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Efficacy Of Essential Oil Enriched With Almond Oil Against Streptococcus Mutans, PorphyromonasGingivalis, FusobacteriumNucleatum- An In Vitro Study.

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ABSTRACT

INTRODUCTION: Oral health is known to serve as a pathway for maintaining the general systemic health. The poor oral health is mainly due to the microorganisms which contributes combined action in formation of dental plaque and calculus which on long term irregular improper maintenance phases leads to destruction of the tooth supporting structures.The present study contemplates about the antimicrobial activity of essential oils against the bacteria's which destructs the supporting structures in oral cavity.

MATERIALS AND METHODS: An in vitro study is conducted for assessing the antimicrobial effects of the essential oils. Oil samples are prepared in required concentration and specific culture plate media for growth of the selected organisms are placed in specified environment. The microbial activity is checked by measuring the zone of inhibition from the inoculated culture plates.

RESULTS: Zone of inhibition for all the three organisms in all four samples are noted and higher zones of antimicrobial activity are seen in orange oil enriched in almond oil and Lavender oil enriched in almond oil against the organisms.

CONCLUSION: The results indicates that Essential oils are promising to be an antibacterial agent the chemical composition of the oils apparently determines the antimicrobial property. The major components in the essential oils individually pertaining to its natural source are responsible for the antibacterial actions.

KEY WORDS: Essential oil, Almond oil, Anti-microbial activity, Oral bacteria.

I. INTRODUCTION

A healthy state is a platform for acquiring healthy mind and well-being in an individual. In order maintain general health remerging of natural products and substitutes in clinical practices are making a viable role from historic periods into the modern era[.1] It's considered that the natural derivatives show results which are comparative and significant to that of medicine products which are obtained from man-made chemical sources. In particular the antimicrobial activity of the plant extracts has formed the basis of many applications in raw, processed food preservation, natural medicine and pharmacological applications.[2]Since synthetic antimicrobial drugs which are used for longer duration against diseases in humans the drawback of the prolonged use is the development of antimicrobial resistance. Due to the increased risk of antimicrobial resistance towards multiple drugs recent studies emphasize use of alternative natural substitutes especially obtained from the plant sources. The volatile compounds from the plant extract source particularly known as essential oils which are also called as secondary metabolite primarily is used in Aromatherapy, Cosmetics and Medicinal liquids are considered to show antimicrobialactivity.[3] The medicinal properties exerted by them act as Antimicrobials, Antibiotics, Anti-inflammatory and in also alterations in psychological mood stimulations. Oral health is known to serve as a pathway for maintaining the general systemic health. The poor oral health is mainly due to the microorganisms which contributes combined action in formation of dental plaque and calculus

which on long term irregular improper maintenance phases leads to destruction of the tooth supporting structures. [4]

Gingivitis is the inflammation of the gums and surrounding supportive tissues of the teeth due to the accumulation of plaque and calculus in which the periodontal microflora plays a vital role. Main contribution is by organisms *Streptococcus mutans, Porphyromonasgingivalis and Fusobacteriumnucleatum* in destruction of the gingival compartment.[5]

To overcome the adverse effects and restricted use of the synthetic preparations essential oils with antimicrobial properties are targeted towards the periodontal flora. Since many essential oils show antimicrobial properties, the predominant action of essential oil towards facultative anaerobes are used in this study. The aim of the study is to check for the antimicrobial properties of essential oils combination against *Streptococcus mutans, Porphyromonasgingivalis and Fusobacteriumnucleatum*. The essential oils due to its higher concentration gradient when used on sensitive mucosa it cannot be applied or exposed directly so a carrier oil is used in which the essential oils are mixed. Carrier oils are those which form the base in which the main ingredient oil is added to show its effect with known concentration gradient usually coconut oil is used but an alternative to it is almond oil which also shows similar properties. In this study almond oil is used as carrier oil in which individual concentrations of essential oils like Lavender oil,orange oil and Eucalyptous oil are used and the antimicrobial of individual concentrations are checked.

II. MATERIALS AND METHODS

The study is an Invitro study which is done under a closed laboratorial condition in which three organisms were isolated and checked for the antimicrobial activity. The ethical approval was obtained from the institutional ethical committee. The study setting was an invitro environment which was done under a private laboratory in which the organisms were cultured and tested for the antimicrobial efficacy.

ORGANISM AND GROWTH CONDITIONS PREPARATION METHODS

Microorganisms The microbial strains employed in the biological assays were Gram – **positive** bacteria: *Streptococcus mutans* and Gram – **negative** bacteria: *Porphyromonasgingivalis* and*Fusobacteriumnucleatum* Obtained from Rontgen Laboratory, Thanjavur, Tamil Nadu. The collected organisms were further confirmed their biochemical characterization as Gram staining, Indole test and Catalase.

