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Effect of a Novel PolyHerbal Mouthwash on Dental Biofilm Induced Gingivitis

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Abstract:

Background: Dental biofilm plays a crucial role in dental caries and periodontal disease development. A mouthwash is a chemical agent used to enhance oral hygiene. Side-effects of Chlorhexidine have compelled to look out for herbal alternatives. The aim of the study was to evaluate the clinical and microbiological effectiveness of a herbal mouthwash containing Myristica fragnans (nutmeg), Trigonella foenum-graecum (fenugreek), Cinnamomum zeilanicum (cinnamon) against Chlorhexidine mouthwash.

Materials and Methods: 60 patients with Dental biofilm induced gingivitis participated in this study and were randomly divided into 2 groups (Group I– Gingivitis patients were provided with prepared herbal mouthwash, Group II- Gingivitis patients were provided with commercially available Chlorhexidine mouthwash)**.** Plaque Index, Gingival Index & Sulcus Bleeding Index were recorded at the baseline and 30 days after using the mouthwash. Supragingival plaque samples were taken for microbial examination (CFU) on day 0 and on day 30.

Results: Intragroup comparison for the clinical parameters showed statistically significant reduction in both the Groups ($p<0.0001$, $p<0.0001$). Intergroup comparison for clinical parameters, there was no statistical significance seen after 30days ($p<0.1029$ and $P<0.1026$). Intragroup comparison for microbial analysis showed significance reduction in both the groups after 30 days ($p<0.0001$, $p<0.0001$). On Intergroup comparison for microbial analysis, both the groups showed reduction after 30 days without significance ($p<0.31227$).

Conclusion: Herbal mouthwash showed similar antimicrobial activity when compared to 0.2% Chlorhexidine mouthwash that could be used as a substitute to chlorhexidine.

Keywords: Biofilm induced Gingivitis, Chemical plaque control, Chlorhexidine, Herbal alternatives, Colony Forming Units.

INTRODUCTION:

Dental biofilm is one of the major reasons for Periodontal disease & Dental caries that are considered as “Posh oral infections”. Mouthrinse helps to maintain oral biologic equilibrium when used along with mechanical tooth brushing and Dental flossing, as mechanical plaque control methods cannot solely maintain oral health [1, 2]. Copious chemicals have been tried to control supragingival plaque deposition and gingivitis [3]. Chlorhexidine, a bisguanide is considered benchmark of antimicrobial rinses due to its high substantivity and wide range of activity. Studies have demonstrated the effectiveness of rinsing with chlorhexidine antimicrobial mouthrinse in significantly reducing bacterial plaque [4]. Studies found chlorhexidine to be dose dependent in inhibiting plaque. Lower concentrations were deficient in plaque reduction [5]. Chlorhexidine is not a ‘Miraculous solution’ as it has definite after effects like taste alteration, mucosal irritation, dry mouth, tooth discoloration, cytotoxic effect [6]. Based on the ill-effects of chlorhexidine, World Health Organization admonished researchers to probe the potential of herbs and plant extracts [7]. Changes in microbial, genetic & metabolic processes, along with the extracellular matrix by microorganisms, are thought to resist the actions of antimicrobials. Herbs, when used exclusively or in amalgamation were proven safe against various health issues. Many plant-derived antimicrobials were found to be effective in treating periodontal disease [8]. Lately, herbal products are incorporated in dentifrice, mouthwash, gum paints, gum astringents and oral gels to avert biofilm formation [9]. In dentistry, Herbal concentrates were used to lessen inflammation, prevent dental caries, to heal gingivitis & periodontitis, and as antimicrobials &

antioxidants. The herbs used in this study were Nutmeg (Myristica fragnans), Fenugreek (Trigonella foenum-graecum) and Cinnamon (Cinnamomum zeylanicum). The efficacy of all the three herbs were proven individually. The combined effect of the herbs was not studied previously. Thus the aim of the study was evaluate the clinical and anti-microbial efficacy of herbal mouthwash containing Nutmeg, Fenugreek and Cinnamon against commercially available Chlorhexidine mouthwash.

