

Antimicrobial Efficacy Of Oregano Oil, Thyme Oil and Helichrysum Oil Against Oral Pathogens: An In Vitro Study

Research Article

Sushanthi^{1*}, Pradeep Kumar Rathinavelu², Meignana Arumugham³

¹Post-Graduate Student, Department of Public Health Dentistry, Saveetha Dental College, Saveetha University, Saveetha Institute of Medical and Technical Science, Chennai, India.

²Professor and Head of the Department (Admin), Department of Public Health Dentistry, Saveetha Dental College, Saveetha University, Saveetha Institute of Medical and Technical Science, Chennai, India.

³Professor and Head of the Department (Academics), Department of Public Health Dentistry, Saveetha Dental College, Saveetha University, Saveetha Institute of Medical and Technical Science, Chennai, India.

Abstract

Essential oils are concentrated natural extracts derived from plants, which were proved to be good sources of bioactive compounds with antioxidative and antimicrobial properties. Aim of the study is to determine the antimicrobial activity of thyme oil, oregano oil and helichrysum oil individually and also all three combined as a fourth group against *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans*. Agar well diffusion method was used to determine the antibacterial activity of different concentrations of the chosen essential oils. Individually, at 100µl concentrations against *S. Mutans* Thyme oil and Helichrysum oil are effective in inhibition followed by all three combinations. At 100µl concentrations against *S. Aureus* combination oil holds the highest zone of inhibition followed by Oregano oil. Thyme oil was most effective against *E. Faecalis* and least effective was helichrysum oil. Thyme oil was more effective against *C. Albicans* followed by Oregano oil and combination oil where as helichrysum oil was the least effective oil against oral pathogens.

Keywords: Thyme Oil; Essential Oil; Helichrysum Oil; Oregano Oil; *Streptococcus Mutans*.

Introduction

Since the late twelfth century, essential oils which are also called volatile oils derived from natural plant extract, have been used as alternative medicines and set off wide spread in the second half of the sixteenth century [1]. In consideration of developments which we achieved in the scientific field, the medicinal properties of plants have encountered a huge interest on account of its less toxic nature, better pharmacological activities and economic viability [1, 2]. Essential oils, a group of compounds can be termed as additives obtained from the plants. In recent times, natural products are on a competitive track among food industries in which direct addition of natural plants and oils to their products

or little contribution to add a synergistic effect. It has also been documented that straight addition of aromatic plant essential oils and extracts to foodstuffs provides an antioxidant or antimicrobial effect to their products [3].

Essential oils can be extracted from the plant parts like leaves, flowers, barks, seeds etc. Oils cannot be directly grinded from the above mentioned parts but it has to undergo multiple complex purifying methods like distillation, hydrodistillation, solvent extraction etc [4].

Specifically the antimicrobial activity of essential and extracts has been the base for many applications in various industries, includ-

*Corresponding Author:

Sushanthi,
Post-Graduate Student, Department of Public Health Dentistry, Saveetha University, Saveetha Institute of Medical and Technical Science, Saveetha Dental College and Hospitals, 162, Ponnammalee high road, Velapanchavadi, Chennai-600077, India.
Tel: 6383746057
Email Id: sushaantheesuresh@gmail.com

Received: April 28, 2021

Accepted: May 28, 2021

Published: May 30, 2021

Citation: Sushanthi, Pradeep Kumar Rathinavelu, Meignana Arumugham. Antimicrobial Efficacy Of Oregano Oil, Thyme Oil and Helichrysum Oil Against Oral Pathogens: An In Vitro Study. *Int J Dentistry Oral Sci.* 2021;08(05):2615-2619. doi: <http://dx.doi.org/10.19070/2377-8075-21000512>

Copyright: Sushanthi©2021. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

ing food preservation technology, pharmaceuticals, alternative medicine and natural therapies [5, 6]. Essential oils were successful in treating several oral conditions including pain, gingivitis and even to reduce postoperative pain after extraction. This induced the interest in research to innovate the antibacterial activity of certain plant essential oils in their particular specialism.

