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Comparative Evaluation of Antimicrobial activity of Tulsi extract [Ocimum Sanctum (Linn.)] and Curcumin extract [Curcuma Longa] with 0.2% Chlorhexidine on Periodontal Pathogens: An in-vitro study

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Abstract---BACKGROUND: Periodontal disease is associated with colonization of specific bacteria in the subgingival area. Widespread use of antimicrobials has resulted in emergence of various side effects and drug resistance. Phytomedicine has been used in many clinical conditions and it appears to be a suitable alternative to manage conditions affecting the oral cavity. AIM: Present study aims to assess the in-vitro antimicrobial efficacy of Tulsi extract and Curcumin extract on Periodontal pathogens. MATERIALS & METHODS: Ethanolic extract of 100% w/v of Tulsi and Curcumin was prepared by cold extraction technique. Prepared extracts were further diluted to obtain solutions of 5 different concentrations to be tested (0.5%, 1%, 2% and 5%). Obtained solutions and controls (0.2% Chlorhexidine

and Distilled water) were subjected to the microbiological investigation against Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. Agar well diffusion method was employed to determine the concentration at which Tulsi and Curcumin gave an inhibition zone, similar to Chlorhexidine. Data was analysed using one-way analysis of variance and post-hoc test for inter-group and intra-group comparisons. RESULTS: At 10% concentration both Tulsi and Curcumin extract have demonstrated comparable antimicrobial activity against Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. However, the difference in zone of inhibition demonstrated in comparison with 0.2% Chlorhexidine were statistically highly significant. CONCLUSION: The antimicrobial efficacy demonstrated by both extracts suggest it's possible use as an effective and affordable "adjunct" along with standard care in management of Periodontal conditions.

Keywords---Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Periodontitis, Tulsi extract, Curcumin extract, Ocimum Sanctum, Curcuma Longa, Periodontal disease, Herbal extracts, Zone of inhibition.

Introduction

Periodontal disease is a chronic infectious disease of the oral cavity that is resultant of various polymicrobial interactions. The noxious by-products released by these bacteria are responsible for triggering an inflammatory process in the periodontal tissues, which ultimately leads to destruction of the periodontal structures and tooth loss.[1] Among the 700 identified bacterial species in the oral cavity, only a small number of species are closely related to the initial incidence and continued persistence of periodontitis.[2] Levels of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and other anaerobic bacteria have been commonly seen to increase in aggressive as well as chronic periodontitis.[3] The incidence and prevalence of chronic periodontitis increases with age, but early onset forms of periodontitis are typically aggressive and rapidly progressive causing periodontal destruction and rapid bone loss. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythia are strongly associated with unsuccessful periodontal therapy and progression of aggressive periodontitis.

Chlorhexidine has been used as a gold standard antiplaque agent ever since it was first investigated in 1969 by Schroeder. Its efficiency is resultant of excellent substantivity and di-cationic activity at pH levels above 3.5 which prevents plaque accumulation for hours after use.[4] However, prolonged use of chlorhexidine has also been associated with brown staining of teeth, restorations or dorsum of tongue, unusual or unpleasant taste perturbation with decreased taste sensation and mucosal erosion. In the last few decades, an increase in the use of natural products like essential oils and herbal extracts has been seen in medicine and dentistry. Tulsi also considered to be the queen of herbs has proven its antimicrobial activity with almost no side effects against Candida albicans,

Staphylococcus aureus, E coli and more.[5] Likewise, Curcumin is a polyphenolic compound that possesses a variety of biological activities which have gained significant attention of researches across the globe. Several studies have reported broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, anti-fungal and anti-malarial activities.[6,7] Thus, keeping the aforementioned findings in view, this in-vitro study was conducted to estimate the antimicrobial efficacy of different concentrations of Tulsi and Curcumin extract and compared with 0.2% Chlorhexidine against periodontal pathogens (Aggregatibacter actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg)).

Material and Methods

The present study employed and in-vitro design. Ethical clearance was obtained from Bharati Vidyapeeth Dental College & Hospital, Navi Mumbai, Scientific Review Board and Institutional Ethics Committee.