Preparation of media

Mutans-Sanguis Agar Medium

Mutans-Sanguis Agar is recommended for the isolation of *Streptococcus mutans (Table 1)*

Preparation of medium:

Suspend 98.1 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at15 lbs pressure (121°C) for 15 minutes. Mix well and pour into Petri plates.

Brucella Agar Base with Hemin and Vitamin K

Brucella Agar Base with Hemin and Vitamin Kis recommended for the isolation of *Porphyromonasgingivalis. (Table 2)*

Preparation of medium:

Suspend 43.12 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5% v/v sterile defibrinated sheep blood. Mix well before pouring into sterile Petri plates.

L. D. Agar

L. D. Agar is recommended for the isolation of *Fusobacteriumnucleatum. (Table 3)*

Preparation of medium:

Suspend 33.22 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Preparation of 24 hours broth culture

A 25 guagenichrome wire is bend for making a loop of 1mm diameter and inoculated in free flame to carry each of the microorganisms and was suspended in about 5ml of physiological saline in a tube. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8˚C until use.

Preparation of sample solutions for the experiment

Essential oils are compounds derived from the plants through steam distillation process or by cold pressing techniques. The oil captures the plants scent and flavor which is termed as “essence”. The essential oils obtained from different plant sources shows uniqueness in their properties and potentiality towards the benefits. Essential oils from Gyoshicoroporation (Nature’s Absolutes) were purchased for the study. The essential oils are obtained from the flower of *Lavandula angustifolia* steam distillation for Lavender oil, Orange peel *Citrus x sinensis* steam distillation for Orange oil and *Eucalyptus globulus* leaf steam distillation for Eucalyptus oil. The main

ingredients responsible for the antimicrobial activity of the essential oils are listed in Table 4.. The ratio of concentration in each oil sample in the study is taken as 1:0.5ml where 1ml of Almond oil contains 0.5ml of essential oils. Each sample standardization was made by adding 10 drops of Essential oil individually in 10ml of Almond oil by Lavender oil , Orange oil and Eucalyptus oils individually and also to determine that almond oil itself has any antimicrobial potential its included as a control separately without any additional components shown below.

SAMPLES	COMBINATION
A	Almond oil + Lavender oil
B	Almond oil + Orange oil
C	Almond oil + Eucalyptous oil
D	Almond oil

Determination of Antimicrobial activity by Zone of Inhibition.

The antimicrobial activity was performed by well diffusion method (NCCLS, 1993; Awoyinka*et al.,* 2007). Petri plates were prepared by pouring 30 ml of medium for bacteria. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial broth culture and used to evenly inoculate the entire surface of the agar plate and allowed for 10 mints. Briefly, inoculums containing *Streptococcus mutans, Porphyromonasgingivalis* and *Fusobacteriumnucleatum* species of bacteria were spread on respective agar plates. Using micropipette tips to prepare the well (7 mm diameter) and 100µl of each sample added on the respective well of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1). Each sample was tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

III. RESULTS

Four different, commercially available essential oils lavender oil, Orange oil, Eucalyptus oil and Almond oil were tested for the antimicrobial activity against the strains of Gram positive Anaerobes and Facultative anaerobe bacteria.

The antimicrobial potential of test compounds was estimated by measuring the zone of inhibition (Table:5). All the four samples showed a great potential against the three strains of bacteria. In all three organisms the zone of inhibition was observed in all four samples and higher zones for *Streptococcus mutans* was measured as 10.25±0.72mm (Fig:1) in Mutans-Sanguis Agar medium by Almond enriched orange oil. On *Porphyromonasgingivalis* 14.25±1.00mm zone of inhibition (Fig:2) Brucella Agar Base with Hemin and Vitamin K medium by Almond enriched orange oil and in *Fusobacteriumnucleatum* 12.75±0.89mm zone of inhibition in (Fig:3) L. D. Agar medium by Almond enriched lavender oil. There was also antibacterial effects by Almond enriched eucalyptous oil and Almond oil sample with minimal zones of inhibition in all three organisms in general.

