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A Comparative Study on the efficacy of plaque removal of three natural toothbrushes - an *in-vitro* study

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Abstract

Objective: The present study attempts to assess the effectiveness of natural fibres including Luffa acutangula (Ridge gourd), Cocos nucifera (Coconut), and Bambusa vulgaris (Bamboo), when they are modified to suit the requirements of a toothbrush, and analyse if they are able to elicit any cleansing property against biofilm formed on the surface of teeth.

Methods: The regular nylon toothbrushes were customized to incorporate bristles made of Bamboo fibres, Ridge Gourd fibres and Coconut fibres. These were assessed on the toothbrush simulator, for their plaque removal ability on the surface of biofilm coated teeth samples. They were also tested for their wear rates post brushing. The Turesky modification of Quigley and Hein index was used to assess the plaque indices of the teeth pre- and post-brushing. The wear indices were measured based on a formula given by Rawles et al. The results were then statistically analysed on the SPSS software v26 (IBM.inc., USA).

Result: It can be inferred from the study that in terms of efficacy of plaque removal, the nylon brushes were most efficacious. However, among the natural fibre brushes, Bamboo and Coconut were equally efficacious while Ridge Gourd was the least efficacious. In terms of resistance to wear, Bamboo bristles were even more effective than the commercial nylon brushes. The Coconut and Ridge Gourd fibre brushes were progressively less resistant to wear.

Conclusion: Natural toothbrushes do show a comparable antibiofilm effect, with differing rates of wear based on the nature of the bristles. However, additional studies would be required in order to implement these results and assess their feasibility in the clinical setting, in-vivo.

Keywords: Toothbrushes, Coconut, Ridge Gourd, Bamboo, Plaque, Biofilm, Innovative technique, Eco-friendly study

Introduction

Toothbrushes are an essential self cleansing tool that enables biofilm removal, which in turn is crucial for the maintenance of oral hygiene [1]. Their mechanical abrasive nature coupled with the cleansing properties of dentifrices used along with them, are the most sought out resort to daily oral hygiene maintenance [2]. However, achieving and maintaining oral hygiene among their patients, is a constant trial for dentists [3]. Innumerable periodontal diseases originate due to the long term breaches in oral health maintenance. Gingivitis, halitosis, periodontitis and gum abscesses [4] are just a few such periodontal inflictions observed commonly among patients by dentists. In patients requiring prosthodontic treatment, post treatment, there is a greater chance of lapses in routine oral health maintenance [5]. The root cause for these lies in allowing bacteria to thrive in the oral cavity, in such a way that the oral ecosystem is disturbed [6]. Thus, devising measures of maintaining oral hygiene are always welcomed by dental professionals, in view of promoting oral health care.

A disturbed oral ecosystem ultimately leads to the formation of biofilm, and hence a cascade of oral health issues. The surface of teeth undergo demineralisation as a result of biofilm formation over it [7]. This is facilitated by the cumulative effect of sugary foods, bacteria and the tooth surface [8]. Added to this, prolonged periods of neglecting the necessary cleansing of the tooth surface allows biofilm formation to occur [9]. These finally result in dental caries. A host of periodontal problems are also resulted in, as discussed earlier. Hence, periodic cleansing of the teeth with a suitable instrument is necessary for optimum oral hygiene maintenance.

At present, advised means of establishing oral health care can be classified into mechanical and chemical models. Mechanical methods include the use of toothbrushes, interdental brushes, floss etc. while chemical methods involve the use of mouthwashes, dentifrices, mouth gels, etc. [10] Among these, the toothbrush is an easily accessible, easy to use and effective tool in removing biofilm formed over the tooth surface, as established in several previous studies [11,12]. Considerations regarding the brushing motion, the type of toothpaste used, and the nature of the bristles, influence the level of cleansability elicited by the brush used [13]. Similarly, optimum cleansing using brushes is observed in only specific surfaces of the teeth, including the buccal, lingual or palatal and occlusal surfaces [14]. The proximal surfaces require other cleansing aids like interdental brushes, for cleaning [15]. Besides, the inclination of the patient to take concerted efforts in maintaining oral hygiene plays a considerable role in maximising the efficacy of the technique of brushing for oral hygiene maintenance [16]. Hence, among the overall population, there is no single accepted method, superior to others, considering oral hygiene maintenance. It is totally dependent on the user's comfort and preference, so as to obtain maximum results in establishing good oral hygiene.