Thymus vulgaris is a species of evergreen plant belonging to Lamiaceae family originating from Mediterranean regions has numerous medicinal values and in recent times it turns wonders in dentistry too. *Thymus vulgaris* leaves oil or extract has also been used in the treatment of sore throat, tonsillitis, gum diseases, rheumatism, and arthritis [7]. Thymol and carvacrol contents of the oil were believed to be responsible for the antimicrobial activities of *Thymus vulgaris* oil [8]. This essential oil has been considered as an antiseptic, antimicrobial, antispasmodic, antioxidant, and antitussive agent.

Helichrysum italicum is a typically Mediterranean plant. Basically, essential oil is present in all green parts of the plant. This plant oil has been known for its good anti inflammatory and antimicrobial activity against *Staphylococcus aureus* strains, reducing both their growth and some of the enzymes such as coagulase, DNase, thermonuclease, and lipase [9]. The main components of this essential oil were α -pinene, neryl acetate, α -cedrene, nerol, α -curcumene, γ -curcumene, and geranyl acetate.

Oregano essential oil (OEO) is well known for its antimicrobial properties, as well as its antifungal and antioxidant actions. The major constituents, carvacrol (55-85%) and thymol (0-5-10%), have the most potent antimicrobial activity due to their phenolic structure [10]. Selectivity against Gram-negative bacteria but with lesser activity against Gram-positive *Lactobacillus* and *Bifidobacterium* has been observed.

So the aim of the study is to determine the antimicrobial activity of thyme oil, oregano oil and helichrysum oil individually and also all three combined as a fourth group against *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans*.

Materials and Methods

Essential Oils

Three most commonly used and promising essential oils were brought from the commercial market to check for their antimicrobial against oral pathogens. Thyme oil (*Thymus vulgaris*), Helichrysum oil (*Helichrysum italicum*), Oregano oil (*Origanum vulgare*) were the chosen essential oils for this study. We tried to get the original oil with good quality with no added artificial or synthetic compounds.

Figure 1. a. Antimicrobial activity of Thyme oil against C. Albicans. b. Antimicrobial activity of Helichrysum oil against E. Faecalis.

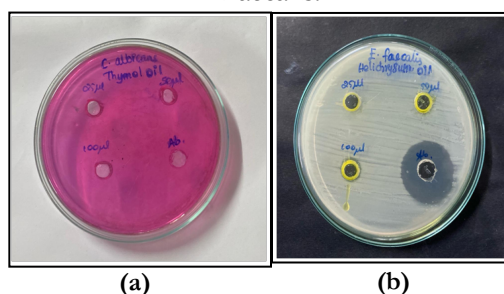


Figure 2. a. Antimicrobial activity of Helichrysum oil against S. Mutans. b. Antimicrobial activity of Oregano oil against S. Mutans.

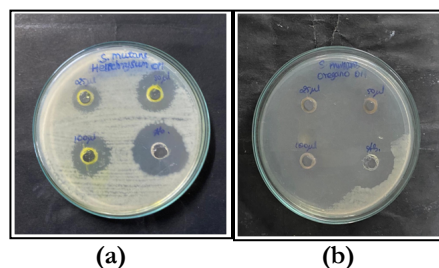
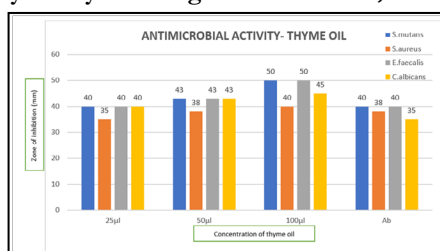


Figure 3. Antimicrobial activity of thyme oil against S. Mutans, S. Aureus, E. Faecalis, C. Albicans.



Micro Organisms

The most commonly found oral microorganisms were chosen for the study. Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans were selected. Microorganisms were obtained from the Department of Pharmacology and Nanotechnology, Saveetha University.

