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Anti-bacterial activity of three essential oils - An *in vitro* study

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ABSTRACT

To conduct a study regarding the antibacterial activity of essential oils against bacteria causing Caries. Essential oils are distillates of the volatile compounds of a plant's secondary metabolism and may act as photoprotective agents. Their curative effect has been known since antiquity. It is based on a variety of pharmacological properties which are specific for each plant species. The mouth contains a variety of oral bacteria, but only a few species of bacteria are believed to cause dental caries. Antibacterial activity of the three essential oils, Rosemary oil, Holy basil oil, Thyme oil was screened against Streptococcus mutans, using disc diffusion technique. The rosemary oil was more effective against Streptococcus mutans with a zone of inhibition of 52 mm diameter (at concentration 200 µl), Rosemary oil showed a zone of inhibition of 44 mm diameter and with thyme oil, the zone diameter was 30 mm. The results of this study showed that the essential oils at different concentrations exhibited antibacterial activity against the bacterial species tested.

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INTRODUCTION

The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Sreevidhya. T. M, Geetha. R. V. 2014). Despite the implementation of measures to control and treat dental caries with fluoride; they remain the most prevalent dental disease in many countries (Brasil, Ministério da Saúde. 2011). Caries is a multifactorial infectious disease caused by the accumulation of biofilm on the tooth surface (P. D. Marsh. 2003). 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 (W. H. Bowen, S. M. Amsbaugh *et al.,* 1980, J. K. Kajfasz, I. Rivera-Ramos *et al.,* 2010). Streptococcus mutans is considered the most cariogenic of all oral streptococci (D. Ajdić, W. M. McShan, R. E. McLaughlin 2002). S. mutans can colonize the tooth surface and 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 (T. M. S. Alves, C. A. Silva 2010). Streptococcus mutants are usually found in mouth, intestine and pharynx. Various factors which are present in dental caries are adherence to enamel surfaces, production of acidic metabolites, ability to synthesize extracellular polysaccharides. Two organisms which have an important role in dental caries are Streptococcus mutans and Streptococcus sobrinus. Risk of cavities increases when there is an acidic environment which is produced by Lactobacilli. Usually, the presence of Streptococcus mutans will lead to dental caries after 12 - 24 months.

Several products have been used to control dental caries, such as fluoride, chlorhexidine, and their associations (J. D. Bader, D. A. Shugars, *et al.,* 2001). However, natural products have contributed significantly to the discovery of chemical structures to create new medicaments to be used as innovative therapeutic agents against this prevalent disease (J. Clardy and C. Walsh, 2004. J. G. Jeon, P. L. Rosalen, *et al.,* 2011). 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. The antimicrobial activity of essential oils is due to a number of small terpenoids and phenol compounds. Essential oils such as tea tree oil, lavender oil, thyme oil, peppermint oil, holy basil oil, rosemary oil and eugenol oil have been traditionally used by people for various purposes in different parts of the world. Most common essential oils such as lavender, peppermint, tea tree oil, patchouli, and eucalyptus are distilled. Raw plant material, consisting of the flowers, leaves, wood, bark, roots, seeds, or peel, is put into an alembic (distillation apparatus) over water. As the water is heated, the steam passes through the plant material, vaporising the volatile compounds. The vapours flow through a coil, where they condense back to liquid, which is then collected in the receiving vessel.

Most oils are distilled in a single process. One exception is ylang-ylang (Cananga odorata) which is purified through fractional distillation. An example of the medicinal value of essential oils is thymol, isomeric with carvacrol and found in oil of the common spice thyme. Thymol is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides such as carvacrol (M. Simões. 2011)(V. F. Furletti, I. P. Teixeira, G. Obando-Pereda *et al.,* 2011)

Taken by mouth, many essential oils can be dangerous in high concentrations. Typical effects begin with a burning feeling, followed by salivation. In the stomach, the effect is carminative, relaxing the gastric sphincter and encouraging eructation (belching). Further down the gut, the effect typically is antispasmodic (Soares, I.H.; Loreto *et al.,* 2014). Typical ingredients for such applications include eucalyptus oils, menthol, capsaicin, anise, and camphor.