Preparation of Tulsi extract and Curcumin extract

Tulsi and Curcumin extract of 100% w/v concentration were prepared at Phyto Life Sciences P. Ltd. in Ahmedabad, Gujarat. Both extracts were prepared using cold extraction technique. Tulsi extract was prepared using dried ground Tulsi leaves macerated with 100% ethanol which was allowed to stand still for 3 days. This decoction was filtered using Whatman filter paper to obtain a clear filtrate which was reduced to a low temperature to obtain a solid residue. Similarly, Curcumin extract was prepared using dried ground curcumin root. Both extracts were tested and certified for its authenticity and toxicity by a pharmacognosist.

Preparation of dilutions of Tulsi and Curcumin extract

A stock solution of 10% concentration was prepared for each extract by weighing 10mg of compound (Tulsi and Curcumin extract respectively) using a high precision balance scale and dissolving it in 1ml of Dimethyl sulfoxide solvent. This stock solution was used for preparing further dilutions (5%, 2%, 1% and 0.5% concentration) of both Tulsi extract and Curcumin extract using distilled water. 1ml of stock solution was transferred to a micro test tube using a pre calibrated micropipette to which 1mL of distilled water was added to obtain a 5% concentration solution of Tulsi and Curcumin extracts. 200µL of the stock solution of each extract was similarly added to 800µL of distilled water to obtain a 2% concentration solution of Tulsi and Curcumin extracts. Likewise, 100µL and 50µL of pipetted stock solutions were added to 900µL and 950µL of distilled water respectively to obtain 1% and 0.5% concentration solutions of both extracts. (Fig.: 1a, 1b)

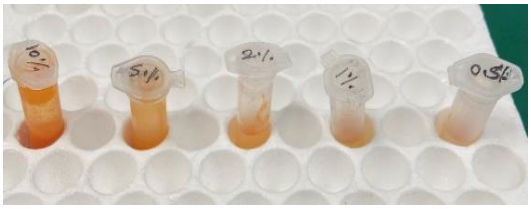


Figure 1a. Prepared dilutions for Curcumin extract

Figure 1.b. Prepared dilutions for Tulsi extract

Microbiological assay

Agar well diffusion method was used to determine the antimicrobial activity of Tulsi leaves extract in vitro. Brain Heart Infusion media was used to culture different micro-organisms examined in this study. Colonies of microorganisms were transferred to the agar plates using a swab, and their turbidity was visually adjusted with the broth to equal that of a 0.5 McFarland turbidity standard. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. The entire surface of agar plate was then swabbed 3 times with the cotton swab, transferring the inoculum, while the plates were rotated by approximately 60° between streaks to ensure even distribution. The overall procedure of inoculum preparation and inoculation of culture media remained the same for both bacteria. Each bacterium was inoculated on four agar plates for five respective concentrations (0.5%, 1%, 2%, 5%, and 10%) of the Tulsi extract and Curcumin extract, Positive and Negative control. Therefore, a total of 24 plates were inoculated to test both the bacteria.

The inoculated plates were allowed to stand for at least 3min but no longer than 15min, before making wells for different compounds to be tested. A hollow tube of 5mm diameter was heated and pressed above the inoculated agar plates. It was removed immediately by making a well in the plate; likewise, five wells on each plate were made for each concentration of Tulsi and Curcumin extract and two wells were made on agar plates for control group; one each for positive control and negative control. Each well received 5 µl of respective compound assigned for it. Plates were incubated at 37°C in an incubator within 15 min of compound application. Incubation was done for 24 h for both bacteria. After the incubation period, plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of inhibition zone was measured to the nearest whole millimetre by using a Vernier Calliper. The microbiological procedure was repeated 4 times for each bacterium, and corresponding four values of zones of inhibition (ZOI) for each concentration of Tulsi extract and Curcumin extract along with 0.2% Chlorhexidine and Distilled water were obtained for both bacteria.