MATERIALS AND METHODS:

This study was framed and conducted in the Department of Periodontics & Implantology, Thai Moogambigai Dental College & hospital. The study was registered in the university research committee [Dr. MGRDU/TMDCH/2018-19/22101]. This study was approved by the human subject ethics board of Dr. MGR University & research institute, Chennai and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Herbal mouthwash was formulated at Faculty of Pharmacy and microbiological analysis was done in the Department of Microbiology, ACS Medical College & Hospital, Chennai.

PARTICIPANTS:

A total of 80 patients with biofilm induced gingivitis who came to the Department of Periodontics & Implantology were assessed and 60 patients satisfying the eligibility criteria were randomly selected by lottery method. Patients within the age group of 20 to 45 years (32 female & 28 male) having a minimum of 20 natural dentition with moderate biofilm induced gingivitis were included in the study. Patients who underwent oral prophylaxis within 6 months, non biofilm induced gingivitis and Periodontitis, history of using mouth rinse and other inter dental aid, pregnant women, lactating mothers, smokers, patients with systemic diseases, patients who were under antibiotic & NSAID's for past 6 months were excluded from the study. All participants were motivated and described about the study protocol. Informed consent was obtained from all the participants in their mother tongue. All patients reported promptly at the follow-up time. No patient developed allergy or burning sensation to mouthwash. Participants were divided into two groups:

Group I: 30 patients were provided with herbal mouthwash

Group II: 30 patients were provided with Chlorhexidine mouthwash

Plaque Index [Silness J & Loe H, 1964], Gingival Index [Loe H & Silness J 1963] & Sulcus Bleeding Index [Muhlemann & son 1971] were recorded at baseline and 30 days after using the mouthwash. All the participants underwent Supragingival Scaling after baseline measurements. Supragingival plaque samples were collected with a sterile hand scaler from the buccal surface of upper molar and lingual surface of lower molar and were transported to the microbiology lab in phosphate buffered Saline for quantification of microorganisms in the sample by Colony Forming Units.

PREPARATION OF HERBAL EXTRACT:

The herbal extract preparation was carried out at Department of Pharmacognosy, Faculty of Pharmacy, ACS Medical College & Hospital, Chennai.

The nutmeg seeds, fenugreek seeds, cinnamon bark were procured from IMPCOPS Ltd., (Chennai, India) and were air-dried at room temperature for 7 days. The plant products were weighed and grounded using high speed grinder into fine powder. It was stored in separate air tight containers and kept away from heat, moisture, and sunlight. Dry powder of all the three components were weighed (50 g each) and taken in equal ratio (1:1:1) and was extracted by hydro- alcohol solution (50:50), using a soxhlet apparatus for 3 days **(Figure 1)**. The liquid extract was then concentrated by placing the petridish in an electric water bath at 40^{o} C till remaining extract dried completely **(Figure 2)**. Dried extract was scraped out and employed for preparation of mouthwash **(Figure 3)**.

Figure 1: Soxhlet extraction of the herbal components.

Figure 2: Herbal extract placed on hot water bath

Figure 3 : Herbal extract dissolved for mouthwash formulation

Figure 4: . Formulated herbal mouthwash

PREPARATION OF MOUTHWASH

The mouthwash preparation was carried out at Pharmaceuticals laboratory, Faculty of Pharmacy, ACS Medical College & Hospital, Chennai.

Composition

Herbal extract – 1%, Ethanol -0.2%, Glycerol- 2%, Propylene glycol- 2%, Sorbitol- 15%, Sodium saccharin- 0.3%, Menthol- 0.07%, Peppermint oil- 0.05%, Methyl paraben- 0.05%, Amaranth - 0.1%, Distilled water- 100 ml.

Preparation

Herbal extract was dissolved in ethanol using magnetic stirrer for 15 minutes. Glycerol, Propylene glycol was then added to the ethanol mixture**.** Sorbitol & Sodium saccharin were added as sweetening agent. Menthol, Peppermint oil was used as flavoring agent, Amaranth as coloring agent, Methyl paraben as preservative and distilled water as vehicle. The final mouthwash was transferred into clean dry containers and stored at cool dry place until use **(Figure 4)**.