IV. DISCUSSION

Essential plant products like oils, extracts, and powders have historically been used in medicine to prevent infectious diseases as topical preparations because they are thought to have antiseptic and antibacterial properties.[6] Anecdotal evidence and the use of plants as medicine provide the foundation for the use of essential oils and plant extract in specific medical conditions. Clove, cinnamon, and tea tree were once employed as natural substitutes in herbal medicinal oils.[7]

Numerous studies are carried out to determine and examine the effects of essential oils in the disciplines of dentistry and medicine to treat with a holistic approach. According to a study by Mitscher et al., it is crucial to do scientific research on the plants used in traditional medicine as a possible source of novel antimicrobials.[8]

The resurgence of interest in natural therapies are increasing and demand for effective,safe,natural products and the data on quantitative aspects of plant oils and extracts are required

According to a study by K.A. Hammem et al, essential oils have strong antibacterial action, however varied oil concentrations should be used with caution and with concern for safety.[9] According to a review study by Ruchika Agarwal, although eucalyptus oil has been used extensively and successfully to treat a number of infections, human trials for its safety and potential side effects are still being conducted.[10]

According to G. Bochir et alstudy's eucalyptus essential oils have widespread antibacterial effects on bacteria, fungi, and some viruses. However, while they are likely to be used as antiinfectives in the agriculture and food industries, their medicinal use must be closely regulated due to their high potency and irritating capacity on sensitive mucosa.[11]

According to a study published in the International Journal of Biotechnology, using orange oil has antibacterial effects due to the active ingredient limolene, which is found to be a major component of Citrus x sinensis[12]. As a result, the antimicrobial effects are used to combat medically significant pathogens that cause various infections. It was discovered that lavandula was an effective choice for infection management in an animal investigation using lavender essential oils from Lavandula species[13]. The oil was examined for the antibacterial activities in facultative anaerobic gramme positive and negative pathogens. J. When employed as a medicinal endodontic root canal sealer, eucalyptus and orange oils have similar mechanisms of action, according to Martor et al.[14]

The use of essential oils, according to a study by Andrea Pusharova et al, offers an alternative method for combating microbial contamination, but only when the concentrations of the oils in humans are low enough to be safe. The study also noted that the essential oils have strong disinfectant properties[15].

In conclusion, there are many essential oils that exhibit antimicrobial properties, but very few of them have any effect on the facultative anaerobes, which are the majority of the organisms that cause damage to the oral cavity. Thus, in this study, gingival compartment destruction organisms are the focus of Lavender, Orange, and Eucalyptus oil treatment, and impressive antibacterial effects are shown.

V. CONCLUSION

The results indicates that Essential oils are promising to be an antibacterial agent the chemical composition of the oils apparently determines the antimicrobial property. The major components in the essential oils individually pertaining to its natural source are responsible for the antibacterial actions. Thus all the three essential oils used in this study against the bacteria's Streptococcus mutans , Porphyromonasgingivalis and Fusobacteriumnucleatum exhibited a wide action of antibacterial effects. However the minor components present in the should also be considered because they may produce some synergestic, additive or antagonist actions when individual preparations are made.

REFERENCES

[1]. Ruchika Agarwal 1 Lakshmi.T Eucalyptus oil in dentistry: A mini Review Int. J. Drug Dev. & Res., October -December 2013, 5 (4): 58-61

[2]. Seenivasan Prabuseenivasan, Manickkam Jayakumar and Savarimuthu IgnacimuthuIn vitro antibacterial activity of some plant essential oils BMC Complementary and Alternative Medicine https://doi.org/10.1186/1472-6882-6-39

[3]. NCCLS. (1993) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. PA: NCCLS Publications 25.

[4]. Awoyinka, O., Balogun, I.O., Ogunnowo, A.A. (2007) Phytochemical screening and *in vitro* bioactivity of *Cnidoscolusaconitifolius* (Euphorbiaceae). *J Med Plant Res*; 1(3): 63-65.

[5]. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pac J Trop Biomed*. 2012;2(9):739–742. doi:10.1016/S2221-1691(12)60220-2

[6]. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extractsJApplMicrobiol. 1999 Jun;86(6):985-90.

[7]. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus.Asian Pac J Trop Biomed. 2012 Sep;2(9):739-42. doi: 10.1016/S2221-1691(12)60220-2.

[8]. E. R. Hendry, T. Worthington, B. R. Conway, P. A. LambertAntimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J AntimicrobChemother. 2009 Dec; 64(6): 1219–1225. Published online 2009 Oct 16. doi: 10.1093/jac/dkp362

[9]. Hossain S, Heo H, De Silva BCJ, Wimalasena SHMP, Pathirana HNKS, Heo GJ. Antibacterial activity of essential oil from lavender (*Lavandula angustifolia*) against pet turtle-borne pathogenic bacteria. *Lab Anim Res*. 2017;33(3):195–201. doi:10.5625/lar.2017.33.3.195

[10]. S. Frassinetti, L. Caltavuturo, M. Cini, C. M. Della Croce & B. E. Maserti (2011) Antibacterial and Antioxidant Activity of Essential Oils from *Citrus* spp., Journal of Essential Oil Research, 23:1, 27-31, DOI: 10.1080/10412905.2011.9700427

[11]. Shigeharu Inouye, Toshio Takizawa, Hideyo Yamaguchi, Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact, *Journal of Antimicrobial Chemotherapy*, Volume 47, Issue 5, May 2001, Pages 565–573, https://doi.org/10.1093/jac/47.5.565

[12]. Martos J, Bassotto AP, González-Rodríguez MP, Ferrer-Luque CM. Dissolving efficacy of eucalyptus and orange oil, xylol and chloroform solvents on different root canal sealers.IntEndod J. 2011 Nov;44(11):1024-8. doi: 10.1111/j.1365-2591.2011.01912.x. Epub 2011 Jun 10.

