

Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study

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ABSTRACT

Objectives: This study was aimed to find out the minimum inhibitory concentration (MIC) of five essential oils against oral pathogens and to find out the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of five essential oils against oral pathogens. **Materials and Methods:** The antimicrobial activities by detecting MIC and MBC/MFC of five essential oils such as tea tree oil, lavender oil, thyme oil, peppermint oil and eugenol oil were evaluated against four common oral pathogens by broth dilution method. The strains used for the study were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 90028. **Results:** Out of five essential oils, eugenol oil, peppermint oil, tea tree oil exhibited significant inhibitory effect with mean MIC of 0.62 ± 0.45 , 9.00 ± 15.34 , 17.12 ± 31.25 subsequently. Mean MBC/MFC for tea tree oil was 17.12 ± 31.25 , for lavender oil 151.00 ± 241.82 , for thyme oil 22.00 ± 12.00 , for peppermint oil 9.75 ± 14.88 and for eugenol oil 0.62 ± 0.45 . *E. faecalis* exhibited low degree of sensitivity compared with all essential oils. **Conclusion:** Peppermint, tea tree and thyme oil can act as an effective intracanal antiseptic solution against oral pathogens.

Key words: Antimicrobial activity, essential oils, oral pathogens

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Essential oils and other extracts of plants have evoked interest as sources of natural products.^[1] 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.^[2] The antimicrobial activity of essential oils is due to a number of small terpenoids and phenol compounds.^[3] Several of these are classified as generally recognized as safe.^[4] Essential oils such

as tea tree oil, lavender oil, thyme oil, peppermint oil and eugenol oil have been traditionally used by people for various purposes in different parts of the world.

The root canal environment after chemomechanical treatment becomes unfavorable for microorganisms; there is reduced oxygen tension, limited nutrient availability and antimicrobial agents that act as driving forces in survival balance of bacteria in the root canal system.^[5] Root canal dentinal tubules harbor microorganisms; also bacterial biofilm may be present at the apical portion of root canal and extra-radicular regions.^[6] Therefore, irrigation with a broad spectrum antiseptic substances and interappointment intracanal medication has become a

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standard regimen in root canal therapy. Many species and herbs exert antimicrobial activity due to their essential oil fractions. For thousands of years clove oil (eugenol) has been used in dentistry. Eugenol has been used topically in dental practice to relieve pain arising from a variety of sources, including pulpitis and dentinal hypersensitivity. Interestingly, eugenol exhibits irritant action in addition to its analgesic effect as found in certain studies.^[7] So in search of plant essential oils possessing antimicrobial activity, this study was undertaken to determine *in vitro*, antimicrobial action of eugenol, thyme oil, tea tree oil, peppermint oil and lavender oil against four pathogenic oral micro-organisms.

In this study, it was aimed to detect *in vitro* antimicrobial efficacy of five essential oils against oral pathogens. Firstly, it was aimed to find out the minimum inhibitory concentration (MIC) of five essential oils against oral pathogens. Secondly, it was aimed to find out the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of five essential oils against oral pathogens.

MATERIALS AND METHODS

We used five essential oils as following:

Tea tree oil, lavender oil, thyme oil, peppermint oil, and eugenol oil.

Essential oils

Five essential oils such as tea tree oil, lavender oil, thyme oil, peppermint oil and eugenol oil were obtained from Arromatantra, Mumbai, India. These

oils were selected based on the literature survey and their use in traditional medicine. Quality of oils was ascertained to be pure.

Test organism

Microorganisms were obtained from the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. The strains used for the study were *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028). Stock cultures of bacteria were used for the study.

MIC and MBC/MFC determination

The antimicrobial activities by detecting MIC of five essential oils such as tea tree oil, lavender oil, thyme oil, peppermint oil and eugenol oil were evaluated against four common oral pathogens by broth dilution method [Figure 1].^[8] MBC was detected by subculturing onto blood agar from the tube showing no turbidity (i.e., MIC) and the next tube to it. The blood agar plates were incubated at 37°C overnight for 18-20 h and on the next day the readings were taken. For MFC, subcultures were done on Sabouraud's dextrose agar plates following the same procedure [Figures 2-5]. Various concentrations of five essential oils used against oral pathogens were in doubling dilutions [Table 1]. Results were analyzed statistically by using the one-way analysis of variance.

