A Comparative Evaluation of the Antiplaque And Antigingivitis Effects of Herbal Extracts Incorporated In Dentifrices: A Double Blinded Randomized Controlled Trial

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ABSTRACT

Introduction: The control of supragingival plaque is thus a crucial factor in maintaining the health of periodontal tissue. Various chemical agents have been advocated as a measure for plaque control However, an increase in the research of herbal medicine has facilitated the growth of alternative and more biocompatible strategies. The rationale behind the present clinical trial was to compare the effectiveness of 5 different dentifrices that have incorporated these herbal extracts, in reducing gingival bleeding and plaque accumulation measured by gingival bleeding index and plaque index respectively and comparing their efficacy to that of commercial non herbal toothpaste.

Materials and Method: The present study was a double blinded randomized controlled trial which incorporated 420 participants into 6 groups namely Group I Aloe Vera based dentifrices, Group II- Triphala based dentifrice, Group III- *Ocimum sanctum* (Tulsi) based dentifrice, Group IV- *Punica granatum Linn* (Pomegranate) based dentifrice, Group V- *Acacia catechu* (AC) based dentifrice and Group VI- A commercially available dentifrice. After the procurement of the material, extracts were obtained and incorporated into dentifrices. Patients were provided with a soft bristle brush and the tooth paste. They were asked to brush twice a day and not use any other intra oral cleansing aid. Follow up was done after one month and 2 months. The gingival bleeding index used was by Carter and Barner 1974 and the plaque index used was by (Loe and Silness**)**

Results and Conclusion: All the 5 herbal extracts showed significant reduction in both gingival bleeding index and plaque indices with a 2 month follow up, compared to the commercially available toothpaste. Aloe Vera showed the highest efficacy and pomegranate showed the least in comparison to other herbal extracts. However all the extracts were more effective compared to commercially available toothpaste, and could be considered as a more

biocompatible alternative. However, further studies are required to standardize the concentration and methods to commercially incorporate these extracts.

Key Words: Herbal Medicine, Dentifrice, Dental Plaque, Gingivitis, Aloe Vera, Ocimum sanctum, Punica granatum, Acacia catechu, Triphala

INTRODUCTION

Plaque is a complex biofilm that consists of bacterial colonies of diverse species adherent on the tooth surface within the oral cavity [1]. Plaque formulations vary from tooth to tooth and even based on their location on the tooth [2]. The initial colonizers are gram positive, coccoid, facultative microbes that adhere to the enamel salivary pellicle and are eventually replaced by gram negative, filamentous microbes which become the dominant species in mature plaque [3]. The study by Ritz et al demonstrates the progression of microbial species in plaque from streptococci (pioneer species) to Actinomyces to gram negative anaerobes as the plaque matures [4]. Mutans streptococci, being the etiological factor in dental caries, have gained considerable interest. The change in the formulation of dental plaque as it matures is accompanied by alterations in the gingival tissue adjacent to it. A direct correlation has been established between gingivitis, periodontitis and plaque through epidemiological studies. The non specific plaque hypothesis establishes the mass of the plaque as the major contributing factor in inflammatory periodontal diseases and regards the qualitative features of microbiota within the plaque as a factor of minor importance [5]. However, later it was established that a degree of specificity exists in the microbiota observed in healthy tissue and that observed in gingivitis and periodontitis [6].

The control of supragingival plaque is thus a crucial factor in maintaining the health of periodontal tissue. A combination of both chemical and mechanical control of plaque is essential for maintaining oral health [7,8]. Various chemical agents have been advocated as a measure for plaque control [9]. However an increase in the research of herbal medicine has facilitated the growth of alternative and more biocompatible strategies. Comparative studies have tested herbal and conventional oral products for their ability to control plaque and gingivitis. Current evidence based on systematic review and meta analysis suggests that herbal tooth paste is as effective as non herbal toothpaste in plaque control. However the evidence is derived from studies with low certainty of evidence [10].

Herbal extracts of Aloe Vera, Triphala, *Ocimum sanctum* (Tulsi), *Punica granatum Linn* (Pomegranate), and *Acacia catechu* (AC), have been extensively studied for their medicinal value in ayurveda due to their antimicrobial and anti-inflammatory properties. There have been no clinical studies that have compared their effectiveness in reducing gingival and periodontal inflammation. The rationale behind the present clinical trial was to compare the effectiveness of 5 different dentifrices that have incorporated these herbal extracts, in reducing gingival bleeding and plaque accumulation measured by gingival bleeding index and plaque index respectively and comparing their efficacy to that of commercial non herbal toothpaste.