Statistical analysis

The values obtained for zone of inhibition were compared within the group and for both extracts and positive control for each of the two bacteria. Statistical analysis was done using IBM SPSS software (Version 20.0 Chicago IL, USA) to calculate Mean and Standard Deviation. Data for zone of inhibition was expressed as mean, median with standard deviation (SD) and standard error of mean (SEM), 95%

confidence intervals (C.I.). The scores were compared for intergroup and intragroup differences using Analysis of variance (ANOVA) and Post Hoc tests. Statistical significance level was established at $P < 0.05$.

Results

The least zone of inhibition was observed for negative control (Fig. 2a) and lower concentrations (0.5%, 1%, 2% and 5%) of Tulsi extract and Curcumin extract as both Aa and Pg were resistant while the widest zone of inhibition was observed for positive control i.e., 0.2% CHX (20mm for Pg, 16mm for Aa) (Fig 2b).

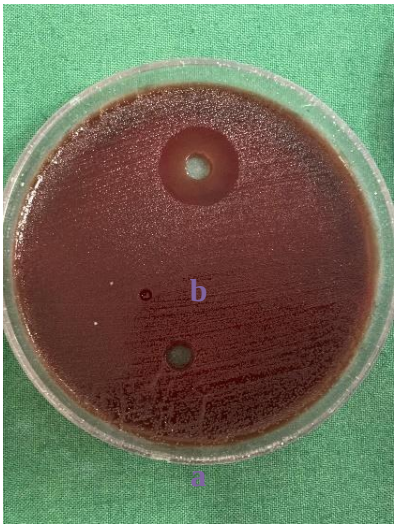


Figure 2.a: no zone of inhibition seen with respect to the negative control, 2.b: confluent zone of inhibition observed for 0.2% chlorhexidine

At 10% concentration Tulsi extract showed a considerable ZOI (13 mm) for both Aggregatibacter actinomycetemcomitans (Fig. 3a) and Porphyromonas gingivalis (Fig. 3b). Similarly, Curcumin extract at 10% concentration showed a considerable ZOI (10 mm) against both Aggregatibacter actinomycetemcomitans (Fig. 4a) and Porphyromonas gingivalis (Fig. 4b). Graph 1 represents the mean zone of inhibition in mm for 10% concentration of Tulsi and Curcumin extract, positive and negative control against Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans respectively.

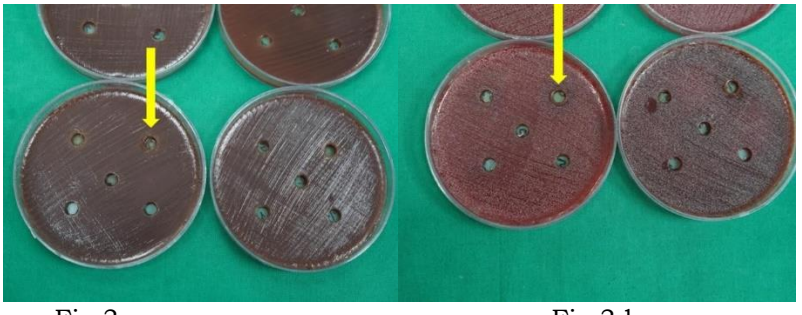


Fig.3.a Fig.3.b

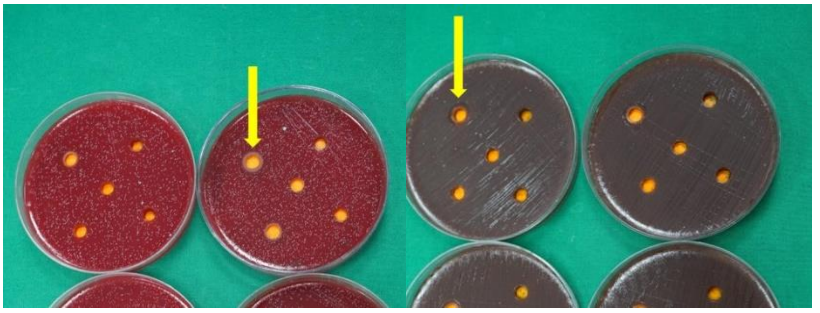
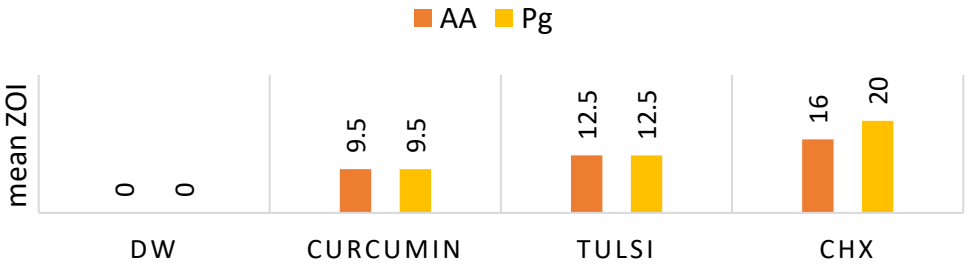


