: After swabbing, four wells were formed on each plate using a gel puncher. To three wells, respective oil was loaded in the concentration range of 25 µL, 50 µL, and 100 µL [24]. To the fourth well, an antibiotic (amoxicillin) was loaded which was used as a control. The plates were then incubated at 37 °C for 24 hour and after incubation, the zone of inhibition was measured and calculated. Each oil was tested against the three microorganisms.

Measurement of zone of inhibition: A ruler was held against the back of the petri-plate and the zones of inhibition were measured in millimeter.

STATISTICAL ANALYSIS

Data were entered in a Microsoft Excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) software (version 23.0). Descriptive statistics were used to express the zone of inhibition. Kruskal Wallis test was used to compare the difference in the anti-microbial activity of the essential oils at 25 µL, 50 µL and 100 µL. Mann-Whitney U test was used to make pairwise comparison between the different concentrations of each oil. The p-value less than 0.05 were considered statistically significant.

RESULTS

[Table/Fig-1] depicts the comparison of the zone of inhibition of all the five essential oils against the oral pathogens. For *Streptococcus mutans*, the mean zone of inhibition was highest for turmeric oil and lowest for nutmeg oil at 25 µL. At 50 µL, the mean zone of inhibition was highest for peppermint oil and lowest for nutmeg oil. At 100 µL, the mean zone of inhibition was highest for both turmeric and peppermint oils and lowest for nutmeg oil. The findings were highly significant (p<0.001). At 25 µL, the mean zone of inhibition of cinnamon oil, turmeric oil, and peppermint oil was higher than the control. At 50 µL and 100 µL, the mean zone of inhibition of all the oils was higher than the control except for nutmeg oil.

For *Candida albicans*, the mean zone of inhibition was highest for cinnamon oil and lowest for peppermint oil at 25 µL. At 50 µL, the mean zone of inhibition was highest for clove oil and lowest for nutmeg oil. At 100 µL, the mean zone of inhibition was highest for clove oil and lowest for nutmeg oil. All the oils were most effective at 100 µL. The findings were highly significant (p<0.001). For all the three concentrations, the mean zone of inhibition for clove oil, cinnamon oil, and turmeric oil was higher than the control.

For *Enterococcus faecalis,* the mean zone of inhibition was highest for clove oil and lowest for nutmeg oil at 25 µL. At 50 µL, the mean zone of inhibition was highest for both cinnamon and turmeric oils and lowest for nutmeg oil. At 100 µL, the mean zone of inhibition was lowest for nutmeg oil. All the oils were most effective at 100 µL. All the findings were highly significant (p<0.001). At 25 µL, the mean zone of inhibition of only clove oil was higher than the control. At 50 µL and 100 µL, the mean zone of inhibition of clove oil, cinnamon oil, and turmeric oil was higher than the control.

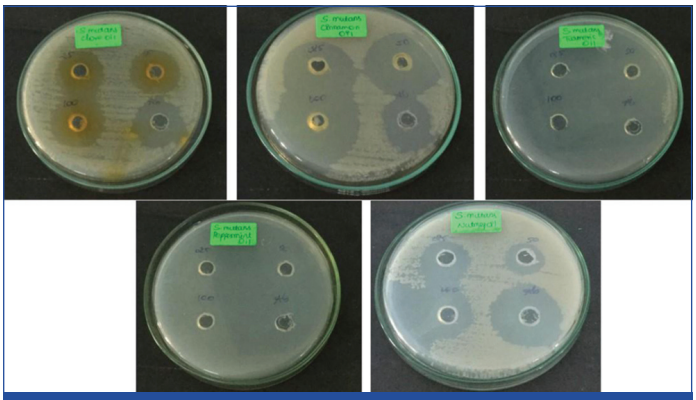
It was also found that as the concentration of the oils increased, the anti-microbial activity increased. All the oils were most effective at 100 µL. Agar plates showing the zone of inhibition against *Streptococcus mutans, Candida albicans*, and *Enterococcus faecalis* have been depicted in [Table/Fig-2-4].