MATERIALS AND METHOD

Study design:

The present study is a double blinded parallel arm randomized control trial.

Ethical approval:

Ethical clearance was obtained from the Institutional review board (IRB) and institutional ethics committee of Dr G.D. Pol Foundation's YMT Dental College and Hospital, Navi Mumbai.

Study groups:

The study groups were divided as follows:

Group I Aloe Vera based dentifrices

Group II- Triphala based dentifrice
Group III- *Ocimum sanctum* (Tulsi) based dentifrice
Group IV- *Punica granatum Linn* (Pomegranate) based dentifrice
Group V- *Acacia catechu* (AC) based dentifrice
Group VI- A commercially available dentifrice

Eligibility criteria:
Inclusion Criteria: Subjects with informed consent, having Gingival Bleeding Index (GBI) score >40% and those with at least 20 natural teeth present in their oral cavity were included in the study.
Exclusion Criteria: Subjects with medical disorders or probing depth >3 mm. Individuals under antimicrobial therapy at least 1 month prior to the study and using mouth rinses or dentifrices containing substances with anti-inflammatory properties. Also smokers and pregnant women were excluded.

Setting and location:
The study was carried out in the department of public health dentistry, Dr GD Pol Foundation's YMT Dental College, Navi Mumbai. Participants recruited were the patients that reported to the department OPD.
Sample size determination:
Sample size was determined using the least mean difference as 0.15 & 0.3 as SEM from a study by Nair and Malaiappan, 2016.[8] The formula used was as follows:
$n=2\ Z\alpha+Z\beta d2$
$n=2\ 1.96+0.84\ 0.30.15$
$= 62.72$
$= 63$
Adding 10% for attrition
Where,
$Z\alpha$ is determined from table values
is standard deviation; $d=x1-x2= 0.15$
n= sample size
Hence 420 participants were recruited into 6 groups. Thus 70 participants were present in each group.
Randomization sequence generation:
Randomization sequence was generated using the lottery method by graph pad using a website (https://www.graphpad.com/quickcalcs/randomize2/) Participants were randomly allocated by lottery method into 6 study groups with 70 subjects in each group.
Allocation Concealment:
Allocation concealment was done using the SNOSE technique (Sequentially Number Opaque Sealed Envelopes) which helped in concealing the sequence until the intervention was assigned.
Blinding:
The laminate tubes of the dentifrices procured were without any labels. Labelling was done by wrapping a paper which had letters A, B, C, D, E, F. Double blinding was done where both the primary investigator and subjects were not aware of the intervention.
Procurement of material:
Aloe Vera leaves, Pomegranate fruits, Tulsi leaves, constituents of Triphala (amalaki, bibhitaki and haritaki) and Acacia catechu were obtained from the botanical garden of Ayurvedic college of YMT group of institutions. Extracts were obtained by processing it with 96% ethanol, drying and filtration.

Preparation of extracts and dentifrice:

Aloe Vera [11]

Fresh aloe Vera leaf gel was dried, powdered, soaked in methanol and ethanol for 24 hours. This was then filtered and dried. This dried extract was further powdered and then dissolved in distilled water. Acetone extract was prepared in a similar manner except that the extracted powder was dissolved in 0.15N NaOH and was further neutralized with 0.15N HCl.

Triphala [12]

The ingredients used in the Triphala Churna were Amlaki (Embellica officinalis), Bibhitaka (Terminalia bellerica), Haritaki (Terminalia chebula). They were cleaned, dried, sieved, powdered and mixed in the ratio of 1:1:1. Ethanol and aqueous extracts were concentrated in a water bath. Extracts were dried under reduced pressure using Rotary Vacuum Evaporator (Equitron® Roteva) and were preserved in the desiccator until further use.

Tulsi

The whole plant was weighed, cleaned, air-dried and weighed again. This was then crushed and mixed with ethanol for the process of maceration. Filtration was done to separate the filtrate and residue which were further processed and subjected to evaporation at 70°C. Extracts were then dried and a dark green residue was obtained. These were refrigerated until further use. Dried extracts were also subjected to phytochemical screening.

Pomegranate

Pomegranate fruits were washed with distilled water and cut manually to separate the arils and peel. The juice was manually extracted and subjected to drying ± 5ºC for 6 hrs or till its moisture content reached ~5-6 %. Seed powder was separately blended with distilled water or 80% methanol followed by filtration, centrifugation to obtain extracts which were kept at -20°C prior to analysis.

