Comparative Efficacy of Four Fruit Extract Mouthrinses on Gingivitis in Orthodontic Patients: A Randomised Clinical Trial

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Aim: To evaluate the efficacy and antimicrobial properties of four herbal mouthrinses on orthodontic patients with gingivitis

Methods and Materials: A total of 60 individuals with fixed orthodontic therapy with established gingivitis were enrolled in this study. Well Diffusion method was used to formulate mouthrinses of freshly prepared fruit extracts from Punica granatum (Pomegranate), Vaccinium macrocarpon (cranberry), Morinda Citrifolia L. (Noni) and Psidium guajava L. (Guava) and distilled water as control. The Plaque and Gingival Index scores were taken into consideration for assessing the effectiveness of mouthrinses against gingivitis.

Results: ANOVA test showed significant difference between the groups for PI and GI score.

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The Post Hoc analysis shows the plaque and gingival index scores were significantly reduced in Group A, Group B, Group C, and Group D than Group E whereas no significant difference exist in the mean PI and GI scores of Group A, B, C, and D (p>0.005).

**Conclusion:** All the tested mouthrinses from fruit extract were equally effective in reducing gingivitis in individuals with fixed orthodontic therapy suggesting that phytotherapeutic agents may be used in the future to inhibit oral microbial growth.

**Keywords:** phytotherapeutic agent; dental plaque; gingivitis; orthodontic appliances.

1. **INTRODUCTION**

Fixed orthodontic therapy increases plaque retention areas and disrupts the oral hygiene maintenance and hence is a risk factor for plaque accumulation [1–3]. Dental plaque is a highly complex bacterial structure that shifts rapidly to gram-negative anaerobic or facultative species that can cause gingival inflammation [4]. Maintenance of an acceptable oral hygiene is of utmost importance in fixed orthodontic [5–6]. Mechanical removal of dental biofilm is still considered the main method [7].

Nevertheless, orthodontic appliances protect the dental plaque from the cleansing effects of brushing and mastication [8], allowing the undisturbed plaque to induce gingivitis, gingival hyperplasia, and periodontitis which itself might interfere with orthodontic outcome by detachment of connective tissue and inhibition of remodelling [6–8]. Hence, a recent literature review suggested that improved antimicrobial solutions are urgently needed to prevent biofilm-related complications of orthodontic treatment [9].

In the midst of growing evidence of the connection between oral health and whole body health, phytotherapeutic agents with their ‘naturally occurring’ active ingredients offers a gentle and enduring way for restoration of health [10]. Various Phytotherapeutic agents have been used alone or in combination and have been scientifically proven to be safe against various oral health problems like bleeding gums, halitosis, mouth ulcers and decay [11].

Fruits like Pomegranate (*Punica granatum*) [12,13], Cranberry (*Vaccinium macrocarpon*) [14,15], Noni (*Morinda citrifolia* L.) [16,17], and Guava (*Psidium guajava* L.) [18,19] has shown antimicrobial properties in oral conditions.

Thus in view of this, the present study was carried out to compare the efficacy and antimicrobial properties of the aforementioned fruits in orthodontic patients with gingivitis.

2. **MATERIALS AND METHODS**

The study was designed and conducted in the Department of Orthodontics, DY Patil Vidyapeeth, India from November 2018 to March 2019. Approval from the Institutional Ethics Committee was obtained before initiating the study.

2.1 **Subject Selection**

This study was conducted on 60 fixed orthodontic patients with established gingivitis.

2.2 **Eligibility Criteria**

The inclusion criteria comprised the subjects' willingness to participate, the indication for bimaxillary fixed non-extraction orthodontic treatment, a lack of numerous or severe dental caries and/or restorations, an absence of severe dental plaque/ severe gingival inflammation/ severe periodontal condition, and the absence of any systemic diseases. The exclusion criteria were taking any medications or mouthrinses, presence of any composite restorations near the gingival margins, and a history of previous orthodontic or periodontal treatments.

2.3 **Study Design**

2.3.1 **Gingival examination**

O'Leary plaque index (PI) using a disclosing agent and Loe and Silness gingival index (GI) was assessed using a William's probe only on the six index teeth. Gingival examination was carried out by single operator, once immediately before the treatment and after 1 week from the intervention.

60 patients were randomly assigned to 5 groups of mouth rinses, consisting of an herbal antiseptic mouth rinse (Pomegranate: Group A; Cranberry: Group B; Noni: Group C; Guava: Group D) and sterile water (Group E: Placebo Control). The investigator handed the mouth rinses to patients in uniform plastic containers placed in amber color bottles.
Patients were instructed orally by the primary investigator and in written to use the mouthrinse twice a day (preferably after each meal), as doses of 15 mL used for 30 seconds. Patients should avoid eating or drinking for about 30 minutes after applying the mouthwash. The same regime was used in the placebo group.