RESULTS

Out of five essential oils, eugenol oil, peppermint oil, tea tree oil exhibited significant inhibitory effect [Table 2] with mean MIC of 0.62 ± 0.45 , 9.00 ± 15.34 ,

Table 1: Different concentrations of essential oils against common oral pathogens

Essential oil	<i>Enterococcus faecalis</i> (µl)	<i>Escherichia coli</i> (µl)	<i>Staphylococcus aureus</i> (µl)	<i>Candida albicans</i> (µl)
Tea tree	1-128	0.25-16	1-16	0.125-4
Lavender	1-512	1-512	1-512	0.5-128
Thyme	1-64	1-16	1-512	0.5-32
Peppermint	1-128	1-16	1-512	0.125-4
Eugenol	0.5-4	0.05-16	0.05-3.2	0.05-0.8

Table 2: MIC and MBC/MFC of five essential oils (µl/ml)

Essential oil	<i>Enterococcus faecalis</i>		<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Candida albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Tea tree	64	64	2	2	1	2	0.5	0.5
Lavender	32	64	128	512	32	64	8	16
Thyme	32	32	2	8	32	32	16	16
Peppermint	32	32	1	4	2	2	0.5	0.5
Eugenol	1	1	1	1	0.4	0.4	0.1	0.1

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration

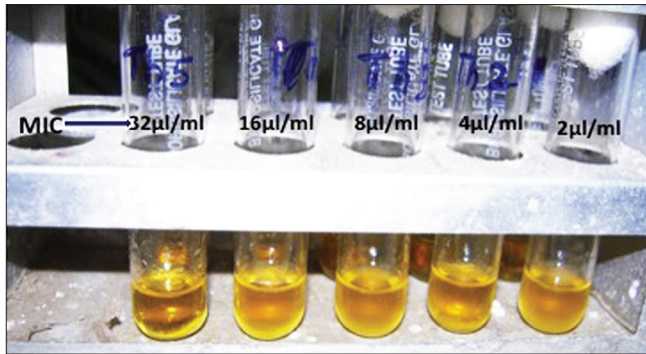


Figure 1: Minimum inhibitory concentration of thyme oil for *Staphylococcus aureus*