Acacia Catechu [13]

It was extracted from heartwood by drying at room temperature, boiling heartwood chips with 10% hydro-alcoholic solution. The specimen was processed for pharmacognostic standardization.

These extracts were then studied in-vitro to test the antimicrobial efficacy i.e. zone of inhibition by Kirby buer method and minimum inhibitory concentration (MIC%).

The extracts were then used to prepare dentifrices with only differing in the composition of their active ingredient, which was the extract with a concentration corresponding to the results obtained according to its MIC. Common ingredients were Silicon dioxide (Abrasive), Glycerol, Sodium lauryl sulphate (detergent), carboxymethylcellulose (binder), methyl-p-oxybenzoate (preservative), Peppermint oil (flavoring agent), Saccharin, Sodium metaphosphate and deionized water.

The prepared dentifrices were then transferred to laminate tubes and crimped to seal.

Intervention protocol:

A clinical examination of type III (ADA) was performed on a fully functional dental unit with proper illumination and instruments (mirror and explorer) which were autoclaved and used only once. All subjects were asked to maintain and fill a compliance sheet and get the used toothpaste packs at every visit which was weighed and assessed for compliance. Patients were provided with a soft bristle brush and the tooth paste. They were asked to brush twice a day and not use any other intra oral cleansing aid. No diet restrictions were given. Frequent reminders through phone calls were made to motivate the participants.

Outcome measures:

Gingival Bleeding Index (Carter and Barner 1974)

Unwaxed dental floss was allowed to pass interdentally to examine susceptible areas. Mouth was divided into six parts, upper and lower left, right and anterior. Bleeding area was

observed under direct vision (buccal or lingual). No attempts were made to quantify the bleeding, it was just recorded as present or absent.

Plaque Index (Loe and Silness**)** [14]

1 No plaque.

2 A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.

3 Moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin that can be seen with the naked eye.

4 Abundance of soft matter within the gingival pocket and/or on the tooth or the gingival margin.

Statistical analysis:

Data obtained was compiled on a MS Office Excel Sheet (2016) and was subjected to statistical analysis using the Statistical package for social sciences (SPSS v 23.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data have been depicted. Normality of data was checked using Shapiro-Wilk test and it was found that data did not follow a normal curve.

Inter group comparison for indices were done using Kruskal-Wallis Analysis of Variance followed by Mann-Whitney U test for group wise comparison. Intra group comparison for indices was done using Friedman's test followed by pair wise comparison using Wilcoxons Signed rank test. For all the statistical tests, $p<0.05$ was considered to be statistically significant, and $p<0.01$ was highly significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

RESULTS

Baseline demographic data:

There were 420 subjects recruited in the study with a distribution of 70 each group. The mean age of the subjects was 38.72 years (±2.62), with a female preponderance.

Intergroup and pair wise comparison of gingival bleeding index (Table 1 and 2):

At baseline, there was a statistical significant difference seen between the groups with highest values observed in group 3 followed by group 1, 6, 5 followed by group 4 and the least score observed in group 2. A pair wise comparison showed no significant differences between the pairs of groups for the values at baseline except between group 2 and group 3, group 3 vs. group 4, group 4 vs. group 6 and group 5 vs. group 6.

Similarly there was a statistical significant difference observed at 1 month with higher values observed in group 6 followed by group 4, group 3 then group 5 and 1 and least in group 2. A pair wise comparison showed a significant difference between all the pairs of the groups except between group 1 vs. group 5.

Gingival bleeding index scores at 2 months showed higher values in group 6 followed by group 4 & group 5, group 3 then group 2 & least in group 1 with a statistical significant difference. A pair wise comparison showed a significant difference between all the pairs of the groups except group 2 vs. 3, group 2 vs. 5 and group 3 vs. 5.

Intergroup and pair wise comparison of plaque index (Table 1 and 3):

Plaque index scores showed a statistically significant difference between the values at baseline with the highest mean values observed in group 2 followed by group 3 and group 6, followed by group 1 and the least scores observed in group 4 and 5. A pair wise comparison of baseline scores showed a significant difference between the scores of group 1 and 5, group 2 with 4, 5 and 6.

At one month the highest mean scores were observed in group 6 followed by group 3 then group 4 which was followed by group 1 and 2 and the least scores were observed in group 5.