Different essential oils may have drastically different pharmacology. Some act as locally anaesthetic counterirritants and, thereby, exert an antitussive effect (Soares, I.H.; Loreto *et al.,* 2014)(Mandras, Narcissa; Nostro *et al.,* 2016).

Some essential oils, such as those of juniper and agathosma, are valued for their diuretic effects (Sapeika, Norman 1963). With relatively recent concerns about the overuse of antibacterial agents (Haneke, Karen E 2002), many essential oils have seen a resurgence in off-label use for such properties and are being examined for this use clinically (Watt, John Mitchell; *et al.,* 1962). Essential oils (EOs) are important for their detected antibacterial activity including that against S. Mutans (Levy, Stuart B. (2001, Singh, G.; Kapoor, I. P. S.2002., M. A. Botelho, N. A. P. Nogueira, G. M. Bastos *et al.,* 2007., F. Silva 2005). The presence of complex chemical structures constituted of several groups, such as terpenes and terpenoids, aromatic and aliphatic constituents, all characterized by low molecular weight, may explain their successful bacteriostatic and bactericidal action (Bakkali, S. Averbeck, et al. 2008).

Thyme (Thyme Vulgaris) is a perennial herb which is hosting mainly in Mediterranean countries. The medicinal part of thyme is flowering part and branches. Thyme plant is a strong antispasmodic, antiseptic, aromatic smell, slightly bitter. The main chemical components are athujone, a-pinene, camphene, b-pinene, pcymene, a-terpinene, linalool, borneol, caryophyllene, thymol and carvacrol. The health benefits of thyme are undeniable; thyme oil alone, or when used in a mixture of other natural health-promoting compounds, has been found to relieve the following conditions. Nail fungus, Parasites, Muscle pain, Chronic fatigue, hair loss, Depression, Fatigue, Headache, insomnia, snoring, and Skin problems. As an antioxidant, thyme protects the body from the effects of ageing. As a stellar digestive herb, thyme can enhance appetite and digestion while stimulating the liver. One of the characteristics of pure essential oils is their ability to penetrate mucous membranes. Therefore, their supragingival use yields a beneficial subgingival effect.

Rosemary (Rosmarinus officinalis L) initially grows in southern Europe. Rosmarinus officinalis L.(Lamiaceae) is a small evergreen plant which grows wild in most Mediterranean countries. The main producers are Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa. Essential oils of R. officinalis, known as rosemary oils, are obtained by steam distillation of the fresh leaves and twigs, and the yields range from 0.5 to 1.0 %. It is almost colourless to pale yellow liquid with a characteristic, refreshing and pleasant odour. The major constituents of the rosemary essential oil reported in literature being α- pinene, 1, 8-cineol and camphor; associated with variable amounts of camphene, limonene, borneol, verbenone, etc. The rosemary oil is used as a

seasoning for foodstuffs, such as meat salami and sauces, but due to its chemical active constituents properties, it is used as an antioxidant (for food preserving), antibacterial and antifungal agents against some spoilage organisms. The oil is also used in traditional medicine as tonic pulmonary antiseptic, choleretic and cholagogues agents. It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing of a wide range of diseases such a diabetic mellitus (DM), respiratory disorders, stomach problems and inflammatory diseases. Its herb and oil are commonly used as spice and flavouring agents in food processing for its desirable flavour, high antioxidant activity and lately as an antimicrobial agent. Studies reveal that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries.

Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine. Of these, *Ocimum tenuiflorum*, also known as *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities. It has been one of the most valued and a holistic herb used over the years in traditional medicine in India, and almost every part of the plant has been found to possess therapeutic properties. Traditionally, Tulsi is used in different forms; aqueous extracts from the leaves (fresh or dried as powder) are used in herbal teas or mixed with other herbs or honey to enhance the medicinal value. Traditional uses of Tulsi aqueous extracts include the treatment of different types of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart disease. Oils extracted from Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers; and as anti-asthmatic, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents.