[Table/Fig-5] depicts the pairwise comparison of various concentrations of the essential oils. There was significant difference between 25% and 50% concentration of all the essential oils (p<0.5) except for turmeric oil against *Streptococcus mutans* and nutmeg

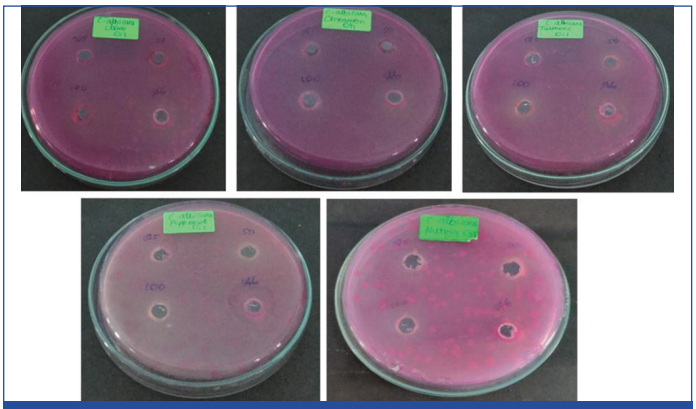
Micro-organism	Concentration	Zone of Inhibition (Mean±Standard deviation in mm)					p-value	Antibiotic (control)
		Clove oil	Cinnamon oil	Turmeric oil	Peppermint oil	Nutmeg oil		
Streptococcus mutans	25 µL	24.60±0.28	33.50±0.50	38.00±0.38	37.60±0.57	17.80±0.28	**<0.001***	26.00±0.38
	50 µL	28.40±0.38	37.10±0.28	39.60±0.57	40.00±1.00	20.20±0.50	**<0.001***	25.00±0.00
	100 µL	29.80±0.28	39.80±0.28	41.80±0.76	41.80±0.76	24.00±0.00	**<0.001***	28.16±0.28
Candida albicans	25 µL	30.08±0.14	34.83±0.28	25.25±0.25	17.41±0.38	19.33±0.57	**<0.001***	22.10±0.28
	50 µL	40.33±0.57	37.50±0.50	34.83±0.28	22.41±0.38	21.50±0.50	**<0.001***	24.83±0.28
	100 µL	44.75±0.66	40.33±0.57	40.50±0.50	27.16±0.28	24.83±0.28	**<0.001***	35.00±0.50
Enterococcus faecalis	25 µL	30.08±0.62	25.00 ±0.50	14.41±0.36	10.10±0.28	8.91±0.14	**<0.001***	44.30±0.57
	50 µL	32.16±0.16	33.16±0.28	33.16±0.28	12.08±0.38	9.16±0.28	**<0.001***	20.16±0.28
	100 µL	40.33±0.57	40.00±1.00	40.00±1.00	17.00±0.00	9.33±0.38	**<0.001***	28.00±0.00

[Table/Fig-1]: Zone of inhibition against the oral pathogens.
*Kruskal Wallis Test *p<0.001 highly significant

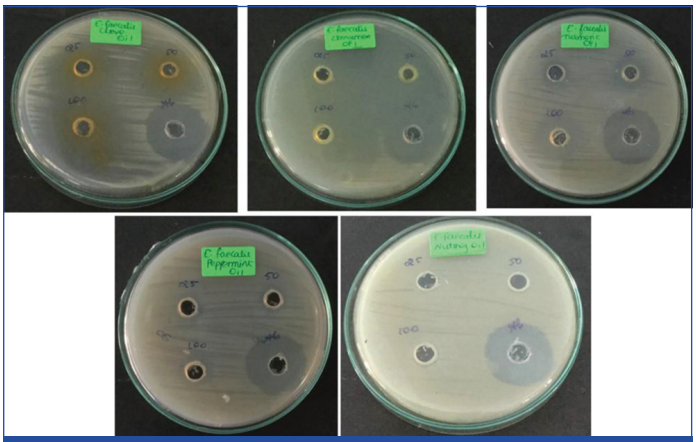
oil against *Enterococcus faecalis*. There was significant difference between 50% and 100% concentration of all the essential oils ($p<0.05$) except for clove and peppermint oil against *Streptococcus mutans* and nutmeg oil against *Enterococcus faecalis.*



[Table/Fig-2]: Agar plates showing the zone of inhibition against *Streptococcus mutans*.



[Table/Fig-3]: Agar plates showing the zone of inhibition against *Candida albicans*.



[Table/Fig-4]: Agar plates showing the zone of inhibition against *Enterococcus faecalis*.