At one month all the pairs of the groups except group 1 vs. 2, group 1 vs. 5, group 2 vs. 5, group 3 vs. 4 and group 3 vs. 6 showed a statistical significant difference.
At 2 months the highest mean scores were observed in group 6 followed by group 4 which was followed by group 3, then group 2 and then group 5. The least scores were observed in group 1. At two months, all the pairs of the groups except group 1 vs. 5, 2 vs. 3 and 2 vs. 5 showed a statistically significant difference.

Intragroup and pair wise comparison of gingival bleeding index within the group at different time intervals (table 4 and 6):

All the groups except group 6 showed a statistical significant difference between the gingival bleeding index scores at baseline, 1 month and 2 month follow up with the highest values observed at baseline and the lowest at 2 month follow up. A pair wise comparison of different time intervals within the groups 1 to 5 showed a statistical significant difference between all the pairs except for 1 month vs 2 month follow up in group 2.

Intragroup and pair wise comparison of plaque index within the group at different time intervals (table 5 and 6):

All the groups except group 6 showed a statistical significant difference between the plaque index scores at baseline, 1 month and 2 month follow up with the highest values observed at baseline and the lowest at 2 month follow up. A pair wise comparison of different time intervals within the groups 1 to 5 showed a statistical significant difference between all the pairs except for 1 month vs. 2 month follow up in group 2 and group 4.

TABLE 1: INTERGROUP COMPARISON OF GINGIVAL BLEEDING INDEX AND PLAQUE INDEX USING THE KRUSKAL WALLIS TEST.

Gingival Bleeding Index					
	Group	**Mean**	**Std. Deviation**	**Median**	**p value of Kruskal-Wallis Test**
Baseline	1	14.06	1.793	14	
	2	13.33	1.886	14	
	3	14.43	1.982	15	
	4	13.50	1.401	13.5	
	5	13.71	1.843	13.5	
	6	13.97	.916	14	0.006
1 month	1	10.63	1.321	10	
	2	9.56	1.923	9	
	3	12.00	.978	12	
	4	12.53	.812	12.5	
	5	10.81	1.804	10	
	6	13.99	1.148	14	0.000
2 month	1	8.44	1.163	9	
	2	9.27	1.424	9	
	3	9.57	.972	9	
	4	10.64	1.842	10	
	5	9.79	1.825	10	
	6	14.03	.978	14	0.000
Plaque Index					
	Group	Mean	Std. Deviation	Median	**p value of Kruskal-Wallis Test**

Baseline	1	2.47	.171	2.49	
	2	2.55	.211	2.58	
	3	2.48	.221	2.475	0.003
	4	2.43	.201	2.45	
	5	2.43	.174	2.39	
	6	2.48	.217	2.44	
1 month	1	2.05	.294	2.035	
	2	2.05	.244	2.01	
	3	2.40	.353	2.37	0.000
	4	2.33	.182	2.35	
	5	2.02	.171	2.08	
	6	2.42	.158	2.45	
2 month	1	1.56	.146	1.605	0.000
	2	1.77	.144	1.68	
	3	1.92	.501	1.98	
	4	2.25	.365	2.245	
	5	1.74	.461	1.65	
	6	2.32	.532	2.47	

TABLE 2: PAIRWISE COMPARISON BETWEEN THE GROUPS FOR GINGIVAL BLEEDING INDEX SCORES

Baseline		
Group	**vs. group**	**p value of Mann-Whitney Test**
2	3	0.001
3	4	0.003
4	6	0.012
5	6	0.008
1 Month Follow up		
Group	**vs. group**	**p value of Mann-Whitney Test**
1	2	0.000
1	3	0.000
1	4	0.000
1	5	0.827
1	6	0.000
2	3	0.000
2	4	0.000
2	5	0.000
2	6	0.000
3	4	0.005
3	5	0.000
3	6	0.000
4	5	0.000
4	6	0.000

5	6	0.000
2 Month Follow up		
Group	**vs. group**	**p value of Mann-Whitney Test**
1	2	0.003
1	3	0.000
1	4	0.000
1	5	0.000
1	6	0.000
2	3	0.068
2	4	0.000
2	5	0.116
2	6	0.000
3	4	0.000
3	5	0.967
3	6	0.000
4	5	0.005
4	6	0.000
5	6	0.000

