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Article

In vitro Study: Determining the Antibacterial Activity of Different Concentrations of Essential Oil against *Streptococcus sanguinis* Isolated from supragingival Plaque

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Abstract: Several mouth diseases are common health issues, including dental caries and periodontal disease. In the absence of prevention, these conditions result in periodontal pockets. It has been proven that periodontal diseases are associated with supra-gingival plaque bacteria, including *Streptococcus sanguinis*. To avoid antibiotic side effects and bacteria becoming increasingly resistant, an alternative to antibacterial agents must be developed to ensure no side effects or increased resistance. As a consequence, natural herbs and Ayurveda drugs have become increasingly important. This research aims to investigate the antibacterial activity of *Eucalyptus Globules* essential oil against *Streptococcus sanguinis* bacteria. An evaluation of the anti-microbial effects of different concentrations of Eucalyptus Globules oil on Streptococcus sanguinis bacteria was conducted using a well-plate approach was also utilized to compare the effects of Eucalyptus Globules oil on bacterial growth in comparison to 0.2% chlorhexidine mouthwash. It was found that Eucalyptus Globules essential oil was highly antibacterial against Streptococcus sanguinis as the concentration of extract increased, with a highly significant difference ($P \leq 0.01$) between all concentrations and chlorhexidine. Thus, this antibacterial activity of Eucalyptus Globules essential oil against Streptococcus sanguinis suggests that it could be used as a natural antibacterial component in treating oral infections.

Keywords: Essential oil, Eucalyptus Globules, Dental plaque, Antibacterial activity.

Introduction

Humans have relied on natural remedies such as plants in pharmaceuticals for thousands of years to treat and alleviate diseases. The ultimate goal has always been to use natural products for therapeutic purposes that have suitable antibacterial, anti-inflammatory, and antitumor activities while avoiding side effects at the desired dosage. As a result, researchers have recently seen a variety of plant extracts as well as pure natural substances used in various treatment procedures in everyday medicine [1]. The most important among them are E.O.s or essential oils. In traditional medicine, essential oils are a class of volatile phytochemicals derived from plants, which have a wide range of biological effects., including immunomodulatory effects and antibacterial properties. Over 17,500 species of plants of different families of angiosperms, such as Zingiberaceae and Myrtaceae, generate E.O.s, although only a tiny percentage of these are commercially available [2]. E.O. is a complex mixture of terpenoids, terpenes, and phenylpropanoids [3]. "Perioceutics" refers to using

pharmacotherapeutic drugs, such as antibacterial therapy, specifically created to treat periodontal disease [4]. Because of their complex composition of multiple active compounds with many targets, E.O. appears to be a viable alternative to synthesized drugs, particularly in light of pathogenic bacteria's increasing resistance. E.O.s can interact with many target sites due to their large number of ingredients, such as destroying the cytoplasm membrane or inhibiting protein production and efflux pumps, among other things. [5]Additionally, E.O.s may improve microorganism susceptibility to antibiotics widely used in dentistry and stimulate the beneficial host response. These are some benefits of employing whole E.O.s rather than purified components. [6] Eucalyptus globulus is a Myrtaceae tree native to southeastern Australia whose leaves produce E.O.s rich in 1.8 cineole. Their anti-microbial effects have been demonstrated against bacteria such as E. coli and Staphylococcus aureus, as well as antibiotic-resistant microbes such as certain vancomycin-resistant enterococci (VREs) (Mulyaningsih et al., 2010). Essential oils and their anti-microbial properties are gaining popularity, but little is known about their efficacy against oral bacteria, especially Streptococcus Sanguinus. In this light, the current research was carried out to investigate if Eucalyptus Globules essential oil might inhibit bacterial growth as well as determine the minimal inhibitory and minimum bactericidal concentrations against Streptococcus sanguinis, a gram-positive bacteria isolated from supragingival dental plaque. The alternative hypothesis is that Eucalyptus essential oil has an antibacterial effect on dental plaque primary colonizers.