2.3.2 Preparation of herbal mouthwash

The extract of all the tested phytotherapeutic agents were prepared using the cold extraction or maceration procedure \[13\]. The powdered dried Noni fruit, Guava leaves, Craneberry and Pomegranate were placed into separate stoppered container with 70:30 hydro-ethanol (70% water and 30% alcohol) for a day, with frequent agitation, filtered and the marc was pressed to obtain a liquid extract. The final volume was adjusted to 100 mL by water base for better hemogenation.

Distilled water (D.W.) was used as control in the study. The various preparation used in the study is shown in Fig. 1.

2.4 Statistical Analysis

The obtained data were compiled systematically. Data collected were coded, computerized and analyzed using Statistical package for Social Sciences (SPSS version 17.0). One-way ANOVA and post hoc Tukey’s HSD test was used to compare the means of plaque and gingival index values between the five mouthwashes.

3. RESULTS

A total of 60 individuals participated in the study out of which 25 were males and 35 were females with the mean age of the participants was 21.59±1.4 years.

The mean plaque index scores of the mouthwashes at baseline were 1.4±0.30, 1.25±0.20, 1.30±0.4, 1.35±0.28 and 1.45±0.35 respectively in Group A, B, C, D and E and no significant difference exit between the mean baseline plaque index scores amongst tested group \(p=0.80\) whereas after 1 week of mouthrinsing it was 1.05±0.25, 0.95±0.10, 1.0±0.25, 1.10±0.20 and 1.40±0.30 respectively and there exist a significant difference between the groups \(p<0.05\). Post Hoc analysis shows the plaque index scores were significantly reduced in Group A, Group B, Group C and Group D than Group E whereas no significant difference exist in the mean plaque index scores of Group A, B, C and D (Table 1).

![Fig. 1. Laboratory preparation of test solution](image-url)
Table 1. Comparison among the Five mouthwashes on plaque index using one-way ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>P -Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prerinsing PI (Baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Between groups A, B, C, D &amp; E</td>
<td>2</td>
<td>0.069</td>
<td>0.033</td>
<td>0.061</td>
<td>0.80(NS)</td>
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<tr>
<td>Within Groups</td>
<td>65</td>
<td>4.25</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>4.30</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Postrinsing PI</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups A, B, C, D &amp; E</td>
<td>2</td>
<td>8.52</td>
<td>4.30</td>
<td>30.64</td>
<td>0.04(S)</td>
</tr>
<tr>
<td>Within Groups</td>
<td>65</td>
<td>8.78</td>
<td>0.15</td>
<td></td>
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<tr>
<td>Total</td>
<td>68</td>
<td>17.25</td>
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<td></td>
</tr>
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</table>

Showing microorganisms detected in Group A and Group B

Table 2. Comparison among the Five mouthwashes on Gingival index using one-way ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
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<th>P -Value</th>
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</thead>
<tbody>
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<td>Prerinsing GI (Baseline)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Between groups A, B, C, D &amp; E</td>
<td>2</td>
<td>0.008</td>
<td>0.009</td>
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</tr>
<tr>
<td>Within Groups</td>
<td>65</td>
<td>2.55</td>
<td>0.063</td>
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</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>2.65</td>
<td></td>
<td></td>
<td>0.90(NS)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Postrinsing GI</th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups A, B, C, D &amp; E</td>
<td>2</td>
<td>2.57</td>
<td>1.29</td>
<td>30.64</td>
<td>0.02(S)</td>
</tr>
<tr>
<td>Within Groups</td>
<td>65</td>
<td>1.92</td>
<td>0.04</td>
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<tr>
<td>Total</td>
<td>68</td>
<td>4.53</td>
<td></td>
<td></td>
<td>43.74</td>
</tr>
</tbody>
</table>

Showing mean values of zone of inhibition in Group A and Group B

The mean gingival index score of the mouthwashes at baseline were 1.50±0.40, 1.40±0.30, 1.45±0.35, 1.55±0.25 and 1.35±0.15 respectively in Group A, B, C, D and E and after 1 week of mouthwashing it was 1.15±0.30, 1.05±0.15, 1.20±0.35, 1.25±0.40 and 1.30±0.25 respectively and there exist a significant difference between the groups (p<0.05). Post Hoc analysis shows the plaque index scores were significantly reduced in Group A ,Group B , Group C and Group D than Group E whereas no significant difference exist in the mean plaque index scores of Group A,B,C and D (Table 2).