Direction for use of mouthwash

Participants of Group I was provided with herbal mouthwash [120 ml] (30 ml X 4 bottles for each patient) and was instructed to use 5ml of the herbal mouthwash (using measuring cap) without dilution twice a day, 30 minutes after brushing. While Participants of Group II were provided with Chlorhexidine mouthwash and were asked to rinse with 5ml of the mouthwash twice a day for 30 days, 30 minutes after brushing. Participants were asked not eat or drink anything immediately after using mouthwash.

Microbial analysis

The collected supra-gingival plaque samples were inoculated on blood agar plate by streak culture method & incubated at 37^0 C for 24 hours & analyzed using automated colony counter for Colony Forming Units [CFU].

Statistical analysis

Data was analyzed using statistical Software Package SPSS version 22 (IBM SPSS Statistics for Windows, version 22.0, IBM Corp.). Intragroup comparison of clinical parameters and microbial analysis at baseline and on 30^{th} day was analyzed using Student's paired t-test. Intergroup comparison of clinical parameters and microbial analysis at baseline and after 30 days was analyzed using independent t-test. P≤0.05 was considered statistically significant.

RESULTS:

In Group I, Plaque Index score was 1.81±0.1 at baseline and 0.48±0.08 on 30^{th} day. Gingival Index was 1.91±0.54 at baseline and 0.65±0.65 on 30^{th} day. Sulcus Bleeding Index was 2.07±0.08 at baseline and 1.06±0.05 on 30^{th} day.

In Group II, Plaque Index score was 1.74 ±0.11 at baseline and 0.49±0.05 on 30^{th} day. Gingival Index was 1.91±0.05 at baseline and 0.66±0.08 on 30^{th} day. Sulcus Bleeding Index was 2.08±0.07 at baseline and 1.03±0.06 on 30^{th} day.

Intragroup comparison of Plaque Index showed statistically significant reduction in Group I & Group II patients (p<0.0001, p<0.0001) (**Table 1**). Inter group comparison of Plaque Index showed difference at baseline and after 30 days which was not significant (p=0.519) (**Table 2**).

Intragroup comparison of Gingival Index, showed a statistical significance in both groups after 30 days (p<0.0001, p<0.0001) (**Table 1**). Intergroup comparison of Gingival Index after 30 days showed difference without statistical significance (p=0.374) (**Table 2**).

Intragroup comparison of Sulcus Bleeding Index, showed a statistical significance in both groups after 30 days (p<0.0001, p<0.0001) (**Table 1**). Intergroup comparison of Sulcus Bleeding Index after 30 days showed difference without statistical significance (p=0.146) (**Table 2**).

Intragroup comparison for microbial analysis showed significant reduction after 30 days in both the groups (p<0.0001, p<0.0001) (**Table 3**). Intergroup comparison showed a higher reduction of colony forming units in Group I patients compared to Group II patients without statistical significance (p=0.611) (**Table 4**).

Table 1: Intra group comparison of clinical parameters at baseline and after 30 days

		Plaque Index*	Gingival Index*	Sulcus Bleeding Index
Group I	Baseline	1.81±0.1	1.91±0.54	2.07±0.08
	30^{th} day	0.48±0.08	0.65±0.65	1.06±0.05
	p- value	<0.0001(S)	<0.0001 (S)	<0.0001(S)
Group II	Baseline	1.74 ±0.11	1.91±0. 05	2.08±0.07
	30^{th} day	0.49±0.05	0.66±0.08	1.03±0.06
	p- value	<0.0001(S)	<0.0001(S)	<0.0001(S)

*****Mean ±SD

S – Significant

P≤0.05 is considered significant

Table 2: Inter group comparison of clinical parameters at Baseline and after 30 days