Materials And Methods

An in-vitro microbiological study was undertaken following approval of the protocol at the Department of Periodontology and then by the Research Ethics Committee at the College of Dentistry/University of Baghdad.

Essential oil extract preparation

Eucalyptus Globules essential oil was purchased from Eden Garden. San Clemente.EXP.DATE.2022

Sample collection

This study involves collecting a plaque sample from subjects. A sample was taken from supragingival plaque using a sterilized Gracy curette, a cotton roll, and air spray to isolate the tooth and prevent contamination by saliva. The sample was transferred to 3 ml of Brain Heart Infusion broth (BHIB) and transported to the laboratory [7]. According to the exclusion criteria, the patient should not have taken antibiotics for at least one month before the trial.

Isolation of Streptococcus sanguinis

To optimize sample dispersion, the transported material was thoroughly mixed in a vortex mixer and allowed to vibrate for 10–20 seconds before inserting (5–6) small sterile glass beads (110–150 μm) into a small tube [8]. A few of these inclusions were streaked within duplicate on plates containing mitis salivarius agar medium, a selective medium for isolating streptococci spp. The plate was incubated anaerobically for 48 hours at 37°C, followed by aerobic incubation at 37°C for 24 hours[9]

Confirmation of Streptococcus sanguinus

Streptococcus sanguinis was identified upon microscopic examination (Gram stain), colony morphology (on blood agar and in Mitis Salivaris Agar Medium) and conventional PCR.

In - vitro experiments

An Antibacterial Activity of various Concentrations of Eucalyptus essential oil on Streptococcus sanguinis:

In this study, tests were conducted using the agar well-diffusion method to measure the oil's antibacterial activity. The oil had been produced in four concentrations of dimethyl sulfoxide (DMSO): 25%, 50%, 75%, and 100%. Wells in Mueller-Hinton-Agar (MHA) plates were created and filled with 100 µl of the various concentrations of the essential oil, and other wells were made, one with 0.2% chlorhexidine as a positive control and the other with distal water as a negative control. Then, at 37°C, 24 hours were spent incubating the plates aerobically.[10]

Statistical analysis

The Statistical Package for Social Science (SPSS) software version 25 was used to analyze the data. The inhibitory zones were compared using an analysis of variance (ANOVA) test between different essential oil concentrations and chlorhexidine mouthwash concentrations.

High significant differences (HSD): The Tukey's test was used to determine whether there was a statistically significant difference between the two concentrations of the tested oil.

Results

Antibacterial activity

The ager well-diffusion method can be used as it is a qualitative test suitable to measure the sensitivity of bacteria to various concentrations of essential oil. The inhibition zone diameter expresses the agent diffusion, determining the antibacterial activity power of the tested oil.

Antibacterial activity of various concentrations of Eucalyptus Globules essential oil against Streptococcus sanguinis:

In vitro, the sensitivity of S. sanguinis to different concentrations of Eucalyptus oil, distal water (D.W.), and chlorhexidine mouthwash (0.2%) was tested using the agar well diffusion method. S. sanguinis was sensitive to all oil concentrations. Growth inhibition zones were created when the antibacterial activity of Eucalyptus oil was established against S. sanguinis. (A clear area around the wells without any bacterial growth). Antibacterial activity (diameter of S. sanguinis inhibition zone) increased as Eucalyptus oil concentrations increased. An inhibition zone of greater than 8 mm indicates the sensitivity of the tested bacteria to the Eucalyptus oil. [11] 100% of the oil showed a large inhibition zone. In contrast, D.W. (negative control) showed no inhibition.

Data distribution

In the first instance, the Shapiro-Wilk test was used to see if the data for all concentrations of Eucalyptus oil and CHX were typically distributed (Table 1). The result shows that data in the different concentrations of Eucalyptus oil and CHX are normally distributed because all p values were more significant than the

0.05 level of significance ($p > 0.05$), allowing the use of traditional statistical methods like descriptive statistics expressed by mean and standard deviation, or inferential statistical methods like parametric hypothesis.

