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Original Article

Evaluation of Antibacterial Activity of Lemongrass Oil Against Oral Clinical Isolates – An *In vitro* Study

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ABSTRACT

Background: There are 1,200 oral species out of which 400 are potentially important and 20 organisms are periopathic. Periodontal flora plays the important role in initiation and progression of periodontal diseases. There are various conservative and surgical procedures to treat and prevent periodontal diseases. Local drug delivery agents reduce microbial burden, blocks collagenase activity and inhibits bone loss; out of which tetracycline is common in use. Unwanted side effects and resistance of microorganisms towards antibiotics due to their widespread use have modified the general prescription about their efficacy. Various researches elucidate that herbal extracts exhibit wide range of antibacterial activity. In recent years, Lemongrass essential oil gains scientific interest as it targets even the periodontal pathogens. The purpose of this study is to comparatively evaluate the antibacterial activity of lemongrass essential oil with that of tetracycline. **Aim and objective:** To comparatively evaluate the antibacterial activity of lemongrass essential oil with tetracycline against Streptococcus mutans, Staphylococcus epidermidis and Lactobacillus and to determine the minimal inhibitory concentration of lemongrass essential oil. **Study design:** *In vitro.* **Materials and methods:** It is an *in vitro* study done to demonstrate the antimicrobial activity of lemongrass against the oral microbes. Based on their involvement in various clinical conditions Streptococcus mutans, Staphylococcus epidermidis and Lactobacillus were selected for the study. These organisms were inoculated to a solid media and incubated overnight aerobically at 37°C to obtain a pure culture. The culture was made as a suspension in sterile saline with the turbidity matching 0.5 Macfarland standard. This is used to make a lawn culture on the Mueller Hinton Agar. Antimicrobial effect of tetracycline was tested using standard disc of doxycycline 30 mcg (Himedia, SD012) and sterile disc was used to prepare lemongrass essential oil which contained 10 µl, 15 µl and 20µl. In each category 5 discs were tested to get a mean zone of inhibition. After 24 hours of incubation the zone of inhibition was measure in mm using a scale. The measured zone size was tabulated and compared among the groups. **Results:** The minimal inhibitory concentration of lemon grass essential oil was estimated to be 10µl. Statistically significant zone of inhibition and antibacterial zone was greater in lemongrass essential oil than tetracycline for Streptococcus mutans and Staphylococcus epidermis. **Conclusion:** Lemongrass essential oil showed higher antibacterial activity than tetracycline. Hence, it can be used as a good alternative to tetracycline or adjunctive in the treatment of periodontitis.

Key words: Antibacterial, Tetracycline, Lemongrass essential oil, Minimal Inhibitory concentration.

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INTRODUCTION

Periodontal diseases i.e the infections of the periodontium compromise the bacterial etiology, an immune response and tissue destruction.[1] The goals of periodontal treatment is primarily focused on eliminating the microorganisms and their by products by means of mechanical instrumentation like scaling and root planning.[2] Mechanical instrumentation fails to eliminate the penetrating bacteria completely from the sulcus and the surrounding tissue. The role of certain specific bacteria in the etiology of progression of periodontal disease has directed the use of antimicrobial agents in periodontal therapy.[3-5]

Local delivery of antibacterial agents into periodontal pockets has been extensively studied since 1979.[6] This mode of drug delivery avoids most of the problems associated with systemic therapy limiting the drug to its target site and hence achieving a much higher concentration. Local drug delivery has gained acceptance and popularity due to decreased risk in development of resistant flora, opportunist infection and side effects.[7]

Various antimicrobial drugs (eg; tetracycline, minocycline, clindamycin) have been used as adjuncts to mechanical treatment.[8] The proven efficacy of tetracycline group of drugs in the management of periodontal diseases may have a number of additional properties which include collagenase inhibition, anti inflammatory action, inhibition of bone resorption and their ability to promote attachment of fibroblasts to root surfaces.[9] The side effects and resistance of microorganisms