		Plaque Index*		Gingival Index*		Sulcus Bleeding Index	
			p- value		p- value		p- value
Baseline	Group I	1.81±0.1	0.1864(NS)	1.91±0.54	0.50002(NS)	2.07±0.08	0.587(NS)
	Group II	1.74±0.6		1.91±0. 05		2.08±0.07	
After30 days			p- value		p- value		p- value
	Group I	0.48±0.08	0.1029(NS)	0.65±0.65	0.1026 (NS)	1.06±0.05	0.1413(NS)
	Group II	0.49±0.05		0.66±0.08		1.03±0.06	

*mean ± SD
NS – Non significant
P ≤0.05 is considered significant

Table 3: Intragroup comparison of CFU at Baseline and after 30 days

	Group I †	Group II †
Baseline	26.88±7.59	7.05±0.649
After 30 days	26.5±6.25	7.2±0.154
p-value	<0.0001(S)	<0.0001(S)

† COLONY FORMING UNITS / ml
S – Significant
P ≤0.05 is considered significant

Table 4: Intergroup comparison of CFU at baseline and after 30 days

	Baseline †	After 30 days †
Group I	26.88±7.59	7.05±0.649
Group II	26.5±6.25	7.2±0.154
p-value	0.3913 (NS)	0.31227 (NS)

† COLONY FORMING UNITS / ml
NS- Non significant
P ≤0.05 is considered significant

DISCUSSION

Disequilibrium between microorganisms and oral hygiene practice results in disease progression. Herbal products are reconsidered as an alternative to chemicals as they are efficient, safe for long term usage without side-effects and cost effective. [10] Chlorhexidine (CHX) is the most effective chemical agent studied for plaque control till date. Herbal mouthwashes possess components having anti-inflammatory, antimicrobial, and antioxidant activity that enhance oral hygiene comparatively with chlorhexidine mouthwash [11]. Herbal products for the control of gingivitis tend to have fewer side effects and are generally cheaper than synthetic products [12]. The herbs used in this study are fenugreek, nutmeg & cinnamon. The properties of all three herbs have been studied individually and were proven to be effective against oral pathogens. Earlier studies showed that ethanolic extract had better efficacy compared to aqueous extracts of herbs as aqueous extract had shorter shelf life. The decreased activity of aqueous extract could be due to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts but is inactive in methanol and ethanol extracts. Additionally, ethanol was found to penetrate the cellular membrane more easily to extract the intracellular ingredients from the plant material. Properties of the three herbals used in combination had a synergistic effect which was studied in-vitro at Department of Pharmacognosy, ACS medical college & hospital, Chennai before planning to formulate this mouthwash.

In this study, Plaque Index, Gingival Index and Sulcus Bleeding Index scores were recorded on Day 0 & Day 30. Supragingival plaque sample was collected to quantify colony forming units on Day 0 & Day 30. The result of the study showed significant difference in both the groups with regards to clinical parameters at baseline and after 30 days. Intragroup comparison with respect to microbial analysis showed significant difference in both the groups after 30 days while intergroup comparison showed difference in microbial analysis without statistical significance.

Intragroup comparison of Plaque Index score in group I showed significant reduction after 30 days ($p<0.0001$) which could be due to antimicrobial components of the herbal mouthwash. Saponin, a component of fenugreek and cinnamon has a lipophilic property that causes damage to bacterial cell membrane. The antibacterial activity shown by fenugreek seed extracts may be due to its flavonoid content. [13] Flavonoid destroys the bacteria cytoplasmic membrane and causes leakage of important metabolites that inactivate bacterial enzyme. [14] Chlorhexidine inhibits plaque as a result of an immediate bactericidal action and a prolonged bacteriostatic action. This could be the reason for significant reduction in plaque scores at baseline and after 30 days in Group II ($p<0.0001$). In Group I, reduction in Gingival Index and

Sulcus Bleeding Index score could be due to the anti inflammatory effect of flavanoids, saponins, maceglinin that inhibited the production of phorbol-12-myristate-13-acetate-induced inflammatory cytokines such as IL-1α, IL-1β, IL-2, IL-6, tumor necrosis factor (TNF)-α. [15] Awang et al reported that cinnamaldehyde, an essential oil of cinnamon had prominent antimicrobial activity[16]. In vitro study on Cinnamon extract were proved effective against *S. mutans*, but its effectiveness as a mouthwash to reduce dental plaque is yet to be proven. [17] In contrast, Gupta. D & Jain.K [7] has proven cinnamon mouthwash to be equally effective against periodontal pathogens when compared to CHX.