Fig.4.a Fig.4.b

Figure 3.a: ZOI seen for 10% Tulsi extract against Aa, 3.b: ZOI seen for 10% Tulsi extract against Pg, 4.a: ZOI seen for 10% Tulsi extract against Aa, 4.b: ZOI seen for 10% Tulsi extract against Pg



Graph 1. Mean ZOI for Aa and Pg at 10% concentration of Tulsi and Curcumin extract, 0.2% Chlorhexidine and Negative control

Mean zone of inhibition for 10% concentration of Tulsi extract (12.50 ± 0.577), 10% concentration of Curcumin extract (9.50 ± 1.000) and 0.2% Chlorhexidine (20.00 ± 0.000) were calculated for statistical analysis (Tab. 1).

Table 1
Mean scores for ZOI for Tulsi extract, Curcumin extract and Chlorhexidine

		N	Mean	Median	SD	SEM
Pg	Tulsi	4	12.50	12.50	.577	.289
	Curcumin	4	9.50	10.00	1.000	.500
	Chlorhexidine	4	20.00	20.00	.000	.000
	Total	12	14.00		4.651	1.343
Aa	Tulsi	4	12.50	12.50	.577	.289
	Curcumin	4	9.50	10.00	1.000	.500
	Chlorhexidine	4	16.00	16.00	.000	.000
	Total	12	12.67		2.839	.820

One-way analysis of variance test was performed for statistical analysis. It revealed a statistically significant difference between all the groups for antimicrobial efficacy against both the bacteria. ($P < 0.001$) (Tab. 2). Post-hoc test revealed a significant difference in the antimicrobial efficacy between 0.2%

Chlorhexidine and 10% Tulsi extract, 0.2% Chlorhexidine and 10% Curcumin extract and between 10% Tulsi extract and 10% Curcumin extract against Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis (Tab. 3).

Table 2
Inter group and Intra group analysis of antimicrobial efficacy

		Sum of Squares	df	Mean Square	F	Sig.
Pg	Inter group analysis	234.000	2	117.000	263.250	.000
	Intra group analysis	4.000	9	.444		
	Total	238.000	11			
Aa	Inter group analysis	84.667	2	42.333	95.250	.000
	Intra group analysis	4.000	9	.444		
	Total	88.667	11			

Table 3
Multiple comparisons for antimicrobial efficacy

Dependent Variable	(I) groups	(J) groups	Mean difference (I-J)	Std. Error	Sig.
Pg	tulsi	Curcumin	3.000*	.471	.000
		Chlorhexidine	-7.500*	.471	.000
	curcumin	Tulsi	-3.000*	.471	.000
		Chlorhexidine	-10.500*	.471	.000
	chlorhexidine	Tulsi	7.500*	.471	.000
		Curcumin	10.500*	.471	.000
Aa	tulsi	Curcumin	3.000*	.471	.000
		Chlorhexidine	-3.500*	.471	.000
	curcumin	Tulsi	-3.500*	.471	.000
		Chlorhexidine	-6.500*	.471	.000
	chlorhexidine	Tulsi	3.500*	.471	.000
		Curcumin	6.500*	.471	.000

Thus, maximum inhibitory effect was seen with 0.2% Chlorhexidine against Aggregatibacter actinomycetemcomitans (20mm) and Porphyromonas gingivalis (16mm) and at 10% concentration the least inhibitory effect was seen with Curcumin against Aggregatibacter actinomycetemcomitans (8mm) and Porphyromonas gingivalis (8mm).