Antimicrobial Analysis

By well diffusion method, the antibacterial activity of thyme oil, oregano oil and helichrysum oil and a combination of all the above three essential oil as a fourth one were studied against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans. Secondary cultures of microbial suspension were dispersed evenly on the surface of Mullen Hinton agar and Rose Bengal agar plates using a sterile spreader. Four wells were made in each plate by a well cutter. Different concentrations of each essential oil (25µl, 50µl, 100µl) were incorporated through a sterile micropipette in to the wells created on the agar plate using sterile cork borer. The plates were then incubated at 37°C for 24 hours to 48 hours. Commercial antibiotic ampicillin (50mg/ml) was used as a positive control for S. Mutans, S. Aureus, E. Faecalis and fluconazole (50mg/ml) was used as positive control for Candida albicans. The zone of inhibition (mm) was recorded for each plate and compared with control. All the tests were replicated in triplicate for analysis.

Results

Agar well diffusion method was used to determine the antibacterial activity of different concentrations of the chosen essential oils like thyme oil, oregano oil, Helichrysum oil and all three combinations of it against S. Mutans, S. Aureus, E. Faecalis, C. Albicans (Figure 1 and 2). Antimicrobial efficacy of thyme oil against oral pathogens in different concentrations was shown in Figure 3. Antimicrobial efficacy Helichrysum oil against oral pathogens in different concentrations was shown in Figure 4. Antimicrobial efficacy Oregano oil against oral pathogens in different concentrations was shown in Figure 5. Antimicrobial efficacy of all three essential oils against oral pathogens in different concentrations was shown in Figure 6. Mean of inhibition was found to be increased as the concentration of the essential oils gets increased.

At 100µl concentration for thyme oil, the maximum zone of inhibition for S. Mutans, S. Aureus, E. Faecalis, C. Albicans was higher when compared with their respective antibiotic. At 100µl concentration for helichrysum oil, the maximum zone of inhibition for C. Albicans was found higher than fluconazole. When compared with their respective antibiotics for oregano oil, at 100µl concentrations the maximum zone of inhibition was found higher against all four oral pathogens. When all the three essential oils are combined, it gives an absolute synergistic effect where the maximum zone of inhibition was found at 100µl compared with antibiotics.

Individually, at 100µl concentrations against S. Mutans Thyme

Figure 4. Antimicrobial activity of Helichrysum oil against S. Mutans, S. Aureus, E. Faecalis, C. Albicans.

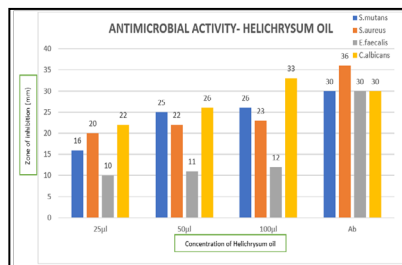


Figure 5. Antimicrobial activity of Oregano oil against S. Mutans, S. Aureus, E. Faecalis, C. Albicans.

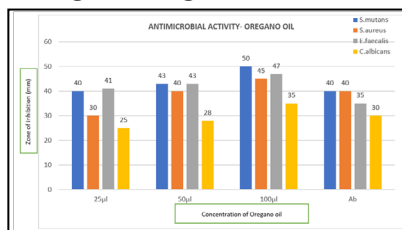
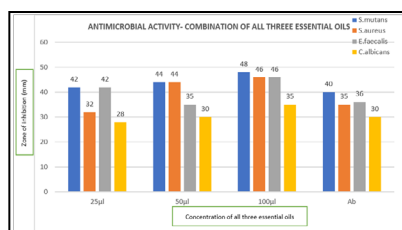


Figure 6. Antimicrobial activity of combination of all three essential oils against S. Mutans, S. Aureus, E. Faecalis, C. Albicans.



oil and Helichrysum oil are effective in inhibition followed by all three combinations. At 100 μ l concentrations against *S. Aureus* combination oil holds the highest zone of inhibition followed by Oregano oil. Thyme oil was most effective against *E. Faecalis* and least effective was helichrysum oil. Thyme oil was more effective against *C. Albicans* followed by Oregano oil and combination oil where as helichrysum oil was the least effective oil against oral pathogens.