The effectiveness of fenugreek extract against Helicobacter pylori showed damage to the cell by disrupting the cell membrane leading to cell lysis [18]. Scopoletin, a coumarin derivative is responsible for the anti-microbial activity of fenugreek against prokaryotes [19]. Michael D & Kumawat D has shown the broad spectrum activity of fenugreek seeds which could be due to the ability of flavonoid compounds in the extract to complex with cell wall causing cell wall rupture. [20] Various extracts and the essential oil of nutmeg seeds have presented strong antimicrobial activity against gram-positive and gram-negative bacteria, as well as a variety of fungi. Macelignan, a component in mace of nutmeg possessed antibacterial effect against oral microbes [21]. Trimyristin and myristic acid were found to be the chief antibacterial principles isolated from nutmeg seeds. [22] These components of nutmeg provide its antimicrobial property. Ethanolic extracts of Myristica fragrans were effective against gram-positive organisms like S. mutans, S. salivarius and few Gram-negative bacteria like A. actinomycetemcomitans, P, gingivalis and F. nucleatum [23].

Our study showed that herbal mouthwash was equally efficacious compared to Chlorhexidine due to antimicrobial property of all the herbs incorporated in the mouthwash. Combining several such herbal extracts in single mouthwash would certainly have more benefits in reducing the oral pathogens. Despite being potent antimicrobial & anti-plaque agent, long term use of chlorhexidine is limited [24.] Studies have shown that chlorhexidine lack plaque inhibitory properties at lower concentrations. [25] Our results were similar to the results of Chatterjee [26] who compared 1% turmeric mouthwash with 2% Chlorhexidine and found turmeric mouthwash to be as effective as CHX in reducing the Gingival and Plaque Index. Similarly Sahana [27] compared triphala mouthwash with CHX and found that both mouthwashes were equally effective in reducing the Plaque and Gingival scores. In our study, patient did not report with burning sensation or taste alteration in test group (herbal mouthwash. To the best of author's knowledge, this was the first study to combine nutmeg, fenugreek and cinnamon in single mouthwash against Chlorhexidine mouthwash.

Limitations:

The study was conducted on lesser population for a shorter course of time which could not explain about the long term effectiveness of the herbal mouthwash. Substantivity of the herbal mouthwashes is not proven till today. Long term study with larger population on substantivity of the herbal mouthwashes could have provided better result and enhanced decision making on mouthwash selection.

CONCLUSION:

A proper maintenance of oral health is essential for wellbeing. Though healing potential in plants is an ancient concept, lately it has gained renewed interest and importance. Within the limitations of the study, the newly formulated herbal mouthwash when compared to 0.2% Chlorhexidine mouthwash showed similar antimicrobial activity. Combining several such herbal extracts in single mouthwash would certainly have more benefits in reducing the pathogenic oral microorganisms. Herbal mouthwash has many advantages along with equal efficacy compared to the gold standard (CHX). Herbal mouthwash could be used as replacement for chemical mouthwash for prolonged usage without side effects. It is advisable to shift to herbal mouthwashes as they are better alternative to Chlorhexidine.

Key findings of the study:

Herbal mouthwash showed similar antmicrobial activity when compared to 0.2% Chlorhexidine mouthwash.

List of each author's contribution:

Dr. Gomathi.G.D – Conceptulisation, sample collection, analysis of data, drafting the manuscript.

Dr. Gopalakrishnan- Conceptulisation, drafting the manuscript, critical revision

Dr. Uma Sudhakar- drafting the manuscript, critical revision

Dr. S. Nandhakumar- mouthwash preparation and formulation

Dr. Hari Krishnan Narayanaswamy- mouthwash formulation

Dr. Nimisha Mithradas- sample collection

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Conflict of Interest

The authors also report no conflict of interest related to this study.

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