A one-way Analysis of Variance (ANOVA) statistical test was used to compare different concentrations of Eucalyptus oil and control agents. Significant high differences ($P \leq 0.01$) were found among different concentrations of oil and control agents, as shown in Table 2 and Figure 1.

Because the differences between different concentrations of Eucalyptus oil and the control agents were highly significant, a statistical comparison between each pair of different concentrations of the oil and the control agents was performed using Tukey's test (HSD), as shown in (Table 3). The results demonstrated that there were highly significant differences ($P \leq 0.01$) between all concentrations of Eucalyptus oil and with CHX, except 25% and CHX; the change was not statistically significant ($P > 0.05$).

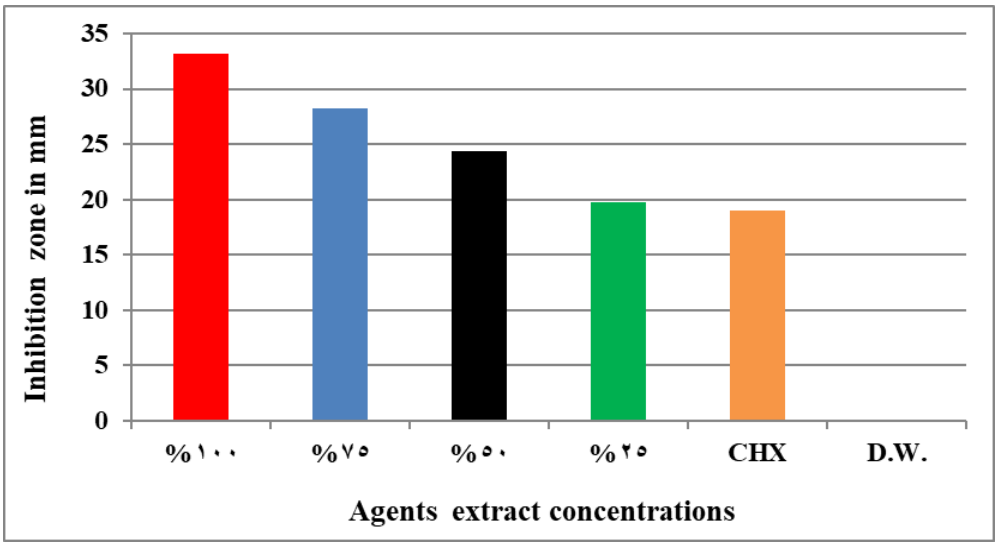

Figure 1. Mean values of *S. sanguinis* inhibition zones of different concentrations of *Eucalyptus* oil, CHX and D.W.

Concentrations %	Shapiro –wilk	D.F.	p-value
100%	0.879	10	0.126
75%	0.972	10	0.911
50%	0.943	10	0.591
25%	0.875	10	0.115
0.2%CHX	0.918	10	0.341

*D.F. = Degree of freedom

Table 1. Shapiro-Wilk test for normality distribution of data of all concentrations.

Con. %	Descriptive statistics					ANOVA		
	N	Mean	S.D.	Mini.	Maxi.	F -test	P -value	Des.
100 %	10	33.2	2.150	31	38	503.556	0.000	H.S.
75%	10	28.2	1.989	25	32			
50%	10	24.4	1.713	22	27			
25%	10	19.8	1.39	18	22			
0.2%CHX	10	19	1.491	17	21			
D.W.	10	0	0	0	0			

Note…. Con : concentration . Des :Description

Table 2. The statistical analysis of *S. sanguinis* inhibition zones by different concentrations of *Eucalyptus* oil, CHX and D.W.

Concentration %		Mean differences	P-value	Description
100%	75%	5	0.000	H.S.
	50%	8.8	0.000	H.S.
	25%	13.4	0.000	H.S.
	CHX	14.2	0.000	H.S.
	D.W.	33.2	0.000	H.S.
75%	50%	3.8	0.000	H.S.
	25%	8.4	0.000	H.S.
	CHX	9.2	0.000	H.S.
	D.W.	28.2	0.000	H.S.
50%	25%	4.6	0.000	H.S.
	CHX	5.4	0.000	H.S.
	D.W.	24.4	0.000	H.S.
25%	CHX	0.8	0.877	N.S.
	D.W.	19.8	0.000	H.S.
CHX	D.W.	19	0.000	H.S.

