

## Validating Antibacterial Efficacy of Essential Oils Combinations against Dental Caries Pathogens

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### ABSTRACT

**Aim:** To study the antibacterial efficacy of combination of essential oils (EOC) consisting of Clove oil, Cinnamon oil, Turmeric oil and Neem oil against dental caries pathogens *S. mutans* and *L. acidophilus* with different concentrations and at different time intervals.

**Material and Methods:** Antibacterial efficacy of dental caries microbes was studied to determine the effectiveness of combination of essential oils at reducing bacterial growth of *S. mutans* and *L. acidophilus* using selective enrichment media. The antimicrobial activity of EOC was determined by the standard serial dilution by pour plate technique. Essential oils such as clove oil, cinnamon oil, turmeric oil, neem oil were of food grade (edible).

**Results:** EOC showed 96.2% reduction of *S. mutans* at a concentration of 100 µl after 4 minutes whereas 96.8% reduction of *L. acidophilus* at 50 µl after 4 mins.

**Conclusions:** It is obvious that present study has revealed the importance of EOC to control caries causing *S. mutans* and *L. acidophilus*. This scientific information can serve as an important platform for the development of inexpensive safe and effective natural medicines. The long-range goal of this study is to develop a consumable agent in oral hygiene products.

### INTRODUCTION

Dental diseases are recognized as a major public health problem throughout the world. Teeth and their supporting structure the gum (gingival) are subjected to infection by cariogenic bacteria that causes cavity and periodontitis, which if left untreated can eventually lead to gingivitis. Recent study suggests that such chronic low-grade localized infection such as periodontitis contribute to heart disease and coronary heart disease [1,2]. Dental caries is a multifactorial disease caused by the interaction of dietary sugars, dental biofilm and the host's dental tissue within the oral environment [3]. It is the cumulative result of consecutive cycles of demineralization and remineralization at the interface between the biofilm and the tooth surface [4,5].

Despite the implementation of measures to control and treat dental caries with fluoride, they remain the most prevalent dental disease in many countries [6]. Caries are a multifactorial infectious disease caused by accumulation of biofilm on tooth surface [7]. Manifestations of the disease occur when there is an imbalance between the biofilm and the host due to changes in biofilm matrix pH caused by diet, microorganisms, or salivary flow and their components [8-10].

The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans [11]. Among the major cariogenic bacteria, *Streptococcus* spp. specially *S. mutans* found in a greater number followed by *Actinomyces* spp. and *Lactobacilli* spp [12,13]. *Streptococcus mutans* is considered the most cariogenic of all oral streptococci [14]. *S. mutans* is able to colonize the tooth surface and to produce large amounts of extra and intra-cellular polysaccharides. This microorganism is also highly acidogenic and aciduric, and it metabolizes several salivary glycoproteins, thus being responsible for the initial stage of oral biofilm formation and caries lesions. [15]

Dental caries remains the most prevalent chronic disease in children. The Centers for Disease Control and Prevention (CDC) reported that from 1999 through 2004, 42 percent of children aged 2 to 11 years experienced dental caries in their primary teeth, the trend in younger children aged 2 to 4 years has increased over time, and 59 percent of adolescents aged 12 to 19 years experienced dental caries in their permanent teeth. The critical pH is significantly higher for children than adults. Children have a greater driving force for demineralization in a more acidic oral environment and a decreased driving force for remineralisation at normal oral pH. This puts children at greater risk for demineralization than adults [16,17].

Essential oils also called volatile oils, are aromatic oily liquids obtained from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. An estimated 3000 essential oils are known, of which 300 are commercially important in the fragrance market [18]. The antimicrobial activity of essential oils is due to a number of small terpenoids and phenol compounds. Several of these are classified as generally recognized as safe [19-21].

The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. 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 use as alternative remedies for the treatment of many infectious diseases [22]. World Health Organisation (WHO) noted that a considerable part of the world's population depends on traditional medicine for primary care. Many essential oils have been advocated for use in complementary medicine for bacterial and fungal infections including boils, acne, gingivitis and vaginal candidiasis [23,24].

**Cinnamon oil (*Cinnamomum camphora*):** It acts to prevent gingivitis caused by poor oral hygiene, oral thrush, and gum disease. It equally works as an anti-parasitic and antioxidant. The antibacterial and antifungal properties are incredible for the teeth and gum, and if you're not keen about menthol based oils, then this works as a perfect alternative to improving your oral hygiene [25].

**Clove oil (*Eugenia caryophyllus*):** Cloves have been used by humans for medicinal applications for over 2000 years, being chewed to alleviate the pain of toothache and are also widely used as disinfectant root canals in temporary fillings and as an oral anesthetic [26,27].

**Turmeric oil (*Curcuma longa*):** The anti-inflammatory property of curcumin has been studied and demonstrated significant reduction of inflammation. Numerous studies have demonstrated that turmeric mouthwash can be effectively used as an adjunct to mechanical plaque control in prevention of dental plaque and gingivitis in dentistry applications [28].