Streptococcus mutans		
Essential oil	**25% vs 50% (p-value)**	**50% vs 100% (p-value)**
Clove oil	**0.01***	0.06
Cinnamon oil	**0.01***	**0.01***
Turmeric oil	0.07	**0.02***
Peppermint oil	**0.01***	0.07
Nutmeg oil	**0.01***	**0.01***
Candida albicans		
Clove oil	**0.01***	**0.01***
Cinnamon oil	**0.01***	**0.01***
Turmeric oil	**0.01***	**0.01***
Peppermint oil	**0.01***	**0.01***
Nutmeg oil	**0.01***	**0.01***
Enterococcus faecalis		
Clove oil	**0.02***	**0.01***
Cinnamon oil	**0.01***	**0.01***
Turmeric oil	**0.01***	**0.01***
Peppermint oil	**0.02***	**0.01***
Nutmeg oil	0.4	0.9

[Table/Fig-5]: Pairwise comparison of various concentrations of the essential oils. *Mann-Whitney U test *p<0.05 statistically significant

[Table/Fig-6] depicts the pairwise comparison of 100% concentration of the essential oils. For *Streptococcus mutans*, there was a significant difference in the anti-bacterial activity of 100% concentration of the oils except for turmeric and peppermint oil pair. For *Candida albicans*, there was a significant difference in the anti-bacterial activity of 100% concentration of the oils except for cinnamon and turmeric oil pair. For *Enterococcus faecalis*, there was a significant difference in the anti-bacterial activity of 100% concentration of the oils except for clove and cinnamon oil pair, clove and turmeric oil pair, and cinnamon and turmeric oil pair.

Streptococcus mutans				
	Cinnamon oil	**Turmeric oil**	**Peppermint oil**	**Nutmeg oil**
Clove oil	**0.01***	**0.01***	**0.01***	**0.01***
Cinnamon oil	-	**0.02***	**0.02***	**0.01***
Turmeric oil	-	-	0.9	**0.01***
Peppermint oil	-	-	-	**0.01***
Candida albicans				
	Cinnamon oil	**Turmeric oil**	**Peppermint oil**	**Nutmeg oil**
Clove oil	**0.01***	**0.01***	**0.01***	**0.01***
Cinnamon oil	-	0.5	**0.01***	**0.01***
Turmeric oil	-	-	**0.01***	**0.01***
Peppermint oil	-	-	-	**0.01***
Enterococcus faecalis				
	Cinnamon oil	**Turmeric oil**	**Peppermint oil**	**Nutmeg oil**
Clove oil	0.9	0.9	**0.01***	**0.01***
Cinnamon oil	-	0.9	**0.01***	**0.01***
Turmeric oil	-	-	**0.01***	**0.01***
Peppermint oil	-	-	-	**0.01***

[Table/Fig-6]: Pairwise comparison at 100% concentration of essential oils. *Mann-Whitney U test *p<0.05 statistically significant

DISCUSSION

In the present study, the anti-microbial activity of clove, cinnamon, turmeric, peppermint, and nutmeg oils against oral pathogens was assessed and compared with each other at 25 µL, 50 µL, and 100 µL. Peppermint and turmeric oils were found to be most effective against *Streptococcus mutans*. Clove oil was found to be most effective against *Candida albicans and Enterococcus faecalis.* The least effective oil against all three micro-organisms was nutmeg oil. Also, it was found that as the concentration of the oils increased, the anti-microbial activity increased (highest at 100 µL).

The key phytochemicals present in clove oil are eugenol (70-85%), eugenyl acetate (15%), and β-caryophyllene (5-12%) [25]. The presence of high-level eugenol in it could be the reason for its anti-microbial property [26]. *Candida albicans* was most susceptible to clove oil among the three oral pathogens. The anti-fungal activity of clove oil has also been also proved in a study conducted by Pinto E et al., [27]. However, the anti-microbial activity of clove oil against *Streptococcus mutans* in a study conducted by Gupta C et al., was much lower than the present study (13 mm) [17]. The reason could be the use of a different strain of *Streptococcus mutans* in this study.

The key phytochemicals present in cinnamon oil are tannins, alkaloids, flavonoids, and phenols [28]. The key chemical component found

Author's name/year/ place of study	Oil	Micro-organism used	Zone of inhibition	Conclusion	Similar/contrary to present study's findings
Gupta C et al.,/2011/ India [17]	Clove oil Cinnamon oil	*Streptococcus mutans*	13 mm 24 mm	Anti-microbial activity of cinnamon oil was better than clove oil against S mutans	Similar to present study's findings
Wiwattanarattanabut K et al.,/2017/Thailand [31]	Cinnamon oil Peppermint oil	*Streptococcus mutans*	32.17±1.32 11.33±1.03	Anti-microbial activity of cinnamon oil was better than peppermint oil against S mutans	Contrary to present study's findings
Mohammed NA and Habil NY/2015/Iraq [35]	Turmeric oil	*Streptococcus mutans*	9.7 mm	Anti-microbial activity of turmeric oil was lower than antibiotic	Contrary to present study's findings
Iyer M et al.,/2017/India [41]	Nutmeg oil	*Candida albicans*	12±0.15	Anti-microbial activity of nutmeg oil was better than control	Contrary to present study's findings
Present study	Clove oil, cinnamon oil, turmeric oil, peppermint oil, nutmeg oil	*Streptococcus mutans* *Candida albicans* *Enterococcus faecalis*	Clove oil-29.80±0.28 Cinnamon oil-39.80±0.28 Turmeric oil-41.80±0.76 Peppermint oil-41.80±0.76 Nutmeg oil-24.00±0.00 Clove oil-44.75±0.66 Cinnamon oil-40.33±0.57 Turmeric oil-40.50±0.50 Peppermint oil-27.16±0.28 Nutmeg oil-24.83±0.28 Clove oil-40.33±0.57 Cinnamon oil-40.00±1.00 Turmeric oil-40.00±1.00 Peppermint oil-17.00±0.00 Nutmeg oil-9.33±0.38	Anti-microbial activity of turmeric and peppermint oil was most effective against *S mutans* Anti-microbial activity of clove oil was most effective against *C albicans* Anti-microbial activity of clove oil was most effective against *E faecalis*	