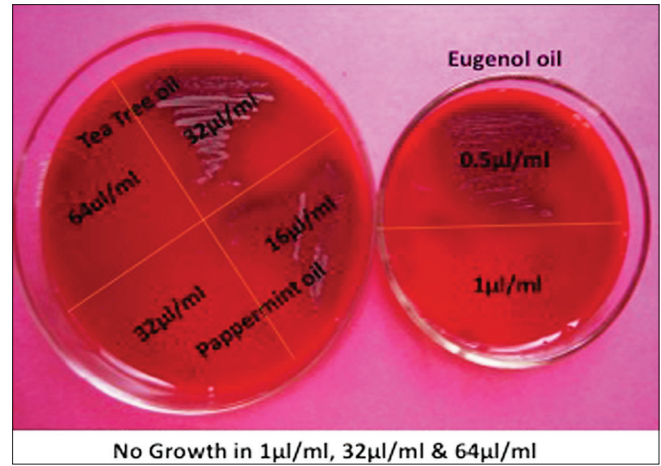


Figure 2: Minimum bactericidal concentration for *Enterococcus fecalis*

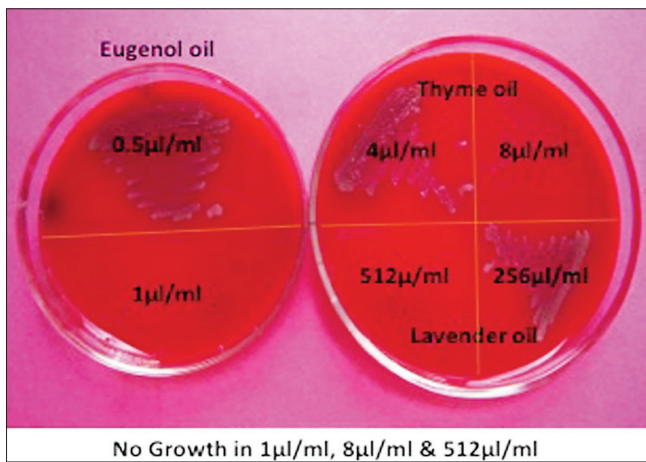


Figure 3: Minimum bactericidal concentration for *Escherichia coli*

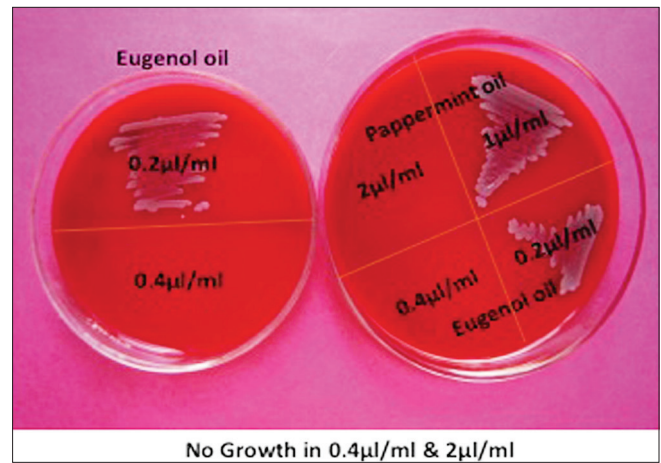


Figure 4: Minimum bactericidal concentration for *Staphylococcus aureus*

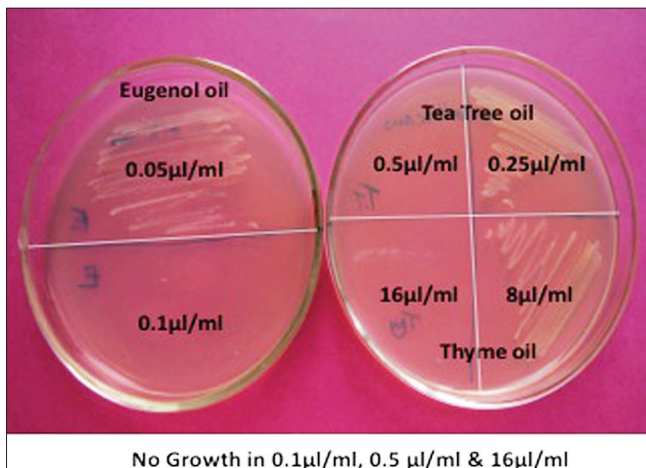


Figure 5: Minimum fungicidal concentration for *Candida albicans*

17.12 ± 31.25 subsequently. There was no statistical significant difference in five essential oils ($F = 1.61$, $P = 0.221$) [Table 3]. Mean MBC/MFC for tea tree oil was 17.12 ± 31.25, for lavender oil 151.00 ± 241.82, for thyme oil 22.00 ± 12.00, for peppermint oil 9.75 ± 14.88 and for eugenol oil 0.62 ± 0.45 ($F = 1.30$,

$P = 0.312$) [Table 4]. Eugenol showed antimicrobial activity at the lowest concentration compared with all essential oils i.e., for *C. albicans*: MIC, MFC: 0.1 µl/ml, for *S. aureus*: MIC, MBC: 0.4 µl/ml, for *E. coli* and for *E. faecalis*: MIC, MBC: 1 µl/ml respectively [Figures 2-5].

DISCUSSION

Tea tree oil is the volatile essential oil derived mainly from the Australian native plant, *Melaleuca alternifolia*. Tea tree oil is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. Terpenes are volatile, aromatic hydrocarbons and may be considered polymers of isoprene, which has the formula C_5H_8 .^[9] Antibacterial activity in literature appeared from 1940 to 1980.^[10-13] From the early 1990s onward, many reports describing the antimicrobial activity of tea tree oil appeared in the scientific literature.^[9]

Antimicrobial activity of tea tree oil is due to terpinen-4-ol, α -terpineol and 1,8-, which cause

Table 3: MIC of five essential oils

Essential oil	N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum	P value
					Lower bound	Upper bound			
Tea tree	4	17.12	31.25	15.62	-32.615	66.86	0.5	64	*P=0.221
Lavender	4	34	22.97	11.48	-2.56	70.56	8	64	
Thyme	4	20.5	14.45	7.22	-2.5	43.5	2	32	
Peppermint	4	9	15.34	7.67	-15.41	33.41	1	32	
Eugenol	4	0.62	0.45	0.22	-0.09	1.34	0.1	1	