**Neem Oil (*Azadirachta indica*):** Neem is a natural antibacterial agent. It have been reported that the active components from a bark containing neem stick have appeared to inhibit virulence factors of oral streptococci related with dental plaque formation [29].

## MATERIALS AND METHODS

**Essential Oils Combination (EOC):** The concentration of essential oils used was Cinnamon Oil-0.6%, Clove oil-0.2%, Neem oil-0.2%, Turmeric oil-0.8%. A combined mixture of these oils (EOC) was prepared and from this 10 µl, 20 µl, 50 µl, 100 µl and 200 µl were used for further analysis.

### Samples Collection

This study was conducted at National Collateral Management Services Limited (NCML), Mumbai, India. The samples used in this study were collected from the tooth of a volunteer diagnosed with dental caries. The infected area of the tooth was swabbed with sterile cotton wool and saliva transferred to a sterile screw capped tube that contained 0.1% of NaCl with aseptic precautions, vortex mixed for 1minute, to disperse the bacteria.

### Optimization of Saliva Sample

Dental caries saliva samples were diluted with 0.1% sterile NaCl as per experimental convenience to 106 CFU/ml and were used for further studies. Mitis-Salivarius Bacitracin (MSB) agar for the recovery of *S. mutans* and De Man, Rogosa

and Sharpe (MRS) agar for *L. acidophilus* was used as selective media. Molten sterile agar held at 45°C was poured in plates and allowed to solidify and then incubated at 37°C for 48 hours.

To initiate the experiments blank 1 ml of saliva sample was plated out and poured in MSB and MRS agar medium as control. Experiments was further was performed with 10 µl, 20 µl, 50 µl, 100 µl and 200 µl of EOC concentration was added and plated at different time intervals of 0 min, 2 min, 4 min, 7 min, 10 min, 2 hrs, 4 hrs using pour plate technique in both MSB and MRS medium. All these plated samples were incubated at 37°C for 48 hours. All experiments were performed in triplicates.

After the incubation, all plates were counted to achieve final counts per time accounting for dilution factors. At different concentrations and at different time points, the percent reduction of bacterial growth was calculated by the following formula.

$$CFU/ml = \text{Colony count} \times \text{dilution factor}$$

$$\% \text{ reduction} = \left[ \frac{\text{Initial CFU/ml} - \text{CFU/ml at time interval}}{\text{Initial CFU/ml}} \right] \times 100$$

## RESULTS

*Streptococcus mutans* is a facultative anaerobic, gram-positive coccus (round bacterium) commonly found in the human oral cavity and is a significant contributor to tooth decay. Antibacterial efficacy of EOC against *S. mutans* was performed with 10 µl, 20 µl, 50 µl 100µl and 200 µl at different time intervals of 2 min, 5min, 7min, 10 min, 2 hrs and 4 hrs and incubated at 37°C for 48 hrs. CFU/ml and % reduction of *S. mutans* for control and with EOC at different concentrations and time intervals are shown in **Table 1**.

**Table 1.** CFU/ml and % reduction of *S. mutans* at different concentrations and time intervals.

<i>S. mutans</i>											
Time in Minutes	Concentration of EOC										
	Control (CFU/ml)	10 µl		20 µl		50 µl		100 µl		200 µl	
		CFU/ml	% Reduction								
0	692	682	1.45	619	10.55	449	35.12	391	43.5	298	56.94
2	694	639	7.93	606	12.68	387	44.24	87	87.46	227	67.29
4	692	599	13.44	529	23.55	350	49.42	55	92.05	20	97.11
7	698	581	16.76	521	25.36	308	55.87	26	96.28	7	99
10	701	485	30.81	462	34.09	240	65.76	16	97.72	0	100
120	690	23	96.67	4	99.42	0	100	0	100	0	100
240	699	0	100	0	100	0	100	0	100	0	100

*Lactobacillus acidophilus* is a gram positive bacterium in the genus *Lactobacillus*. *L. acidophilus* is a homofermentative, microaerophilic species, fermenting sugars into lactic acid, and grows readily at rather low pH values (below pH 5.0). Antibacterial efficacy of EOC against *L. acidophilus* was performed with 10 µl, 20µl, 50 µl 100µl and 200 µl at different time intervals of 2 min, 5 min, 7 min, 10 min, 2 hrs and 4 hrs and incubated at 37oC for 48 hrs. CFU/ml and % reduction of *L.acidophilus* for control and with EOC at different concentrations and time intervals are shown in **Table 2**.

Table 2. CFU/ml and % reduction of *L. acidophilus* at different concentrations and time intervals.