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to antibiotics have altered the general perception of the capabilities of these antimicrobial agents.[10]

Research in phytoscience, an emerging multidisciplinary science, has revealed various medicinal plants possessing antimicrobial activity with fewer side effects and reduced toxicity. Extracts of herbal plants offer a new choice for optimal antimicrobial therapy against various oral microorganisms.[11]

Cymbogoncitratus (lemongrass) is a medicinal plant used for treating different diseases. The plant is commonly used for its antiseptic, antiemetic, anti rheumatic, analgesic, antispasmodic and antipyretic properties.[12] Its chemical components like phenol and flavanoid were reported to show many anti- oxidant and anti- inflammatory activities.[13] In the present study, an attempt was made to evaluate the antibacterial activity of lemongrass essential oil compared to tetracycline against Streptococcus mutans, Staphylococcus epidermidis & Lactobacillus and to measure the minimal inhibitory concentration of lemongrass essential oil.

MATERIALS AND METHODS

This *in vitro* study was done to evaluate the antimicrobial activity of lemongrass against the oral microbes. The test organisms were isolated from the salivary samples of clinical isolates. The organisms were selected based on their pathogenic role in different oral complications. The isolated organisms were identified and confirmed by standard microbiological procedure. Based on their involvement in various clinical conditions Streptococcus mutans, Staphylococcus epidermidis and Lactobacillus were selected for the study.

The isolated organisms were inoculated to a solid media and incubated overnight aerobically at 37°C to obtain a pure culture. The pure culture were isolated and made as a suspension in sterile saline with the turbidity matching 0.5 Macfarland standard. This pure culture is used to make a lawn culture on the Mueller Hinton Agar. Antimicrobial effect of lemongrass essential oil was compared against tetracycline (doxycycline 30 mcg - Himedia, SD012) as it is considered as standard drug of choice. Stock solutions of lemongrass essential oil were prepared and serial dilutions were done to obtain the concentrations of 10 µl, 15 µl and 20 µl respectively. In each category 5 discs were tested to get a mean zone of inhibition. After 24 hours of incubation the zone of inhibition was measure in mm using a scale. The measured zone size was tabulated and compared among the groups.

Statistical analysis

Statistical analysis of the data was performed by using Statistical Package. ANOVA analysis and Post hoc tests were used to test the mean change in scores within each group.

RESULTS

Table 1.

In Table 2 the mean score of lemongrass essential oil 10ul against Streptococcus mutans (46.20) and Staphylococcus epidermis (30.00) is greater than the other groups indicating lemongrass essential oil showed greater antibacterial zone than doxycycline 30 mcg (Himedia, SD012). It also shows that the doxycycline 30 mcg (High media, SD012) has a greater mean score (18.20) than lemongrass essential oil against Lactobacillus.

Table 3 shows that significance is at 0.000 level P<0.001 hence proving that there is 99.9% significant difference between 2 groups [doxycycline30 mcg (Himedia, SD012) and lemongrass essential oil] for all three organisms.

Post hoc tests (Table 4) shows multiple comparisons between each group, where lemongrass essential oil 10ul shows significant difference with lemongrass essential oil 20ul and standard disc of doxycycline (P<0.001) against Streptococcus mutans. It also indicates that the lemongrass essential oil -10 ul showed significant difference with lemongrass essential oil-20 ul (P value-0.000) against Staphylococcus epidermidis. It also suggests that Lactobacillus alone exhibited significant difference with doxycycline disc30 mcg (Himedia, SD012) when compared to other groups as the p value is 0.000.

DISCUSSION

Various studies on the microbial etiology of periodontitis are available and it is commonly accepted that, the consortium of bacteria, not a single microorganism is involved in the disease pathogenesis.[14] This profound finding opens up a wide array of treatments and prevention for periodontal disease. Based on Koch's postulates several

Table 1: Mean zone of inhibition of the 3 organisms tested against lemongrass and tetracycline.

