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Antibacterial efficacy of Oregano Oil (*ORIGANUM VULGARE*) on the type of bacteria and its count in Dental Aerosols

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ABSTRACT

Dental aerosol disinfection should be considered as one of the most serious issues as they have very harmful microbial flora which could lead to multiple serious infections that commonly infect the operator, assistant, or the patients. Essential oils of nature like oregano have been proven recently to possess a greater action against gram-positive and gram-negative bacteria, Candida species, Streptococcus mutans, which are considered as the most prevalent oral microbial flora. Hence this study is aimed to determine the antibacterial effect of the oregano oil (oreganum vulgare) on dental aerosol bacteria around the dental unit during the dental procedure. Dental chairs with patients during treatment were included for the study where 5 agar plates with and without oregano application was placed in each chair at a distance of 1 – 1.5 m from the patient's mouth for 48hours and were incubated. Types of bacteria and its count were evaluated. Results showed significant contamination of bacteria in all plates without oregano oil application. Oregano represents an antibacterial source that can be as effective as modern medicine against pathogenic microorganisms especially the gram-positive and gram-negative bacteria which are usually present in dental aerosols.

Keywords - Dental Aerosol, Oregano Oil, Antibacterial Activity, Oral Bacteria

Introduction

Dentists, dental hygienists, and oral health care workers practice in a highly contaminated environment, the human mouth where they are exposed to a variety of bacteria, viruses, fungi, and protozoa from many sources[1]. A variety of dental procedures are being carried out in a dental operatory. Whereas the most common procedures that could have high aerosol contamination are endodontic and periodontic procedures since they incorporate usage of headpieces and scalers respectively. These procedures may result in the formation of aerosol and splatter which are commonly contaminated with bacteria, viruses, fungi, and blood. Microorganisms that are present in the mouth and respiratory tract of the patient can be transmitted through the aerosol during dental procedures leading to respiratory infections, skin infections, and other systemic diseases in subjects who are immunocompromised. These aerosols can also contaminate the mucous membrane of the mouth, respiratory passages, eyes of dental professionals as well as the patients and the surrounding surfaces [2].Subsequently, disposable gloves, face masks and eyeglasses have come into widespread use among dental staff and questions have been raised regarding how and where patients with communicable diseases should be treated.[3] Many alterations have been proposed by the dental schools thereafter to provide well-possessed sterilization and disinfection.[4]

Micro-organisms may also colonize dental equipment and water pipes, and form biofilms on their surfaces.[5] Sterilization processes have often been shown to be inadequate in dental primary care.[6] Bacteria and yeasts from these biofilms may contaminate during the dental procedures.[7] Bacterial species such as Pseudomonas aeruginosa,

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Pseudomonas cepacia, Legionella pneumophila, and Mycobacterium chelonae have also been identified in biofilms.[8] The concentration of total bacterial aerosols is associated with clinical working hours in dental surgeries.[9]

Previous research studies have documented increasing rates of antimicrobial resistance of Gram-Negative Bacilli (GNB) (Gupta et al., 2006).[10] Misuse of antibiotics and ineffective infection control have been implicated in the development and spread of resistant GNB pathogens which are associated with increased mortality, morbidity, prolonged hospitalization, and increased costs. Numerous studies provide us with a shred of strong evidence that so many drug-resistant strains of microbes have emerged and proliferated in the world. For example, the occurrence of fluoroquinolone-resistant GNB colonizing community-dwelling people with spinal cord dysfunction is common (Roghmann et al., 2006).[11] It has also been reported that ciprofloxacin-resistant GNB is becoming increasingly important and may cause serious infections in children (Qin et al., 2006).[12]

Multi-drug resistance is common and increasing among GNB, and several strains are being identified that exhibit resistance to commonly used antibiotics, including antipseudomonalpenicillins, cephalosporin, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim-sulfamethoxazole, and carbapenems (McGawan et al., 2006).[13] The impact of antibiotic resistance on the outcome of infections due to GNB remains highly controversial. Therapeutic options for multi-drug resistant GNB strains are limited, for this reason, there is a continuous need for alternative new chemical entities their activities may be identified through a variety of approaches (Combes et al., 2007). [14]