TABLE 3: PAIRWISE COMPARISON BETWEEN THE GROUPS FOR PLAQUE INDEX

Group	**vs. group**	**p value of Mann-Whitney Test**
Baseline		
1	5	0.045
2	4	0.001
2	5	0.000
2	6	0.013
1 Month Follow Up		
1	2	0.713
1	3	0.000
1	4	0.000
1	5	0.227
1	6	0.000
2	3	0.000
2	4	0.000
2	5	0.353
2	6	0.000
3	4	0.172
3	5	0.000
3	6	0.229
4	5	0.000
4	6	0.009
5	6	0.000

2 Month Follow Up		
1	2	0.000
1	3	0.000
1	4	0.000
1	5	0.197
1	6	0.000
2	3	0.241
2	4	0.000
2	5	0.584
2	6	0.000
3	4	0.000
3	5	0.025
3	6	0.000
4	5	0.000
4	6	0.025
5	6	0.000

TABLE 4: INTRA GROUP COMPARISON OF GINGIVAL BLEEDING INDEX SCORES AT DIFFERENT TIME INTERVALS

Group 1	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline	14.00	2.95	0.000
1 month	10.00	2.01	
2 month	9.00	1.04	
Group 2	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline	14.00	2.80	0.000
1 month	9.00	1.50	
2 month	9.00	1.70	
Group 3	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline	15.00	2.73	0.000
1 month	12.00	2.18	
2 month	9.00	1.09	
Group 4	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline	13.50	2.47	0.002
1 month	12.50	2.01	
2 month	10.00	1.51	
Group 5	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline	13.50	2.74	0.000
1 month	10.00	1.86	
2 month	10.00	1.40	
Group 6	**Median**	**Mean rank**	**p value of Friedman Test**

Baseline 1 month 2 month	14.00 14.00 14.00	2.02 1.90 2.08	0.398

TABLE 5: INTRA GROUP COMPARISON OF PLAQUE INDEX SCORES AT DIFFERENT TIME INTERVALS

Group 1	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline 1 month 2 month	2.49 2.04 1.61	2.91 2.00 1.09	0.000
Group 2	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline 1 month 2 month	2.58 2.01 1.68	2.99 1.83 1.19	0.000
Group 3	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline 1 month 2 month	2.48 2.37 1.98	2.37 2.17 1.46	0.000
Group 4	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline 1 month 2 month	2.45 2.35 2.25	2.34 2.10 1.56	0.000
Group 5	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline 1 month 2 month	2.39 2.08 1.65	2.88 1.72 1.40	0.000
Group 6	**Median**	**Mean rank**	**p value of Friedman Test**
Baseline 1 month 2 month	2.44 2.45 2.47	1.95 1.95 2.10	0.577

TABLE 6: PAIRWISE COMPARISON OF GINGIVAL BLEEDING INDEX SCORES AND PLAQUE INDEX SCORES AT DIFFERENT TIME INTERVALS WITHIN EACH GROUP

Gingival bleeding Index		
Groups	**Time pairs**	**p value of Wilcoxon Signed Ranks Test**

Group 1	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.000
Group 2	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.377
Group 3	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.000
Group 4	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.000
Group 5	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.002
Group 6	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.780 1.000 0.366
Plaque Index		
Groups	**Time pairs**	**p value of Wilcoxon Signed Ranks Test**
Group 1	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.000
Group 2	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.000
Group 3	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.292 0.000 0.000
Group 4	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.010 0.000 0.406
Group 5	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.000 0.000 0.000
Group 6	Baseline vs. 1 month Baseline vs. 2 month 1 month vs. 2 month	0.058 0.341 0.902

DISCUSSION

Gingival bleeding index and periodontal index were considered in our study to evaluate effectiveness of 5 dentifrices containing the herbal extracts. In terms of reducing gingival bleeding and plaque index score, Aloe-Vera proved to be the most effective among the 6 groups. Studies have shown it to be as effective in maintaining periodontal health as chlorhexidine when used as mouthwash [15]. A recent systematic review compared Aloe Vera and chlorhexidine for controlling plaque and reducing gingivitis. The evidence supports the use of Aloe Vera as an alternative to chlorhexidine in reducing gingival inflammation and 4 of the 6 studies included in the review suggested the Aloe Vera was as effective as chlorhexidine in reducing plaque however two studies were in support of chlorhexidine. Moreover the use of chlorhexidine was accompanied by side effects such as altered taste sensation and staining which was not observed when Aloe Vera was used [16]. When compared with commercially available toothpaste, like in our study, Aloe-Vera was equally effective in improving both gingival and periodontal health as well as microbial counts in patients [17]. The antimicrobial properties have been attributed to lignin, vitamins, minerals, salicylic acids, amino acids, and saponins. Aloe Vera also contains a latex compound that has been found to be bacteriostatic in nature. [18]