Sl. No	Tetracycline	Lemongrass essentialoil (10 µl)	Lemongrass essential oil (15 µl)	Lemongrass essential oil (20 µl)
Streptococcus mutans	27.4 mm	46.2 mm	41.2 mm	30.4 mm
Staphylococcus epidermidis	26.6 mm	30 mm	26.6 mm	22.8 mm
Lactobacillus	18.2 mm	13.8 mm	14.8 mm	15 mm

Table 2: Mean and standard deviation scores amongst lemongrass essential oil and tetracycline against Streptococcus mutans, Staphylococcus epidermis and Lactobacillus.

	N	Streptococcus Mutans		Staphylococcus epidermidis		Lactobacillus	
		Mean	Std. Dev	Mean	Std. dev	Mean	Std. Dev
Lemongrass essential oil - 10 ul	5	46.20	8.438	30.00	.000	13.80	.837
Lemongrass essential oil - 15 ul	5	41.20	3.834	26.60	1.949	14.80	.837
Lemongrass essential oil - 20 ul	5	30.40	.894	22.80	2.049	15.00	1.000
Doxycycline - Standard disc	5	27.40	1.342	26.60	.548	18.20	.447
Total	20	36.30	8.986	26.50	2.929	15.45	1.849

Table 3: ANOVA analysis between groups against Streptococcus mutans, Staphylococcus epidermidis, Lactobacillus.

	Streptococcus mutans	Staphylococcus epidermidis	Lactobacillus
	Sig.	Sig.	Sig.
Between Groups (Lemongrass and tetracycline)	.000	.000	.000

Table 4: Multiple comparisons between concentrations of lemon grass essential oil and standard disc of doxycycline against Streptococcus mutans, Staphylococcus epidermidis and Lactobacillus (POST HOC TESTS – (Tukey HSD)).

Concentration	Concentration	Streptococcus mutans	Staphylococcus epidermidis	Lactobacillus
		Sig.	Sig.	Sig.
Lemongrass essential oil - 10 ul	Lemongrass essential oil - 15 ul	.365	.009	.243
	Lemongrass essential oil - 20 ul	.000	.000	.127
	Doxycycline – Standard disc	.000	.009	.000
Lemongrass essential oil - 15 ul	Lemongrass essential oil - 10 ul	.365	.009	.243
	Lemongrass essential oil - 20 ul	.011	.004	.979
	Doxycycline – Standard disc	.001	1.000	.000
Lemongrass essential oil - 20 ul	Lemongrass essential oil - 10 ul	.000	.000	.127
	Lemongrass essential oil - 15 ul	.011	.004	.979
	Doxycycline – Standard disc	.747	.004	.000
Doxycycline – Standard disc	Lemongrass essential oil - 10 ul	.000	.009	.000
	Lemongrass essential oil - 15 ul	.001	1.000	.000
	Lemongrass essential oil - 20 ul	.747	.004	.000

periodontopathic bacteria have been nominated and examined in detail all over the world. The specific bacterial characteristics and features help us to formulate effective targeted treatment against periodontitis. Though Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are considered to be the key periodontal pathogens, multiple species co-aggregate to colonize the tooth surface or provide nutrients to other bacteria by metabolization of substrates.[15]

The present study evaluates and compares the antimicrobial effect of lemongrass essential oil and tetracycline against Streptococcus mutans, Staphylococcus epidermidis and Lactobacillus. Periodontitis associated microorganisms can coexist with S. mutans as quorum sensing may enable them to live with key periodontal obligatory anaerobes.[15] Studies suggest that Staphylococcus epidermis belong to the transitory microbiota and found to be the most prevalent one in periodontal pocket and oral cavity.[16] Lactobacilli are known to play an important role in the maintenance of health by stimulating natural immunity and contributing to the balance of microflora. Studies proposed that oral Lactobacilli also suppress the growth of periodontal pathogens.[17] Thus, this study assess the effect of lemongrass essential oil against two non-resident opportunistic microorganisms which represent significant problem in causing and maintaining periodontal infection and one beneficial organism that supplement the host immune system against invading periodontal pathogens.