Discussion

Since classical times, traditional plants and their extracts, oils have been used for varied medicinal purposes but without the exact mechanism of action and complete knowledge about the interaction with ihemicro organisms [1, 11]. Numerous essential oils are available nowadays even commercially.

Thyme (*Thymus vulgaris*) is a very popular herb that plays an important role in world cuisine. It is a flavoring for many meat-based foods. Its essential oil was shown to have antimicrobial, and anti-inflammatory properties [12]. Essential oils of thyme species that contain higher amounts of carvacrol and thymol, present stronger antimicrobial effect [13]. Thyme oil was most effective against *S. Mutans* and *E. Coli* at 100 μ l concentrations with a maximum zone of inhibition. According to Fournomiti et al, thyme oil showed good antimicrobial activity against *E.Coli*, *Klebsiella oxytoca* [11, 14].

Helichrysum italicum as an antimicrobial and anti-inflammatory agent because of its flavonoids and terpenes which were effective against bacteria (e.g. *Staphylococcus aureus*) and its acetophenones, phloroglucinols and terpenoids displayed antifungal action against *Candida albicans* [15, 16]. According to our study results at 100 μ l concentration for helichrysum oil, the maximum zone of inhibition for *C. Albicans* was found higher than fluconazole. When compared with other oils, helichrysum has lower antimicrobial activity.

Among various products of oregano oil thymol and carvacrol [15, 17] are the main components which are responsible for its anti-oxidative, antimicrobial and antifungal effects [18, 10, 19]. A main component of oregano essential oil (more than 60% of the oil composition) is carvacrol, a monoterpenoid phenol, which can also be found in thyme or bergamot, but in lower concentrations [20]. According to the study results, oregano oil shows very good antimicrobial activity against all four oral pathogens chosen for this study. All other essential oils show good antimicrobial activity at 100 μ l concentration but only in oregano oil it shows good antimicrobial activity even at 50 μ l concentration. When we are adding up all three essential oils in equal concentrations we get a synergistic effect and have good antimicrobial activity at 25 μ l concentration, 50 μ l concentration and at 100 μ l concentration when compared with their respective antibiotic.

According to researchers thinking, the antibiotic era may end soon and so a perfect alternative to antibiotics may be of herbal plants and oils. The essential oils, as natural extracts, with low adverse effects, may become reliable alternatives in antimicrobial fight. This could benefit more patients from their local treatments like skin infections due to bacterial infections even to the infections which are hard to control. Further studies are needed in order to find the

proper ways to deliver the active antimicrobial compounds.

Conclusion

The following chosen essential oils have good antimicrobial property against *S. Mutans*, *S. Aureus*, *E. Faecalis* and *C. Albicans*. Among all oregano oil and thyme oil were most effective against oral pathogens, Helichrysum oil when used alone does not show much activity but when added with other essential oils shows good antimicrobial activity.