[13]. Little JW. Complementary and alternative medicine: impact on dentistryOral Surg Oral Med Oral Pathol Oral RadiolEndod. 2004 Aug;98(2):137-45.

[14]. J. Lehrner, G. Marwinski, S. Lehr, P. Johren, L. Deecke Ambient odors of orange and lavender reduce anxiety and improve mood in a dental office. PhysiolBehav. 2005 Sep 15; 86(1-2): 92–95. doi: 10.1016/j.physbeh.2005.06.031

[15]. Takarada, K., Kimizuka, R., Takahashi, N., Honma, K., Okuda, K., &Katô, T. (2004). A comparison of the antibacterial efficacies of essential oils against oral pathogens. *Oral microbiology and immunology, 19 1*, 61-4 .

TABLES:

TABLE 1. Composition of Mutanssanguis Agar Medium

Ingredients	**Gm/Liter**	**Ingredients**	**Gm/Liter**
Casein enzymic hydrolysate	15.000	Sodium acetate	12.000
Yeast extract	5.000	Sucrose	50.000
L-Cystine	0.200	Agar	12.000
Sodium sulphite	0.100		
Sodium chloride	1.000		
Disodium phosphate	0.800		
Sodium bicarbonate	2.000		

Final pH (at 25°C)	7.3±0.2

TABLE 2. Composition of Brucella Agar base with Hemin and Vitamin K

Ingredients	**Gm/Liter**	**Ingredients**	**Gm/Liter**
Casein enzymic hydrolysate	10.000	Sodium bisulphite	0.100
Peptic digest of animal tissue	10.000	Hemin	0.010
Yeast extract	2.000	Vitamin K1	0.010
Dextrose	1.000	Agar	15.000
Sodium chloride	5.000		

TABLE 3. Composition of L.D Agar media

Ingredients	**Gm/Liter**	**Ingredients**	**Gm/Liter**
Casein enzymic hydrolysate	5.000	L-Tryptophan	0.200
Yeast extract	5.000	Vitamin K1	0.010
Sodium chloride	2.500	Hemin	0.010

Sodium sulphite	0.100	Agar	20.000
L-Cystine	0.400		

Final pH (at 25°C) 7.4±0.2

Table 4: Essential oils and their biological active components

ESSENTIAL OILS	BIOLOGICAL ACTIVE CHEMICALS
Almond oil	Mono laureic acid and Alphatocopherol
Lavender oil	Linalol and Lavanolol
Orange oil	Limolene and Cyclohexane
Eucalyptus oil	1,8 Cineole and Cetronyll acetate

Table 5: Antibacterial activity of the microorganisms against the essential oil samples.

Microorganisms	A	B	C	D
Streptococcus mutans (mm)	7.50±0.23	10.25±0.72	8.75±0.61	5.50±0.39
Porphyromonasgingivalis(mm)	11.25±0.79	14.25±1.00	6.50±0.46	5.25±0.37
Fusobacteriumnucleatum (mm)	12.75±0.89	9.25±0.65	7.25±0.51	4.75±0.33

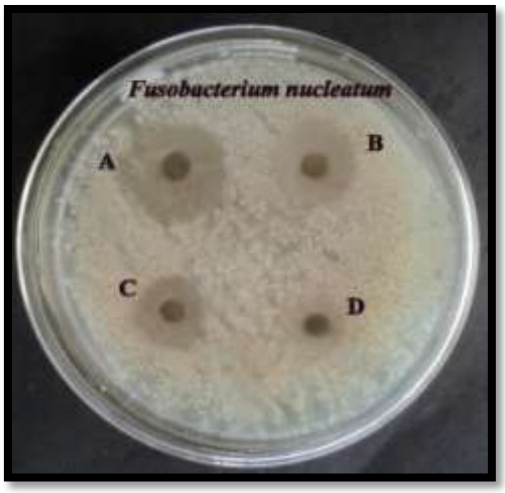
Values were expressed as Mean ± SD

FIGURES:

FIGURE 1: STREPTOCOCCUS MUTANS ANTIBACTERIAL EFFECT ON OIL SAMPLES

FIGURE 2: PORPHYROMONAS GINGIVALIS ANTIBACTERIAL EFFECT ON OIL SAMPLES

FIGURE 3: FUSOBACTERIUM NUCLEATUM ANTIBACTERIAL EFFECT ON OIL SAMPLES



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