*P>0.05, Not significant, MIC: Minimum inhibitory concentration

Table 4: MBC/MFC of five essential oils

Essential oil	N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum	P value
					Lower bound	Upper bound			
Tea tree	4	17.12	31.25	15.62	-32.61	66.86	0.5	64	*P=0.312
Lavender	4	151	241.82	120.91	-233.79	535.79	12	512	
Thyme	4	22	12	6	2.9	41.09	8	32	
Peppermint	4	9.75	14.88	7.44	-13.93	33.43	1	32	
Eugenol	4	0.62	0.45	0.22	-0.09	1.34	0.1	1	

*P>0.05, Not Significant, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration

leakage of 260 nm-light absorbing material and render cells susceptible to sodium chloride.^[14] Thus, tea tree oil causes lysis and the loss of membrane integrity and function manifested by the leakage of ions and the inhibition of respiration.^[9] Antimicrobial resistant isolates of *S. aureus*,^[15,16] *C. albicans*,^[17] and *E. faecium*^[18,19] have been found to have *in vitro* susceptibilities to tea tree oil. In this study, antimicrobial effect of tea tree oil for *C. albicans* (MIC: 0.5 µl/ml, MFC: 0.5 µl/ml) was found at the lowest concentration followed by *S. aureus* (MIC: 1 µl/ml, MBC: 2 µl/ml) and *E. coli* (MIC: 2 µl/ml, MBC: 2 µl/ml), lastly *E. faecalis* (MIC: 64 µl/ml, MBC: 64 µl/ml).

Much of pure lavender oil (*Lavandula officianalis L. angustifolia* [Miller] or *L. vera-Labiatae/Lamiaceae*) comes from Balkans. France still produces the finest quality, but production there is tumbled with the advent of the hybrid. Essential oil of lavender is obtained from the flowering tops by steam distillation method. Its principal constituents include monoterpenes, Oxides, linalyl and geranyl esters, geraniol, linalool etc., It has variety of properties, which includes that it is anti-depressant, hypotensive, soothing, alleviates stress, anxiety and general debility. It is antiseptic and anti-inflammatory for colds, flu and sinusitis and throat infections. It is balancing, antiseptic, anti-inflammatory and regenerative; soothes acne, eczema, dandruff, hair loss, head lice, diaper/nappy rash, sunburn, insect bites and boils; relieves Athlete's

foot and herpes simplex. It has cleansing and calming effect; helps colic, dyspepsia, indigestion, flatulence and gastroenteritis. It is sedative and decongestant; lowers blood pressure reduces palpitations. It has musculo-analgesic and anti-inflammatory effect in neuritis, neuralgia, muscular sprains, cramps, aches and pains. It is also used in inhalations, vaporizers, compresses, bath application or massage.^[20] In the study of Zuzarte *et al.*, 2009^[21] antifungal activity of lavender oil was found with MIC values of 0.32-0.64 µl/ml. In the study of Zuzarte (2011)^[22] candida species, were found to be sensitive to lavender oil with MIC of 0.64 µl/ml. In our study, it was achieved with a higher concentration i.e., 8 µl/ml for *C. albicans*. *S. aureus* and *E. faecalis* were susceptible at the concentration of MIC: 32 µl/ml and MBC: 64 µl/ml respectively. *E. coli* was least susceptible with MIC: 128 µl/ml and MBC: 512 µl/ml.

The name thyme actually comes from the Greek word "thymos," meaning small because of the fragrance of the plant. Thyme belongs to over 300 species of hardy, perennial herbaceous plants and shrubs that are native to Europe, particularly around Mediterranean. It is one of the Hippocrates 400 simple remedies. Essential oil of thyme (*Thymus Spp, T. citriodorits, T. vulgaris-Labiatae/Lamiaceae*) is obtained from the leaves and flowering tops by steam distillation method. Its principal constituents include 20-40% thymol and carvacrol with borneol,

cineol, linalool, menthone, B-cymene, pinene and triterpenic acid. Thyme oil is a tonic stimulant and stomachic and digestive relieves gastritis, enterocolitis and mouth thrush. It is useful for respiratory infections, asthma and bronchitis. It is effective for treating swelling provoked by gout or rheumatic problems, for joint pains, backache and sciatica. Thyme oil is also useful for urinary and vaginal infections, endometritis (*candida*), prostrates and vaginitis.^[20] Thyme oil exhibits antibacterial activity and has been useful in dental practice.^[23] A component of thyme, known as thymol, appears to inhibit growth of oral pathogens in the mouth and in combination with other essential oils, may reduce dental caries.^[24,25] In patients with orthodontic brackets, a dental varnish containing thymol reduced the proportion of *Streptococcus mutans* in supragingival plaque near the bracket.^[26] Thymol is one of the essential oils with antibacterial effects found in Listerine.^[27] In the study of Gislene *et al.*, 2000^[28] Hili *et al.*, 1997^[29] and Nzeako *et al.*, 2006^[30] thyme and clove oil possessed antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans* at various concentration of the extracts. In our study, antimicrobial susceptibility in order of sequence for thyme oil was *E. coli* with MIC: 2 µl/ml, MBC: 8 µl/ml, *C. albicans* with MIC, MFC: 16 µl/ml, *E. faecalis* with MIC, MBC: 32 µl/ml and *S. aureus* with MIC, MBC: 32 µl/ml respectively.