Various studies have been done which reveals lemongrass essential oil's strong anti oxidant property, but very few studies supporting their antibacterial property were present. Shivaraj et al evaluated the efficacy of 2% lemon grass essential oil in a gel form as an adjunct to scaling and root planning in chronic periodontitis patients. The results indicated an improved clinical resolution and destruction which suggested their anti inflammatory and anti microbial property. Increased healing response was also seen which could be due to their anti oxidant property.[18] Another study by Mohd Irfan Naik et al suggested that it is effective against drug resistant organisms (S. Aureus, B. Cereus, B. Subtilis, E. Coli, K. Pneumoniae) and it would be helpful to treat multidrug resistant organism infections.[19] The essential oil of lemongrass at 1000ppm concentration was also found to inhibit fungal growth completely.[20]

It was also proposed that 2% lemon grass essential oil solution can accelerate the gingivitis healing process better, than when used at other concentrations.[21] Lemongrass essential oil mouthwash when used along with nonsurgical treatment in different concentrations (0.1%, 0.25% and 0.5%) implied an additive effect on the prognosis.[22]

Previous studies illustrated that 2% concentration of lemongrass oil was found to be effective in the treatment of periodontitis. But their minimal inhibitory concentrations against those micro organisms were not examined, as most of the studies evaluated its efficacy against periodontal pathogenic bacteria based on the clinical parameters assessment. Literature reveals limited number of studies evaluated the antibacterial effect (by microbiological assessment) of lemongrass essential oil against periodontopathic as well as periodontally beneficial bacteria. Thus, the results of the present study cannot be directly correlated with other studies.

The limitations of this study include *in vitro* assessment of lemongrass antibacterial in clinical isolates, which may differ *in vivo* due to oral microbial ecosystem and interactions. In addition further evaluation on key periodontal pathogens like gram negative anerobes would more precisely define its role in treatment of periodontitis.

Within the limitations of the study, lemongrass essential oil -10µl was found to be the minimal inhibitory concentration against these oral microorganisms. The overall result of the present study interprets that for Streptococcus mutans and Staphylococcus epidermidis, lemongrass essential oil (10µl) showed greater antibacterial zone than Doxycycline 30 mcg (Himedia, SD012) while for Lactobacillus, doxycycline 30 mcg (Himedia, SD012) showed greater antibacterial zone than lemongrass essential oil (Figures 1-3). This clearly suggests that lemongrass

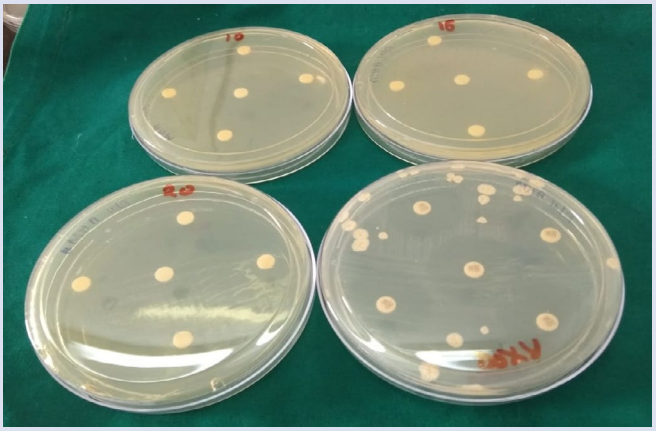

Figure 1: Antibacterial zone of lemongrass essential oil (10 µl, 15 µl, 20 µl) and Doxycycline 30 mcg (Himedia, SD012) against Streptococcus mutans.