The antibacterial activities of essential oils are known for a longer time and many pieces of research on the antibacterial effect of essential oils and their derivatives have been reported. Essential oils from plants provide a rich source of antimicrobial properties. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microorganisms, including foodborne pathogens.[15-20] Essential oils are natural products extracted from vegetal materials. They are used as natural additives in many foods due to their antibacterial, antifungal, antioxidant, and anti-carcinogenic properties.[21,22] The antimicrobial, antiseptic, and other therapeutic applications of plants are well recognized for many years and they are widely used by all civilizations throughout the millennia [23]. Interestingly, according to the World Health Organization, approximately 80% of the world population, mostly in developing countries, still rely on medicinal plants and their extracts for primary health care [24, 25]. There are a few reports on the antimicrobial activity of essential oils or their major constituents .[26,27] Recent studies have shown that essential oils of oregano (*Origanumvulgare*) are among the most active compounds on the strains of *E.coli* when seen in vitro,[28,29] however their antibacterial activity has not been found in the dental aerosol. Hence the present study is aimed to determine the antibacterial effect of the oregano oil on dental aerosol bacteria around the dental unit during the dental procedure.

Materials and Methods

This was a quantitative observational study. Data were collected for one year in two dental school clinics (clinic I - Department of Endodontics and clinic II – Department of Periodontics). Both the dental clinics were sufficed with a good air-conditioned indoor environment with separate cabins which were separated uniformly with dividers which contained 15 chairs each. Both the clinics received more than 80 patients with fallout samples (N 80).

Clinic-I and Clinic-II consisted of 30 chairs and all the chairs were included for the study placing 5 agar plates in

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each chair (150 plates) at a distance of 1 – 1.5 m from the patient's mouth. Apart from these two separate plates were placed as negative controls in the Central Research Laboratory department (CRL) where they did not receive any fallout samples. Sheep blood agar plates were used for sample collection during the procedures. The placement of plates in each chair is mentioned as follows,

1. oregano oil 100%
2. oregano oil 50%
3. oregano oil 25%
4. positive control
5. positive control

Oregano oils were applied to the plates employing the spread plate technique.In Clinic-I and II, the plates were opened for 30 minutes. After sample collection, the plates were transported to the CRL where they were incubated at room temperature 37°C for 48 hours. The colonies were analyzed macro and microscopically, being carried out micro-cultures to observe the bacterial structures, and later the species were identified using Berger's manual. Biochemical tests were done to determine the pathogenicity of different bacteria.

Essential Oil Preparation

The oregano oil (*Origanumvulgare*) was purchased from Thai-China Flavours and Fragrances Co., Ltd., (510/3-4 SoiNgamwongwan 25, Muang, Nonthaburi 11000) shipped through amazon.

For all the concentrations, a standardized mix of a quantity of 150µl was used to perform the spread plate technique and their various concentrations were diluted using Dimethyl Sulfoxide (DMSO) as given below.

Essential oils and DMSO ratio (150 µl)

100% - 150 µl: 0 (Full strength)
50% - 75µl: 75µl (oregano oil: DMSO)
25% - 50µl: 100µl (oregano oil: DMSO)

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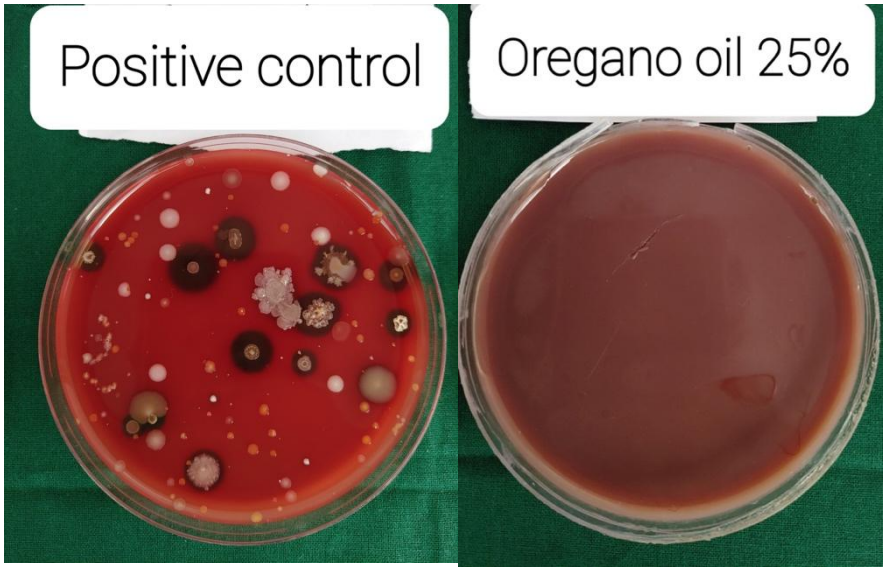

Results

Tables:
Figure 1.