Discussion

Periodontitis continues to be a leading oral disease prevalent in about 67% of the general population with high global economic impact with robust evidence for an association with low socio-economic status.[8,9] Severity of periodontitis however is dependent on multifactorial aspects such as host related risk factors and environmental risk factors both of which are modifiable as well as non-modifiable.[10] There is a considerable difference in the variance of prevalence of aggressive periodontitis across various studies. The pooled prevalence of aggressive periodontitis has been reported to be higher across the African and the South American continents as compared to Europe, North America and Asia which are more developed.[11] The key to maintenance of periodontal health after a clinical therapy involves lifelong supportive care that includes daily removal of the plaque biofilm by the patients with various mechanical and chemical aids supplemented by professional care as and when needed. A large number of studies have even stated patient self-care to be an important aspect in maintaining periodontal health post operatively and reducing the need for professional intervention.[12,13]

Decreased susceptibility or resistance against antimicrobial agents is the result of various intracellular and extracellular mechanisms as a consequence of bacterial phenotypic and genotypic resistance.[14] Therefore, bacterial resistance to various chemotherapeutics also necessitates the need for alternatives that are effective, safe and economical making then additionally beneficial to the general population of developing countries. Considering the availability of data on chlorhexidine which has been studied extensively, has been the gold standard for years and used most commonly as a part of at home regimen for patients with periodontitis; this study was conducted in the quest of identifying Tulsi and Curcumin extract as adjuncts in treatment of periodontitis keeping the side effects of chlorhexidine in mind.

In the present study an attempt was made to obtain information on the antimicrobial efficacy of Tulsi extract and Curcumin extract in comparison with 0.2% Chlorhexidine against two periodontal pathogens which have been most commonly associated with periodontal diseases; Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. Results in this invitro study showed that Tulsi extract and Curcumin extract showed effective inhibition of growth of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis comparable to 0.2% Chlorhexidine. Although many previous studies conducted have shown antimicrobial properties of Tulsi and Curcumin against oral microbiota, and there is evidence claiming phytomedicinal products being effective against periodontal pathogens[15,16,17,18]; To the best of our knowledge, this is the first study till date, to evaluate the antimicrobial efficacy of curcumin extract on periodontal pathogens.

Mallikarjun et al in their invitro study reported 5% Tulsi extract and 10% Tulsi extract to effectively act against Aggregatibacter actinomycetemcomitans and these results demonstrated a comparable efficacy of Tulsi extract to that of doxycycline.[16] Agarwal et al conducted a similar study where in the antimicrobial efficacy of Tulsi extract at 15 different concentrations ranging from 0.5% to 10%

was evaluated against streptococcus mutans in comparison with 0.2% chlorhexidine. Maximum antimicrobial potential of Tulsi extract was observed at a concentration of 4% and higher.[17] Shah et al on comparison of Tulsi extract and Neem extract reported Tulsi extract to have antimicrobial activity against streptococcus mutans and lactobacillus acidophilus at a 25% concentration.[18.] The results of our study demonstrated that the values for zone of inhibition were highest for 0.2% Chlorhexidine however Tulsi extract and Curcumin extract also showed significantly high values for zone of inhibition at 10% concentration. These findings suggest that both Tulsi extract and curcumin extract are efficacious in inhibiting the growth of periodontal pathogens at higher concentrations.

Limitations

Limitations of this study include the use of both extracts at a lower concentration and a lack of pre-treatment and post-treatment analysis of clinical parameters. The microbial parameter chosen in this study was however accurate which helped to determine the role of Tulsi extract and Curcumin extract when used in-vitro to study the inhibition of periodontal pathogens.

Conclusion

In future, commercial availability of herbal extracts in pure form to be used as adjuncts in oral prophylaxis may help in effective plaque control economically. Further studies with larger sample size evaluating antimicrobial efficacy of Tulsi extracts and Curcumin extract at higher concentration are required. Ex-vivo microbiological studies comparing the pre-treatment and post treatment changes in microbial load can give us more insight on significant antimicrobial activity of these extracts.

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