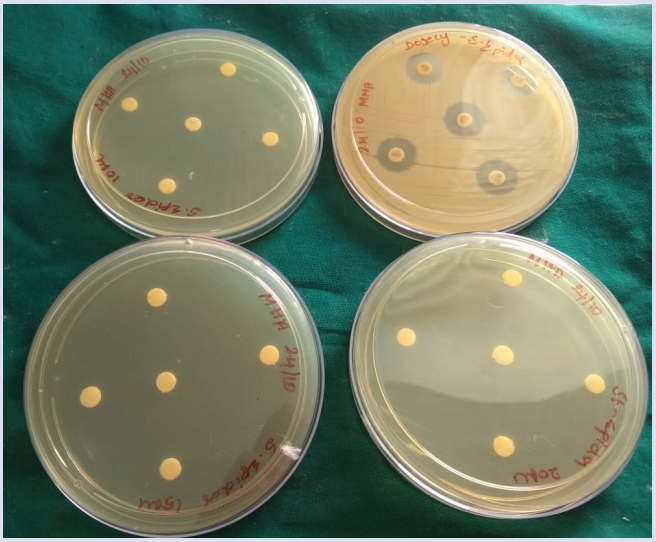

Figure 2: Antibacterial zone of lemongrass essential oil (10 µl, 15 µl, 20 µl) and Doxycycline 30 mcg (Himedia, SD012) against Staphylococcus epidermidis.

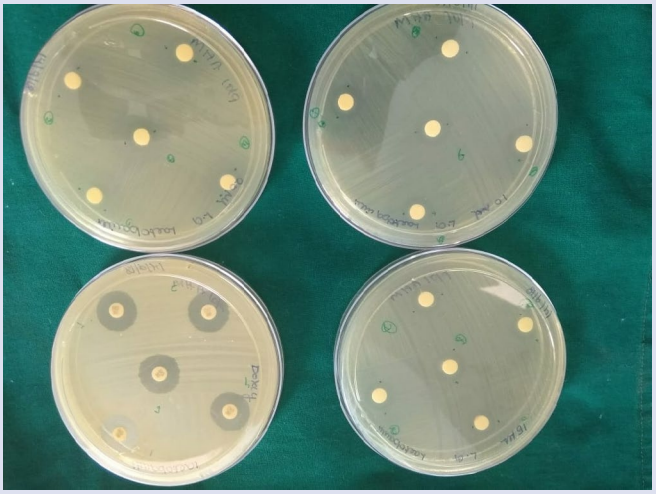

Figure 3: Antibacterial zone of lemongrass essential oil (10 µl, 15 µl, 20 µl) and Doxycycline 30 mcg (Himedia, SD012) against Lactobacillus.

essential oil may be highly effective against Streptococcus mutans and Staphylococcus epidermidis and least effective against Lactobacilli which are a well known probiotic (beneficial species).

On the other hand, doxycycline, commonly used antibiotic apart from lesser efficacy with the periodontopathic bacteria (Streptococcus mutans and Staphylococcus epidermidis), eliminates Lactobacilli from the oral biofilm which creates an unhealthy ecological shift in the oral cavity. Thus, despite of arising problems of antibiotic resistance and the various side effects, this study proved an interesting fact of causing superinfection due to its increased efficacy on beneficial organisms, which usually occurs after a course of antibiotics.

Thus, this study clearly stated that the phytochemical extract of *C. Citratus* (lemongrass) offers new choice of therapy as an adjunct to mechanical instrumentation in the treatment of chronic periodontitis with moderate to deep periodontal pockets.

CONCLUSION

Thus, lemongrass essential oil appears to be an attractive alternative agent that can be used effective in terms of antibacterial property and safe adjunct to mechanical non surgical periodontal therapy than standard concentration of doxycycline 30 mcg. The results of this current study may pave a pathway for the integration of herbal medicine into the modern medical era.

