Research Article

Antibacterial Efficacy of Lemongrass (*Cymbopogon Citratus*) Extract against *S. mutans*

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Abstract

Introduction: Lemongrass (*Cymbopogon citratus* DC. stapf) has beenploughed since numerous years for therapeutical motives. The antibacterial activity of lemongrass oil against a diverse range of organisms was being reported by numerous studies and hence the study was conducted to examine the efficacy of lemongrass extract against as an adjunct in mouthwashes against the most important oral pathogen *S. mutans* in order to prevent plaque development.

To evaluate and compare the zone of inhibition produced by aqueous and alcoholic extract of lemongrass, chlorhexidine and saline using agar well diffusion method. An *in-vitro* study was conducted in the institutional Department of Microbiology to compare the anti-microbial efficacy of aqueous and alcoholic extract of lemongrass, saline and 0.2% Chlorhexidine against *S. mutans*.

Methods and material: Aqueous and alcoholic extracts of lemongrass were prepared and the antimicrobial activity of lemongrass extracts, chlorhexidine and saline were determined by culturing *S. mutans* on Agar well diffusion method and zone of inhibition were measured for analysing inhibitory efficacy of lemongrass against *S. mutans*. Data was analysed.

Statistical package of social sciences version 16. Intergroup comparison done using One-way Analysis of Variance (ANOVA) following Post-hoc pairwise comparison using Tukey's test. Level of significance was set at $p<0.05$.

Results: The intergroup comparison of mean actual Zone of Inhibition (ZOI) against *S. mutans* among 4 study groups was found to be statistically significant ($p<0.05$). Simultaneously, the pairwise comparison among all the pairs shows statistically significant difference in mean zone of inhibition ($p<0.05$).

Conclusion: The alcoholic lemongrass extract showed maximum antimicrobial activity against *S. mutans* and hence could be considered as an effective herbal therapy adjunct to use in mouthrinse, toothpaste or medicam for treating various dental tissues.

Keywords: Antimicrobial efficacy; *Cymbopogon*; Lemongrass; *Streptococcus mutans*

Introduction

Chlorhexidine is the gold standard among chemical plaque control methods. It has antiplaque, anticarious and antibacterial properties. [1] But despite of all these beneficial properties, mouthwash do cause some harmful effects such as taste alterations, mucosal irritation. From last decades, there has been an increasing demand for herbal medicines because of increased concern of consumers over the side-effects of synthetic material. *Cymbopogon citratus* DC. stapf, commonly known as lemongrass is known to have diverse range of antimicrobial activity against multiple microorganisms. [2] Still the literature lacks the evidence of antimicrobial properties of lemongrass hence the study was

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conducted to assess the efficacy of 0.25% lemongrass extract with that of 0.2% chlorhexidine against *S. mutans*.

Methods

The present *in vitro* study was conducted at the department of microbiology of the institution, to compare the anti-microbial efficacy of aqueous and alcoholic extracts of lemongrass, saline and 0.2% chlorhexidine against *S. mutans*. Ethical clearance was sought from the institutional ethical committee. [3] The aqueous extract of lemongrass was prepared by adding ten grams of lemongrass in 100 ml of boiling distilled water and further boiled till the extract obtained was reduced to 10 ml. For the preparation of alcoholic extract of lemongrass ethanol was used. 50 ml of solvent was added to 5 g of the lemongrass. [4] A mortar and pestle was used to grind the lemongrass leaves with the solvent, and this mixture was filtered using Whatsman filter paper to obtain an extract. The extract obtained was reduced to approximately 5 ml over a water bath to obtain a 100% (w/v) concentrated organic extract of lemongrass. [5,6] The culture media was prepared as per the manufacturer's (HIMEDIA) specifications. Sterilization was done by autoclaving at 15 lbs psi (per square inch) for 15 minutes. These media were poured in disposable sterilized plastic petri plates to obtain matrix thickness of 4 mm. [7] Freeze dried cultures of *S. mutans* kept in the refrigerator at 4°C were reconstituted by breaking the glass ampule and adding 500 µl of BHI broth. Later the tube was incubated at 37ºC for 24 hours. The culture *via Streptococcus mutans* was plated on MHA incubated at 37ºC for 24 hours. [8] The purity of culture was confirmed by Gram staining before use.