Table 3. HSD test between each pair of concentrations of *Eucalyptus* oil, CHX and D.W. against *S. sanguinis.*

Discussion

In an oral biofilm, S. Sanguinis is a primary plaque colonialist and is an essential player in dental plaque development. During the initial stage of plaque accumulation, its fimbria and adhesins facilitate its attachment to the acquired pellicle covering the teeth, forming a bacterial layer that can eventually build up

dental plaque. Studies in vitro showed that S. sanguinis can facilitate the adhesion of other periodontal pathogens such as Porphyromonas gingivalis (P. gigivalis) and Fusobacterium nucleatum (F. nucleatum) [12,13]. The field of ethnopharmacology's primary goal is to find and produce effective and accessible agents made from active plant chemicals without side effects. Additionally, antibacterial active substances from the plant extract can be converted into potential anti-microbial agents to inhibit plaque biofilm development [14].

This in vitro experimental study investigated the anti-microbial efficacy of Eucalyptus Globules essential oil against Streptococcus sanguinis. For the treatment of periodontal diseases, anti-microbial therapy such as chlorhexidine, metronidazole, and others is used as a supplement to traditional periodontal therapy scaling and root planning [15,16]. Based on the previous phytochemical analysis findings, Eucalyptus (leaves) contain a vast number of volatile chemicals, particularly terpenes, which concentrate in glands abundantly scattered throughout the leaf parenchyma and bark [17,18]. According to various in vitro studies [19,20], Eucalyptus essential oil has a broad antibacterial spectrum against both gram-positive and gram-negative bacteria. To our knowledge, no reported study evaluates the in vitro effect of Eucalyptus globules essential oil on S. sanguinus. The results of this study, by research [21], in which essential oil of Eucalyptus demonstrated antibacterial activity against gram ve+ bacteria.

Hans et al. studied the anti-microbial efficacy of various essential oils, including Eucalyptus oil, in opposition to oral pathogens and showed that with increased concentration, the inhibitory impact became more significant, with the highest mean inhibition zone. It was 4.5 mm at 100% [22]. Findings from the present study showed that the zone of inhibition of Eucalyptus oil decreased with a decrease in concentration, and the highest zone of inhibition was for S. sanguinus at 100%. These findings are consistent with the results of a previous work on concentration.

The study used chlorhexidine 0.2% mouthwash as a positive control, which has been extensively used in dentistry for its bacteriostatic properties. In the current study, Eucalyptus oil produced an inhibition zone superior to chlorhexidine 0.2% mouthwash, similar to a previous study that determined the antibacterial activity of Eucalyptus and other oils compared to chlorhexidine mouthwash. [23]

A study by Bachheti demonstrated that Eucalyptus oil had marked antibacterial activity against gram-positive bacteria, Staphylococcus strains with inhibition zone (28 mm)) than gram-negative bacteria, Klebsiella pneumoniae and Pseudomonas aeruginosa with inhibition zone (23 mm) [24]. The differences in the findings showed that gram-positive organisms were more susceptible to the action of essential oils extracted from plants than gram-negative- ones. This is because gram ve+ bacteria do not have an outer lipid membrane in their cell wall, which allows entry of hydrophobic extracts that might have an antibacterial action more quickly. Therefore, the hydrophobic components found in the leaves, such as eucalyptol, which is the most abundant ingredient in Eucalyptus globules leaves, could explain the varying inhibitory activities observed, as they can injure the gram-positive cell membrane, causing leakage of constituents and eventually cell death [25,26]

Conclusion

Eucalyptus Globules essential oil exhibited promising antibacterial activity against plaque-forming bacteria, suggesting its potential therapeutic application for preventing and treating periodontal diseases.

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Self-funds it.

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Conflicts of Interest

None.

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