4. DISCUSSION

The present study was carried out to compare the efficacy of four different fruit mouthrinses viz. Noni Fruit, Cranberry, Pomegranate and Guava on gingivitis in patients on fixed orthodontic therapy. To best of our knowledge no study has compared the efficacy of aforementioned fruit extracts together, thereby the present study was carried out.

The effectiveness of Noni fruit mouthrinse could be attributed to the presence of scopoletin, acubin, and alizarin in its fruit . The antibacterial activity of phenolic compounds like scopoletin is known to be better against Gram-positive bacteria than Gram-negative bacteria owing to a difference in their cell wall structure.

The antibacterial and anti-adhesion features of cranberry against oral bacteria has been demonstrated by the presence of certain components like phenolic acids, proanthocyanidins (particularly, A-type proanthocyanidins), anthocyanins, organic acids, and their microbial-derived metabolites which may limit dental caries by inhibiting the production of organic acids by cariogenic

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bacteria, the formation of biofilms by Streptococcus mutans and Streptococcus sobrinus, and the adhesion and coaggregation of a considerable number of other oral species of Streptococcus. Focusing on periodontal diseases, the non-dialyzable constituent fraction of cranberry (NDM) inhibits the formation of P. gingivalis and Fusobacterium nucleatum biofilms, two bacteria species associated with periodontitis. The NDM fraction may also inhibit the adhesion of P. gingivalis to various proteins, including type I collagen and may reduce bacterial coaggregation involving periodontal pathogens [14,15].

Pharmacological properties of pomegranate have a long history, but, in the recent decades, the interest in evaluating therapeutic effects of pomegranate has increased noticeably. Studies show that pomegranate juice has potent antioxidant activity (capability to scavenge free radicals) due to its high polyphenols content, including ellagittannins (hydrolysable tannins) and anthocyanins (condensed tannins). There is a range of phytochemical compounds in pomegranate that have showed antimicrobial activity, but most of the researchers have found that ellagic acid and larger hydrolyzable tannins, such as punicalagin, have the most important activities [12].

Recent study indicates that both pomegranate aril and peel extracts have an effective antimicrobial activity, as evidenced by the inhibitory effect on the bacterial growth of two important human pathogens, including Staphylococcus aureus and Escherichia coli, often involved in foodborne illness. In addition, experimental data strongly support the antibacterial activity of pomegranate extracts against oral pathogen such as S. mutans. However, little is known about the effect of pomegranate extracts on other pathogens involved in tooth decay such as R. dentocariosa, the first bacterium isolated from carious dentin [13].

Compounds of known antimicrobial activity in Guava includes 1,2-Benzenedicarboxylic acid, dibut, Alpha-bisabolol, 1,2-Benzenedicarboxylic acid, buty, hexadeca-2,6,10,14-tetraen, caryophyllene, germacrene, quercetin, quercitin-3-O-α-L-arabinofuranoside, quercetin-3-O-β-D-arabinopyranoside, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin and quercitin-3-O-arabinoside, 11-hydroxy-35-tricont-pentatriacontanoate, hexaeicosan-16-ol, tricosan-17-ene-5-ol, nonacosen-23-ene-3-ol, lupeol and betulnic acid.A recent study used aqueous extracts were tested against cariogenic and dental plaque causative microorganisms (Streptococcus sanguinis, Streptococcus mitis and Actinomyces sp.) and the MIC was determined, varying between 2.61 and 4.69 mg/mL [18,19].

The various indications where our aforementioned phytherapeutic agents can be substituted for commercially available anti plaque agents with equal efficacy are: Patient’s less compliance to chlorhexidine; Healthy, gingivitis and mild periodontitis patient; After periodontal surgery as chemical plaque control agents interferes with fibroblast activity; Patient who are allergic to ingredients of chemical control agents; Patient who have got anterior composite restoration so staining could be prevented.

The strength of the present study is that it corresponds to the actual behavior of prepared extracts in invivo because as they are exposed to the same conditions found in the oral cavity.

The limitations of the study is that it hasn’t assessed the antimicrobial activity of the tested mouthrinses. Substantivity exists or not could not be ascertained in this study. Comparison with the gold standard mouthwash has not been made in our study which could be the future scope of our study. Further researches are needed which focus on various concentration of these fruit extracts.

5. CONCLUSION

In the present study, all the tested phytherapeutic agent showed effective reduction in plaque and gingivitis score in the individuals with fixed orthodontic therapy. Hence could be used as an adjunct to non surgical periodontal therapy. Furthermore, laboratorial studies are needed to support the performance of further clinical investigations with much larger sample size and at various concentrations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of
knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT
As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL
As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES


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