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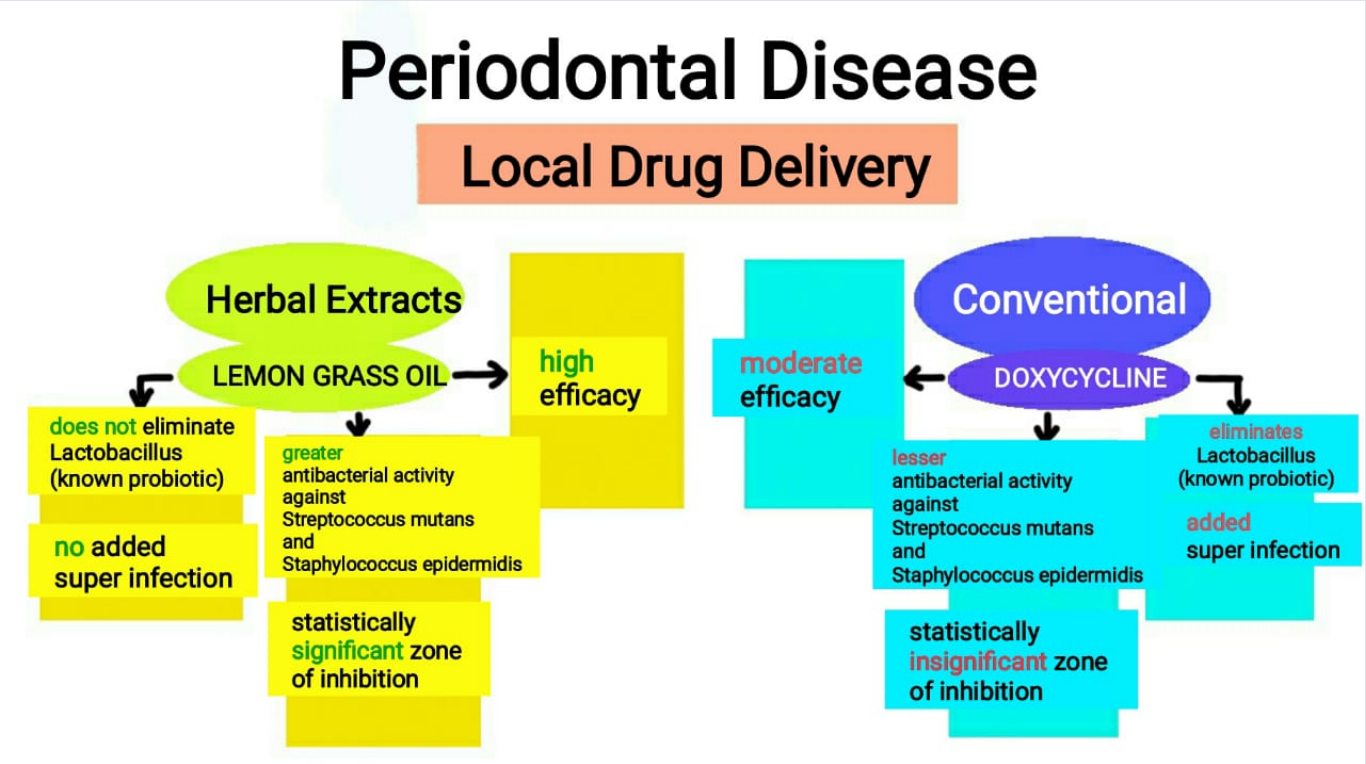
GRAPHICAL ABSTRACT



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Dr. Vanessa Ravel -I have completed my B.D.S at Priyadharshini Dental College at Thiruvallur in the year 2018.I was the topper of the batch and had received the medal for securing the first rank in my college in the final year from Indian Dental Association and also received the best outgoing student award from the college. I have received awards for both best paper and posters. I have won a number of quiz competitions conducted both at college and inter college level. My passion is teaching and research and interests includes reading, exploring and touring.

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