<i>L. acidophilus</i>												
Time in Minutes	Concentration of EOC (in µl)											
	Control (CFU/ml)	10 µl		20 µl		50 µl		100 µl		200 µl		% Reduction
		CFU/ml	% Reduction									
0	66	65	1.52	54	18.18	40	39.39	27	59.09	14	78.79	
2	68	55	19.12	36	47.06	22	67.65	8	88.24	3	95.59	
4	75	49	34.67	37	50.67	3	96	1	98.67	1	98.67	
7	74	48	35.14	8	89.19	0	100	0	100	0	100	
10	69	31	55.07	5	92.75	0	100	0	100	0	100	
120	68	0	100	0	100	0	100	0	100	0	100	
240	66	0	100	0	100	0	100	0	100	0	100	

## DISCUSSION

The benefits of essential oils have been proven extensively for a variety of oral care treatments. Dental caries can be controlled by several strategies used either alone or in combination. These strategies include approaches that involve altering the bacterial flora in the mouth, modifying the diet, increasing the resistance of tooth enamel to acid attack or reversing the demineralization process. The activity of natural products, especially EO, against microorganisms has been recently confirmed by several studies focusing on antimicrobial activity of EO against planktonic cells. However, bacteria growing in biofilms exhibit a specific phenotype and are often, but not always, more resistant to antimicrobial agents than their planktonic counterparts [30,31]. Thus, it is important to search for natural products that have antibiofilm properties and antimicrobial activity against oral pathogens [32]. This study aimed to evaluate the activity of EOC against *S. mutans* and *L. acidophilus*. Cinnamon oil showed highest activity against *S. mutans* using agar well diffusion assay with zone of inhibition 12.51 mm at 100 µl [24]. *Cinnamomum zeylanicum* extract showed MIC (Minimum Inhibitory Concentration) of 250 µl-500 µl against *S. mutans* [10].

As per Zainal-Abidin et al. [33] *C. zeylanicum* (Cinnamon Oil) showed strong growth inhibition against all the pathogenic oral bacteria tested. MIC mean values were found to be 0.21 ± 0.04 to 0.63 ± 0.23 µL/mL (v/v). Both cinnamaldehyde and eugenol standards showed profound activity against both *Streptococci* spp. Cinnamon bark essential oil expressed the strongest inhibitory effect against *S. mutans* (MIC of 0.08% (v/v)) using a broth micro dilution method [34]. Clove oil was effective against *S. mutans* with zone of inhibition at 7.42 mm 100 µl concentration [24]. The Clove extract showed 22 mm zone of inhibition at 1000 µl concentration against *S. mutans* [27]. Antimicrobial study was performed using 40% ethanolic extracts of *Azadirachta indica* (Neem Extract) and *C. longa* (Turmeric). Neem extract showed zone of inhibition 15 mm at 100 mg/ml. Whereas *C. longa* was sensitive at 18 mm zone inhibition at 100 mg/ml [2]. According to Li et al. [35] results showed that curcumin (Turmeric) inhibited the growth of planktonic *S. mutans* with an MIC of 125 M. After treatment with concentrations higher than 125 M, no visible *S. mutans* growth was observed [35]. The neem oil at conc 200 µl was effective against *Streptococcus mutans* with a zone of inhibition of 28 mm diameter [36]. The zone of inhibition was seen with *Streptococcus mutans* measuring 27 mm diameter whereas *Lactobacillus acidophilus* zone of inhibition was found to be 18 mm at 100 µl concentration of neem oil [37]. As per Gupta et al. [38] maximum bactericidal activity was found to be present in the aqueous extract of leaves of *A. indica* plant against *L. acidophilus* with an inhibition length of 16 ± 1 mm at a concentration of 100 µl [38]. The mean zone of inhibition values of 10% ethanolic extract of Cinnamon against *S. mutans* was found to be 9.5 mm, 15.5 mm, 18.5 mm at 10 µl, 20 µl and 30 µl and against *L. acidophilus* it was found to be 7 mm, 8 mm and 9.5 mm respectively by Yadav et al. [39]. Neem oil and Clove oil showed MIC of 0.125 µl/ml and 0.25 µl/ml respectively against *L. acidophilus* [40]. Si et al. [41] studied antimicrobial activity against *L. acidophilus*. Clove oil at a concentration of 300 µg/ml showed inhibition of 2% whereas Cinnamon oil at 200 µg/ml resulted in inhibition of 75% [41]. The antibacterial activity of neem extracts against *Streptococcus mutans* petroleum ether and chloroform extracts of *Azadirachta indica* showed higher activity (18 mm) at 500 µg concentration. Ethanol extract showed lowest activity (14 mm) against this pathogen [42].

The above studies showed effectiveness exceptionally reduction in microbial population of dental caries microbes. Mostly microbial reduction was determined for *S. mutans* and *L. acidophilus*. Combination of EO (EOC) when in contact with dental caries resulted in 92% reduction of *S. mutans* at 4 minutes with 100 µl concentration. Whereas 96.8% reduction was observed for *L. acidophilus* at 4 minutes for 50 µl concentration (Tables 1 and 2).

## CONCLUSION

To conclude, EOC showed exceptional antimicrobial activity against *S. mutans* and *L. acidophilus*. It concedes that a minute concentration of EOC could reduce the dental caries microbes insignificantly. Down the ages essential oils and other plant extracts have potential use as alternative remedies for different dental diseases. This scientific information can serve as an important platform for the development of inexpensive safe and effective all-natural medicines. The long-range goal of this study is to develop a consumable agent in oral hygiene products.

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