[Table/Fig-7]: Results from similar studies [17,31,35,41, Present study].

in cinnamon oil is cinnamaldehyde (60-90%) [29]. The presence of (E)-Cinnamaldehyde, which is the most abundant component of the essential oils of the Cinnamomum species could be the reason for its anti-microbial activity. It was seen that *Candida albicans* was most susceptible to cinnamon oil among the three oral pathogens. Similar findings have been found in previous studies conducted by Firmino DF et al., in which the anti-microbial activity of cinnamon oil against oral pathogens has been assessed [30]. However, the anti-microbial activity of cinnamon oil against *Streptococcus mutans* in a study conducted by Wiwattanarattanabut K et al., was much lower than the present study (32.17 mm) [31]. The reason could be the use of a different strain of *Streptococcus mutans* in this study.

The key phytochemicals present in turmeric oil are curcumin (2-8%), eugenol, turmerin, turmerones [32]. The presence of high levels of curcumin in turmeric could be the reason for its anti-microbial property [33]. It was found that *Streptococcus mutans* was most susceptible to turmeric oil among the three oral pathogens. Anti-microbial activity of turmeric oil against *Streptococcus mutans* and *Enterococcus faecalis* have been proved in previous studies conducted by Suwannakul S et al., and Mohammed NA and Habil NY [34,35]. The findings of anti-microbial activity of turmeric oil against *Enterococcus faecalis* were similar to the findings of a study conducted by Chaitanya BV et al., (Zone of Inhibition=14.42 mm) [36].

The most susceptible micro-organism to peppermint oil was *Streptococcus mutans.* The key compounds present in peppermint oil like menthone, l-menthol, limonene, and menthyl acetate are responsible for its anti-microbial activity [37]. However, previous studies conducted by Thosar N et al., and Nam SH et al., have shown that the anti-microbial activity of peppermint oil is much lesser than other plant derived essential oils [20,38].

The mean zone of inhibition of nutmeg oil against *Streptococcus mutans, Candida albicans* and *Enterococcus faecalis* was highest at 100 µL (24 mm, 24.83 mm, 9.3 mm, respectively). The reason could be due to the presence of macelignan, an active compound present in nutmeg seed [39]. However, the anti-microbial activity of nutmeg oil was found to be much lower than the other essential oils against all three oral pathogens used in the study. This means that the anti-microbial activity of nutmeg oil is not much effective. The most susceptible micro-organism to nutmeg oil was *Candida albicans.* Similar findings were seen in previous studies conducted by Shafiei Z et al., and Iyer M et al., as well [40,41]. Results from similar studies have been described in [Table/Fig-7] [17,31,35,41].

Limitation(s)

Isolating microorganisms from plaque samples would have added more relevance to the findings.

Further research needs to be done to assess the Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of the essential oils. Cell toxicity and safety of the oils at various concentrations needs to be assessed as well. In further studies, in-vivo studies are recommended for assessing people's acceptance values.

CONCLUSION(S)

The most effective oil against *Streptococcus mutans* were peppermint oil and turmeric oil. The most effective oil against *Candida albicans and Enterococcus faecalis* was clove oil. The least effective oil against all three micro-organisms was nutmeg oil. Also, as the concentration of the oils increased, the anti-microbial activity increased. These oils can be incorporated into mouthwashes and can be used as anti-microbial agents instead of using antibiotics. They can also be used as an adjunct to conventional therapy.

Authors' contribution: SM helped with conceptualisation, data collection, data analysis and manuscript preparation; RM helped with data collection, manuscript preparation and RPK helped with manuscript editing and review.

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