The antimicrobial activities of lemongrass extracts and chlorhexidine mouthrinse were determined by culturing the test organisms on Agar well diffusion method. The colony growth from respective cultures was passed in 4 ml of sterile normal saline in a tube. [9,10] The tubes were shaken to have a visible turbidity. Sample size estimation was done by using GPower software (version 3.0). Sample size was estimated for F test and ANOVA: Fixed effects, Omnibus one way for four intervention groups was chosen. A minimum total sample size of 40 plates (10 per group) was found to be sufficient for an effect size of alpha of 0.05 and power of 80%. [11]

The surface of culture media plates was dried by keeping the plates open in incubator for 4 hrs. Normal saline bacterial suspension was flooded on to the surface of dried plates. Extra culture inoculum was decanted off from the surface of the medium back into the culture tubes. The plates were incubated at 37°C for about 45 minutes for the liquid culture to absorb in the matrix. And using the borer which had been flamed red hot and subsequently cooled, wells having a diameter of 6 mm and depth of 4 mm were made in the plates. Four wells were punched in each petri plate. Each well per plate was filled up to the brim, with full strength respective extracts and with marking at the back of the plate. A set of 10 plates was put up for test organism. [12] The plates were kept for 1 hour at room temperature for diffusion of agent through the medium. Afterwards, the plates were incubated at 37°C for 24 hours with lid upwards. This work was carried throughout in laminar air flow station.

Following the incubation, inhibition zone diameters (in mm) were read and measured along the most uniform diameter with the help of scale. The zone of inhibition of growth of test strain was defined by the area where visible growth had been inhibited. All the measurements of zone of inhibition were carried out by a single examiner. [13]

Statistical analysis

The actual diameter of zone of inhibition was calculated by deducting the diameter of wells i.e. 6 mm from the diameter of the zone of inhibition. The value of actual zone diameter according to study groups were tabulated and entered into Microsoft excel. The SPSS software version 16.0 was used for statistical analysis. The normality of the data was checked using the Kolmogorov-Smirnov test and the data was found to be normally distributed. [14] For each test group, the mean and standard deviation of the actual diameters of zone of inhibition were calculated. One-way Analysis of Variance (ANOVA) test was used for comparison of mean of actual zone of inhibition among different groups. Post-hoc pairwise comparison was made using Tukey's test. The level of significance was set at 0.05. Graphs were prepared on Microsoft excel.

Results

The present *in vitro* study was conducted to compare the anti-microbial efficacy of aqueous and alcoholic extracts of lemongrass, saline and commercially available formulation of 0.2% chlorhexidine against *S. mutans* using agar well diffusion method. [15] The extracts used in the study were aqueous and alcoholic extracts of lemongrass, saline and one positive control group i.e. 0.2% chlorhexidine (CHX). The descriptives of actual zone of inhibition (in mm) produced by two (2) test extracts, saline and one control agent against *Streptococcus mutans* are depicted in Table 1. [16] When mean actual ZOI was compared among four study groups using one-way ANOVA test, the overall difference was found to be statistically significant ($P<0.05$). The pairwise comparison of the microbial zone of inhibition of 4 study groups is shown in Table 2. When pairwise comparison was done using post-hoc tukey's test; all the pairs shows statistically significant difference in mean zone of inhibition i.e. maximum ZOI was seen in CHX followed by alcoholic extract of lemongrass followed by aqueous extract and least zone of inhibition was seen in group III.

	N	Mean	Std. Deviation	P value
Group I: Aqueous extract	10	11.08	0.55	<0.0001
Group II: Alcoholic extract	10	14.63	1.08	
Group III: Saline	0	0	0	
Group IV: Chlorhexidine	10	22.73	0.95	

Table 1: Descriptives of actual zone of inhibition (in mm) produced by two test extracts, saline and one control agent against *Streptococcus mutans*.

Group 1	Group 2	Mean difference	Std. error	P value	95% Confidence interval	
					Lower bound	Upper bound
Aqueous extract	Alcoholic extract	-3.55	0.34	<0.0001	-4.48	-2.61
	Saline	11.08	0.34	<0.0001	10.14	12.01
	Chlorhexidine	-11.65	0.34	<0.0001	-12.58	-10.71
Alcoholic extract	Aqueous extract	3.55	0.34	<0.0001	2.61	4.48
	Saline	14.63	0.34	<0.0001	13.69	15.56
	Chlorhexidine	-8.1	0.34	<0.0001	-9.03	-7.16
Saline	Aqueous extract	-11.08	0.34	<0.0001	-12.01	-10.14
	Alcoholic extract	-14.63	0.34	<0.0001	-15.56	-13.69
	Chlorhexidine	-22.73	0.34	<0.0001	-23.66	-21.79
Chlorhexidine	Aqueous extract	11.65	0.34	<0.0001	10.71	12.58
	Alcoholic extract	8.1	0.34	<0.0001	7.16	9.03
	Saline	22.73	0.34	<0.0001	21.79	23.66

Table 2: Pairwise comparison of the microbial zone of inhibition of study groups.

Discussion

Microbial biofilms otherwise called as dental plaque are complex communities of bacteria present in the environment and human body. [17] Dental plaque if not removed regularly and adequately, undergoes maturation resulting in pathogenic bacterial complex which can lead to dental caries, gingivitis, periodontitis and peri-implantitis ultimately leading to impaired oral functioning. Various recent studies have shown a high burden of dental caries globally. [18-20] It is a consequence of interaction among host, micro flora and diet and results in demineralization of tooth surface. The acidogenicity and aciduricity of *Streptococcus mutans* present in dental plaque, to produce acid makes it the main culprit in initiation of dental caries. [21] So, to prevent this disease, plaque control is the main key. Although mechanical plaque control methods are considered as promising one worldwide, but various chemotherapeutic agents have always been used as adjuvants.