Tulsi was the third most effective in reducing gingival bleeding index and fourth most effective against plaque. A previous study has demonstrated significant reduction in microbial counts in children, on merely chewing of Tulsi leaves [19]. An in vitro study comparing the antimicrobial action of Tulsi with chlorhexidine has shown that at low concentrations, *S. mutans* were resistant to the extract however at 4% the extract showed considerable antimicrobial efficacy against the microorganism. However it was observed that chlorhexidine was effective at a concentration of 0.2% showing large zones of inhibitions. [20]. A randomized controlled trial has proven the use of mouthwash with Tulsi extracts to be effective in reducing plaque accumulation, gingival inflammation and bleeding and showing no side effects as compared to chlorhexidine. [21]. When used in the form of a dentifrice also it showed significant improvement in plaque index and gingival index scores when compared to a placebo and fluoridated toothpaste [22]. Antimicrobial properties of Tulsi can be attributed to the presence of eugenol (1-hydroxy-2-methoxy-4-allylbenzene), ursolic acid and carvacrol[18]

In our study Triphala was the second most effective in reducing gingival bleeding and third most effective against plaque. However in contrast to the results obtained in our study, another randomized controlled trial has concluded that Triphala was superior to Aloe Vera when used as a mouthwash in reducing gingivitis [23]. A systematic review of 7 randomized trials has concluded that Triphala reduces gingival inflammatory parameters in gingivitis induced by plaque and is as effective as chlorhexidine with reduced side effects on periodontal tissue [24]. Few studies concluded that Triphala was as effective as chlorhexidine in reducing bacterial counts and gingival and periodontal indices [25–27]. Triphala contains active ingredients like tannins, which restrict adhesion; quinines which provide free radicals and cause inactivation of microbial proteins and flavones, flavonoids, and flavonols, which disrupt microbial membrane, cell walls.

Pomegranate was the least effective among the 5 herbal extracts in reducing gingival bleeding plaque score. Studies have shown pomegranate extract to be effective in reducing plaque and control of plaque induced gingivitis [28–30]. A study in diabetics, evaluating efficacy of pomegranate extracts in form of mouthwash, reported better results in reducing gingival inflammation at 2 weeks compared to chlorhexidine and comparable results in improving plaque and gingival indices at 4 weeks [31]. Although there were not many studies evaluating its efficacy in the form of a dentifrice reported in literature, one unani study highlights promising results in a dentifrice predominantly containing pomegranate extracts

[32]. The pomegranate peel and juice is concentrated with active ingredients such as flavonoids which comprise of anthocyanins, catechins and other complex flavonoids and hydrolyzable tannins such as punicalin, pedunculagin, punicalagin, gallic and ellagic acid. These account for 92% of the antioxidant activity of the fruit. Phenolics present in the peel of pomegranate cause precipitation of bacterial cell membrane proteins resulting in microbial lysis and adding to its antimicrobial property [33].

In our study acacia catechu was the fourth most effective in reducing the gingival bleeding scores and second most effective in reducing plaque accumulation at 2 month follow up. A recent study has shown that acacia catechu is as effective as chlorhexidine with anti-inflammatory and antibacterial effects when used as a herbal mouth rinse [34]. Acacia catechu contains the active ingredient catechu which inhibits Cyclooxygenase and 5-Lipoxygenase and in turn reduces inflammation [35].

Most of the studies which evaluated gingival index as an outcome measure to assess presence of gingivitis have used gingival index given by Loe and Silness [36]. However, we have used the gingival bleed index given by Carter and Barnes 1974, which is a dichotomous index. It is simple and gives similar results as that with Loe and Silness Gingival index; furthermore it is more sensitive in its comparison [37].

Within the limitations of this trial it can be concluded that all the 5 herbal extracts showed significant reduction in both gingival bleeding index and plaque indices with a 2 month follow up, compared to the commercially available toothpaste. Aloe Vera showed the highest efficacy and pomegranate showed the least in comparison to other herbal extracts. However all the extracts were more effective compared to commercially available toothpaste, and could be considered as a more biocompatible alternative. However, further studies are required to standardize the concentration and methods to commercially incorporate these extracts.

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