Essential oil of peppermint (*Mentha piperita-Lamiaceae/Labiatae*) is cultivated on a wide scale in Europe, USA and Japan. It is extensively used in toiletry, food and pharmaceutical industries. A variety of products ranging from toothpastes, mouthwashes and digestive tablets to sweets, ice cream and liquors are flavored with peppermint. Essential oil of peppermint is obtained from the leaves by steam distillation method. Its principal constituents include monoterpenic alcohols mainly menthol (38-48%), ketones mainly menthones (20-30%), some monoterpenes and oxides. It is a good antiseptic, antibacterial and antiviral. It has light, clean, refreshing aroma and is a good insect repellent. It has stimulating and strengthening effect; in treatment of shock, helpful for neuralgia and relief of general debility, headaches and migraines. It has antiseptic and anti-spasmodic effect; in reducing mucus and relieving coughs, sinusitis, throat infections, colds, flu, asthma and bronchitis. It is also used in inhalations, baths or applications. It has got cooling and cleansing effect; soothes itchy skin, relieves inflammation. It has soothing and anti-spasmodic

effect; relieves acidity, heartburn, diarrhea, indigestion and flatulence, also effective for travel sickness and nausea. It has cooling effect in case of varicose veins and hemorrhoids.^[20] Peppermint oil makes the mouth feel fresh and of course, makes the formula taste good. Peppermint oil can also increase salivation, which is useful because dry mouth may result in halitosis.^[31] In our study, antimicrobial effect was achieved at the concentration of 0.5 µl/ml for *C. albicans* and at the concentration of 32 µl/ml for *E. coli*, *S. aureus*, *E. faecalis* (32 µl/ml).

The clove plant grows in warm climates and is cultivated commercially in Tanzania, Sumatra, the Maluku (Molucca) Islands and South America. The tall evergreen plant grows up to 20 m and has leathery leaves. The clove spice is the dried flower bud of *Eugenia caryophyllata* species. Essential oils are obtained from the buds, stems and leaves by steam distillation. The buds or cloves are strongly aromatic. Clove buds yield approximately 15-20% of a volatile oil that is responsible for the characteristic smell and flavor. The bud also contains a tannin complex, a gum and resin and a number of glucosides of sterols. The principal constituent of distilled clove bud oil (60-90%) is eugenol (4-allyl-2-methoxyphenol). The oil also contains about 10% acetyleneugenol and small quantities of gallic acid, sesquiterpenes, furfural and vanillin and methyl-n-amyl ketone. Other constituents include flavonoids, carbohydrates, lipids, oleanolic acid, rhamnetin and vitamins.^[32]

Eugenol is widely used and well-known for its medicinal properties. Traditional uses of clove oil include the use in dental care, as an antiseptic and analgesic.^[33] It is active against oral bacteria associated with dental caries and periodontal disease^[34] and effective against a large number of other bacteria^[35-38] and virus.^[39] Previous studies have reported biological activities of eugenol including antifungal,^[40-42] anti-carcinogenic,^[43] anti-allergic,^[44,45] anti-mutagenic activity,^[46] antioxidant^[47] and insecticidal^[48] properties. In our study, eugenol oil showed antimicrobial activity at the lowest concentration against all organisms compared with all essential oils such as for *C. albicans* with MIC, MFC: 0.1 µl/ml, for *S. aureus* with MIC, MBC: 0.4 µl/ml, for *E. coli* and for *E. faecalis* with MIC, MBC: 1 µl/ml respectively.

CONCLUSION

Hence, this study concludes that apart from traditional use of eugenol, antibacterial effects of essential oils

such as peppermint oil, tea tree oil, thyme oil also can provide an effective intracanal antiseptic solution against oral pathogens.

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