Mouthrinses are frequently recommended for chemical control of plaque especially in areas which are inaccessible to toothbrush. Chlorhexidine is the leading antiplaque agent till date, because of its many ideal properties, and its efficacy. [22] It acts by damaging the cell membrane of prokaryotes and by disrupting the cytoplasmatic constituents. A review concluded that due to the current lack of long term clinical evidence for caries prevention and reported side effects, chlorhexidine rinses should not be recommended for caries prevention as some of cariogenic microorganisms are naturally resistant to chlorhexidine. [23,24] Apart from this, chlorhexidine have some inherent side-effects like taste alteration, extrinsic staining, increases the formation of supragingival calculus and mucosal desquamation. So there was a need to explore various natural products capable of controlling dental caries.

Medicinal plants have been used throughout the world in popular medicine for medical purposes for thousands of years. [25] Recently, they are receiving considerable attention due to their pharmacological effects such as antimicrobial, anticarcinogenic, and antioxidant properties. *Cymbopogon citratus* is a medicinal plant with antimicrobial properties. For the extraction of the active ingredients of this plant there are different methods of preparation of the leaves of *C. citratus*.

The present *in-vitro* study was carried out to assess the effectiveness of lemongrass extract against *S. mutans*. Lemon

grass oil have anti-oxidant properties, which helps in prevention and treatment of periodontitis by increasing the level of thiol anti-oxidants and can be used as an adjunct in mouth washes in order to prevent plaque formation. [26] So far various studies have also been conducted to assess the antimicrobial effect of lemongrass but they have used essential oil form. Thus present *in-vitro* study was initiated where the antimicrobial effect of lemongrass extract was tested against *S. mutans* mainly using lemongrass extract.

In this study, aqueous and alcoholic extracts were prepared from leaves of *Cymbopogon citratus* DC. Stapf and their efficacy against *S. mutans* was analysed using Agar well diffusion method. According to the usefulness of this method is limited to the generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium. In the present study, 2 different forms of extracts of lemongrass (alcoholic and aqueous) were tested against *S. mutans*. Chlorhexidine was taken as positive control and saline as negative. [27] Results of the present study demonstrated that chlorhexidine being the positive control showed the maximum zone of inhibition (22.73 ± 0.95), followed by aqueous lemongrass extract (14.63 ± 1.08). Alcoholic extract of lemongrass comparatively showed lesser zone of inhibition.

One of the non-enzymatic antioxidants found in every cell of the body is Glutathione, also known as sulfhydryl Glutathione (GSH) plays an important role in protection against oxidative stress. According to gargling with 2% and 4% concentrations of lemongrass essential oil increased the salivary GSH levels in moderate gingivitis patients, with the same potency as hexitidine 0.1%, So it can speed-up gingivitis healing process [14]. Antioxidants like that of lemongrass essential oil overcome the ill-effects caused due to Reactive Oxygen Species (ROS) activity. [28] In inflammatory process like gingivitis GSH not only acts as an anti-oxidant but also an immune function modulator. It directly acts as free radical scavenger in detoxification of reactive oxygen and nitrogen species and also avoids the production of pro-inflammatory cytokines. The comparative effects of lemongrass oil and lemongrass extracts of lemongrass probably vary due to difference in concentrations, method of study i.e., zone of inhibition, MIC, MBC, etc,. Lemongrass essential oil could inhibit the growth of several kinds of microrganisms at a concentration lesser than or equal to 2%. The antioxidant activity of lemongrass oil is because of its contents such as citral (neral and geranial) and citronellal. [29]

According to the essential oil of lemongrass has significant antimicrobial potential against oral microorganism S. *mutans, P. intermedia* and *P. gingivalis*. Onawunmi analysed the action of 0.05% to *S. aureus, Bacillus subtilis* and *E. coli* by agar diffusion method and concluded that essential oil of *C. citratus* showed good antimicrobial activity even at low concentrations. [25] The results in the present study were in accordance with the results reported by various authors stating that alcoholic extracts showed better antimicrobial properties than aqueous extracts. Comparison of the data obtained in this study with previously published results could not be done due to the varying composition of plant oils and extracts according to local climatic and environmental conditions. Secondly according to some oils with the same common name may be derived from different plant species reflecting different efficacy against microorganisms.

Conclusion

Aqueous extract of lemongrass found to be the most efficacious when compared to alcoholic extract of lemongrass and saline. It can serve as a longterm herbal antiplaque agent in maintenance phase or as an alternative for the patients looking for natural product mouthrinse, or for the patients who experience side effects due to Chlorhexidine use. Still more evidence is required to approve the significance of aqueous extract of lemongrass on oral health. Thus, further *in vivo* studies would be beneficial to evaluate potential uses of aqueous extracts of lemongrass.

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