The aim of this study is to evaluate the antibacterial activity of three essential oils (THYME OIL, ROSEMARY OIL, and HOLY BASIL OIL) and to analyze its effectiveness against bacteria causing caries.

MATERIALS AND METHODS

Test microorganisms

Bacterial strain used was *Streptococcus mutans.* The organism was isolated using selective media Mutans -Sanguis agar and maintained in nutrient agar slope at 4°C in the Department of Microbiology, Saveetha Dental College.

Methodology

The essential oils, Thyme oil, Rosemary oil and Holy basil oil were loaded on sterile filter paper discs measuring 6mm diameter in the following concentrations 50µl, 100µl and 200 µl respectively. The discs were dried and kept aseptically.

RESULTS AND DISCUSSION

Table 1: Please provide a caption, the table should be in text format

SAMPLES	SAMPLE CONCENTRATION [µL]	ZONE OF INHIBITION [mm]
THYME OIL	200 µL	30mm
	100 µL	22mm
	50µL	12mm
ROSEMARY OIL	200 µL	52mm
	100µL	40mm
	50µL	28mm
HOLY BASIL OIL	200 µL	44mm
	100µL	34mm
	50µL	16mm
CHLORHEXIDINE (CONTROL)	200 µL	28mm
	100µL	20mm
	50µL	11mm

Screening of antibacterial activity (Disc diffusion technique)

Broth culture of the bacterial strain compared to Mac Farland's standard 0.5 was prepared. Lawn culture of the test organisms was made using a sterile cotton swab on the Muller Hinton agar plates, and the plates were dried for 15 minutes. Filter paper discs loaded with different concentrations of the essential oils were placed on the respective plates. The zone of inhibition of growth was measured in millimetres after incubating the plates at 37°C overnight, and All the tests were done in triplicate to minimise the test error.

The antibacterial activity of the essential oils at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the Table. The rosemary oil was more effective against Streptococcus mutans with a zone of inhibition of 52 mm diameter (at conc200 µl), Rosemary oil showed a zone of inhibition of 44 mm diameter and with thyme oil, the zone diameter was 30 mm.

In the study done at Saveetha dental college in the year, 2014 showed that neem oil was more

effective against bacteria causing caries. The three essential oils which were used in that study was Neem oil, Peppermint oil and Thyme oil. While in our study Rosemary oil had increased zone of inhibition and found to be more effective against bacteria causing caries. Anti-bacterial activity of lavender oil, peppermint oil, tea tree oil, thyme oil and eugenol oil was done. The results showed that only eugenol oil, tea tree oil and peppermint oil exhibited the inhibitory effect. In the study done by PrabuSeenivasan in the year 2006, Anti-bacterial activity of 21 essential oils was discussed against various pathogens. Out of 21essential oils, 19 essential oils showed activity against one or more bacteria. Oils like cinnamon oil, rosemary oil, and orange oil showed maximum activity against all the bacterial species tested. On the other hand, eucalyptus oil and camphor oil failed to inhibit any of the tested strains(Prabuseenivasan S, Jayakumar M. 2006). Several studies showed that cinnamon oil, clove oil and rosemary oil had strong and consistent inhibitory effects against various pathogens(Matan N, Rimkeeree H, *et al.,* 2006., Aureli P, Costantini A, Zolea S 1992). Many studies were done in Saveetha Dental College regarding Anti-bacterial activity of various oils against oral pathogens.

CONCLUSION

The results show that all these three essential oils have significant antibacterial activity against bacteria causing caries. Herbs, which are powerful healing agents, must be used appropriately. Herbs contain active ingredients that may interact negatively with prescribed medications or other remedies. It is wise, therefore, to consult a health-care professional in situations in which you question the appropriateness of the herb or its interaction with other remedies. The use of herbs in dentistry should be based on evidence of effectiveness and safety. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The present results, therefore, offer a scientific basis for the traditional use of thyme oil, holy basil oil and rosemary oil.

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