Clinic-I

Type of Bacteria	Bacterial Name	AVG	Percentage
Gram-Positive Rod	Bacillus	10.0	6.66%
Gram-Negative Rod	Escherichia coli	62.0	41.33%
Gram-Positive Cocci	Staphylococcus sp.	48.5	32.33%
Gram-Negative Rod	Klebsiella sp.	29.5	19.66%

Figure 2.

Clinic-II

Type of Bacteria	Bacterial Name	AVG	Percentage
Gram-Positive Rod	Bacillus	10.0	6.66%
Gram-Negative Rod	Escherichia coli	62.0	41.33%

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Gram-Positive Cocci	**Staphylococcus sp.**	**48.5**	**32.33%**
Gram-Negative Rod	**Klebsiella sp.**	**29.5**	**19.66%**

The results showed significant contamination with all positive control samples without essential oil application in both the observation clinics during the procedures. Figures 1 & 2 shows the results of the bacterial fallout collection during dental procedures. The mean density of aerobic oral bacteria was 940 CFU/m^2/h from each plate in Clinic - I and 1260 CFU/ m^2/h from every plate in Clinic - II.

Finally, in Clinic-I we observed the total contamination of Gram-negative rods which consisted of *Escherichia coli* species (55%) and *Klebsiella* species (22%). Apart from that we also found Gram-positive cocci, namely *staphylococcus* species 15%, and Gram-positive rods, namely *Bacillus* species (8%). Whereas in clinic-II the Gram-negative rods were found in abundant and most prominent ones were *Escherichia coli* species (41.33%) followed by Gram-positive cocci, namely *staphylococcus* species (32.33%) and the least two were Gram-negative rods, namely *Klebsiella* species (19.66%) and Gram-positive rods, namely *Bacillus* species (06.66%).

In both the clinics, Escherichia *coli* was found at a higher percentage as compared to other bacteria. Negative Control plates which were placed in the CRL department showed a negative result for all organisms. Contamination was practically non-existent (0%) in plates with essential oil application and also in negative controls.

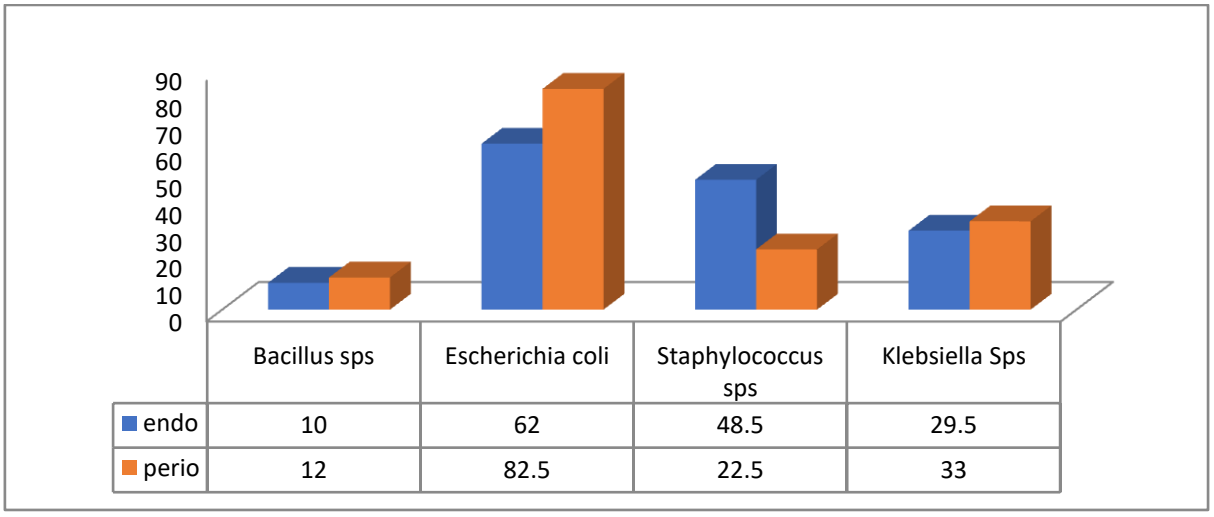

Figure 3. Prominent bacterial counts in Clinic I and Clinic II

Discussion

Aerosols are airborne substances that contain micro-organisms. Bioaerosols are those that occur as a consequence of certain dental treatments which pays the way for the spread of infection. These varieties of infection through splatter from the patient's mouth is considered a significant risk not only to the dentist but also to several other health care providers. Various dental equipment such as dental headpieces, air-water syringes,