References

- Man A, Santacroce L, Jacob R, Mare A, Man L. Antimicrobial Activity of Six Essential Oils Against a Group of Human Pathogens: A Comparative Study. *Pathogens*. 2019 Jan 28;8(1):15. Pubmed PMID: 30696051.
- Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, et al. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol*. 2003 Feb;84(2-3):131-8. Pubmed PMID: 12648805.
- Costa DC, Costa HS, Albuquerque TG, Ramos F, Castilho MC, Sanches-Silva A. Advances in phenolic compounds analysis of aromatic plants and their potential applications. *Trends in Food Science & Technology*. 2015 Oct 1;45(2):336-54.
- Tongnuanchan P, Benjakul S. Essential oils: extraction, bioactivities, and their uses for food preservation. *J Food Sci*. 2014 Jul;79(7):R1231-49. Pubmed PMID: 24888440.
- Soliman SSM, Alsaadi AI, Youssef EG, Khitrov G, Noreddin AM, Husseiny MI, et al. Calli Essential Oils Synergize with Lawsone against Multidrug Resistant Pathogens. *Molecules*. 2017 Dec 20;22(12):2223. Pubmed PMID: 29261103.
- Taiwo MO, Adebayo OS. Plant essential oil: an alternative to emerging multidrug resistant pathogens. *J Microbiol Exp*. 2017;5(5):1-6.
- Tsai ML, Lin CC, Lin WC, Yang CH. Antimicrobial, antioxidant, and anti-inflammatory activities of essential oils from five selected herbs. *Biosci Biotechnol Biochem*. 2011;75(10):1977-83. Pubmed PMID: 21979069.
- Ali NA, Alhamzy EH, Chhetri BK, Dosoky NS, Setzer WN. Chemical composition, antimicrobial, and cytotoxic activities of the essential oil of *Otostegia fruticosa* subsp. *schimperii* from Yemen. *Natural Product Communications*. 2017 Jun;12(6):1934578X1701200634.
- Nostro A, Bisignano G, Angela Cannatelli M, Crisafi G, Paola Germanò M, Alonzo V. Effects of *Helichrysum italicum* extract on growth and enzymatic activity of *Staphylococcus aureus*. *Int J Antimicrob Agents*. 2001 Jun;17(6):517-20. Pubmed PMID: 11397624.
- Ben Arfa A, Combes S, Preziosi-Belloy L, Gontard N, Chalier P. Antimicrobial activity of carvacrol related to its chemical structure. *Lett Appl Microbiol*. 2006 Aug;43(2):149-54. Pubmed PMID: 16869897.
- Fournomiti M, Kimbaris A, Mantzourani I, Plessas S, Theodoridou I, Pappaemanouil V, et al. Antimicrobial activity of essential oils of cultivated oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) against clinical isolates of *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*. *Microb Ecol Health Dis*. 2015 Apr 15;26:23289. Pubmed PMID: 25881620.
- Oliveira JR, de Jesus Viegas D, Martins APR, Carvalho CAT, Soares CP, Camargo SEA, et al. *Thymus vulgaris* L. extract has antimicrobial and anti-inflammatory effects in the absence of cytotoxicity and genotoxicity. *Arch Oral Biol*. 2017 Oct;82:271-279. Pubmed PMID: 28683409.
- Mohtashami S, Rowshan V, Tabrizi L, Babalar M, Ghani A. Summer savory (*Satureja hortensis* L.) essential oil constituent oscillation at different storage conditions. *Industrial crops and products*. 2018 Jan 1;111:226-31.
- Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett Appl Microbiol*. 2003;36(3):162-7. Pubmed PMID: 12581376.
- Antunes Viegas D, Palmeira-de-Oliveira A, Salgueiro L, Martinez-de-Oliveira J, Palmeira-de-Oliveira R. *Helichrysum italicum*: from traditional use to scientific data. *J Ethnopharmacol*. 2014;151(1):54-65. Pubmed PMID: 24239849.
- Mastelic J, Politeo O, Jerkovic I, Radosevic N. Composition and antimicrobial activity of *Helichrysum italicum* essential oil and its terpene and terpenoid fractions. *Chemistry of natural compounds*. 2005 Jan;41(1):35-40.
- Tapiero J, Salamanca G, Marín C. Analysis of volatile compounds and antioxidant activity of the essential oil of oregano (*Origanum vulgare* L.). *Adv Med Plant Res*. 2019;7(2):54-60.

- [18]. Radusienne J, Peculyte D, Janulis V. Variability and antimicrobial activity of *Origanum vulgare* subsp. *vulgare* essential oils. In I International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation 723 2006 Feb 22 (pp. 393-398).
- [19]. Rodriguez-Garcia I, Silva-Espinoza BA, Ortega-Ramirez LA, Leyva JM, Siddiqui MW, Cruz-Valenzuela MR, et al. Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products. *Crit Rev Food Sci Nutr*. 2016 Jul 26;56(10):1717-27. Pubmed PMID: 25763467.
- [20]. De Vincenzi M, Stamatii A, De Vincenzi A, Silano M. Constituents of aromatic plants: carvacrol. *Fitoterapia*. 2004 Dec;75(7-8):801-4. Pubmed PMID: 15567271.