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ultrasonic scalers, and air polishing units are known to produce the aerosols during the procedures.[30] Hence in this study, dental aerosols produced during the dental procedures carried out in Clinic I and Clinic II were considered. it is stated that the dimensions of the particle may vary from 0.001μm to quite 100 μm. the smallest particle size (ranging between 0.5 μm and 10 μm) has the most effective potential to penetrate the respiratory passages and therefore the lungs, possessing the ability to transmit the disease. periodontal disease, being multi-factorial, the oral cavity harbors innumerable bacteria and viruses from the tract, saliva, and bacterial plaque. These microorganisms get aerosolized when coming in contact with the dental equipment. Aerosols might not only contain bacteria but also HIV and mycobacterium tuberculosis.[31] Therefore, in the present study, the bacterial contamination present within the dental aerosols was collected and identified for their varieties and pathogenicity.

According to a study done by Yadav, et al in 2015, Aerosols may accommodate microorganisms like multi-resistant Staphylococcus aureus, influenza, legionnaire's disease, pneumonia, mumps, chickenpox, cytomegalovirus infection, hepatitis B and C viral infection, herpes simplex virus types 1 and 2 infections, human immunodeficiency virus, etc.[32] Usually, diseases like the cold, sinusitis, and pharyngitis of the upper respiratory tract and Pneumonia, Tuberculosis, SARS, and Avian influenza of the lower respiratory tract has been reported with the aerosol contamination, (Ragunath 2016)[33] For microbial air contamination, the appropriate CFU counts of 45100-75000CFU/ m2/h in medical wards were given by Fischer et al.[34] However, the results of the current study showed lesser counts than the suggested values, it also revealed that the positive control plates without oregano oil application showed the presence of different forms of bacteria like Gram-positive cocci, namely Staphylococcus, Gram-negative bacilli, namely Klebsiella, and Escherichia species. This finding was in concurrence with the study done by (Rautemaaet al., 2006) in dental aerosol contamination within a distance of 1- 1.5m.[35]

In a study done by (Force et al., 2000)[36] it is being proved that the employment of oregano is one among the foremost natural therapy for the upkeep of human health for an extended period. The volatile property of oregano oil has been used traditionally for respiratory disorders, indigestion, dental decay, autoimmune disease, and tract disorders. Oregano has been used as antifungal, anticoccidial, antispazmolytic, antibacterial (Ertas et al., 2005)[37], antioxidant (Lamaisonetet al., 1991)[38], antiaggregant (Okazakiet al.,,2002)[39] and anti-inflammatory (Kelmet al., 2000)[40] agent. Hence within the present study, the effectiveness of volatile oil oregano (Origanumvulgare) was accustomed to determine the antibacterial activity at varying concentrations. (Yotovaet al., 2015)[41] concluded that the examined solutions of oregano have antibacterial and bactericidal activity towards both Gram-positive bacteria and Gram-negative bacteria in different concentrations on selected pathogens in clinical isolates. almost like this study, in the present study, the essential oil of oregano was inhibitory against all the bacteria in all three concentrations 100%, 50%, and 25% with absolutely nil growth in any of the agar plates. it was also clearly evident that this oil doesn't only inhibit the growth of fallen bacteria but also possesses a 100% bactericidal action towards the fallen samples even with the lowest concentration of just 25%.

The constituents of oregano oil (Oreganum Vulgare) of essential oil fluctuate from 0.5- 2B up to 7C, and its main components are the isomer phenols carvacrol and thymol, additionally as their precursor monoterpenes p-cymene and γ-terpinene at a lower proportion.[42] The antibacterial effects of oregano oil are associated with the presence of phenolic components carvacrol and thymol. In an exceedingly study by Yamazaki et al[43] carvacrol present in oregano oil had the foremost powerful effect against Listeria monocytogenes, followed by thymol, eugenol, cinnamaldehyde, and isoeugenol. Hence within the present study, it was easy to conclude that the strong antibacterial effect of oregano oil even in its minimal concentration was owing to the presence of carvacrol and thymol.

Conclusion

Ultimately our study demonstrates that oregano represents an antibacterial source that can be as effective as modern medicine against pathogenic microorganisms especially the gram-positive and gram-negative bacteria which are usually present in dental aerosols and a secure alternative to be employed in the prevention and treatment of

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aerosol spread infectious diseases. within the future, this oregano compound can be used collectively of the promising antibacterial agents within the sort of liquid in aerosol contamination of the bacteria in the patient and also with the doctor or any others who are subjected to the aerosols.

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