Several herbal formulations are already present in view of the nature of the dentifrice used for brushing. Neem, meswak, clove and lemon are just a few of the innumerable herbal sources of extracts which have a medicinal value for maintaining oral hygiene [17]. On the same note, there are developments regarding modifications in the type of the toothbrushes used for cleansing, that may enable us to incorporate a natural anti-biofilm effect, apart from the mechanical cleansing effect that it naturally would exhibit [18]. Ancient Indian practices involve the use of Ridge Gourd fibres [19], Coconut fibres [20] and Bamboo fibres [21]

among other mechanical cleansing aids. The present study attempts to assess the effectiveness of these materials when they are modified to suit the requirements of a toothbrush, and analyse if they are able to elicit any cleansing property against biofilm formed on the surface of teeth. Our team has extensive knowledge and research experience that has been translated into high quality publications.[22–34,35–39 40 41]

Study population and methodology

The present study was a pilot study which assessed the ability of three different natural toothbrushes against the regular toothbrush, to remove biofilm formed on the surface of teeth. The chosen natural fibres included Ridge Gourd fibres, Coconut fibres and Bamboo fibres. Brushes made with Bamboo bristles which were commercially available, were procured for the study. The bristles were specified to be made of Bamboo, while the handle of the brush was wooden. On the other hand Coconut fibres and dried Ridge Gourd fibres were procured. These fibres were customised in accordance with the ADA specifications given for a normal toothbrush [42]. Thus, the bristles of a normal toothbrush were removed and replaced with the prepared natural fibres procured (Fig. 3).

Following this, natural teeth, mostly anteriors, were obtained and mounted on blocks of acrylic, for which self-cure acrylic i.e. methyl methacrylate powder and polymethyl methacrylate liquid was used (Fig.1). The mounted teeth with the acrylic blocks were sterilised after which they were immersed in a *S. mutans* broth, with the labial portion of the mounted teeth exposed to the surface of the broth (Fig. 2). They were incubated for 24 hours, at 37 degrees celsius. After the incubation period, a swab from the biofilm on the tooth surface was taken and separately streaked on agar plates for culture and verification of the type of organism formed over the tooth surface.

The teeth were then placed coated with erythrosine dye, which is a commonly used plaque disclosing agent, to check which surface of the tooth has biofilm growing over it (Fig. 4). As all the acrylic blocks were identified to contain biofilm on the tooth surface, they were mounted on the toothbrush simulator (Fig. 5). Brushing action was simulated on the toothbrush simulator to observe any changes to the bristles after brushing for cycles equivalent to a time span of 90 days or 3 months (the arbitrary timespan of use for a normal toothbrush). After brushing, the extent of biofilm was observed using erythrosine dye. The brushes were run for 3000 cycles of which 1000 were run along the X axis, 1000 along the Y axis, 500 in the clockwise direction and 500 in the anticlockwise direction. After the simulation cycles were completed, the acrylic mounts containing the tooth samples were retrieved and stained for assessing the biofilm on them post-brushing. Thus, the pre- and post- brushing plaque scores were calculated using Turesky's modification of the Quigely Hein plaque index [43]. Meanwhile, the wear indices of the brushes were also calculated using the index given by Rawles et al [44]. The results were tabulated and statistically analysed using SPSS v26 (IBM.inc., USA).

Results

In figure 7, the scoring of plaque accumulation on the biofilm cultured surface of the tooth samples are assessed before and after brushing. It is inferred from the graph that the mean plaque score before brushing for all the groups is 5, indicative of plaque accumulation in more than two thirds of the tooth surface. Post brushing, the mean plaque scores showed maximum reduction with nylon bristles giving a post brushing mean plaque score of 0, with almost no plaque. This was followed by Bamboo and Coconut fibres among the natural bristles which each gave post brushing mean plaque scores of 2, with a definitive plaque lining over the cervical portion of the tooth.

Figure 8 is indicative of the wearage of each of the 2 samples of brushes used under a specific material. Among the two Coconut fibre brushes, one gave a WI of 0.21 and the other gave 0.16. Among the two Ridge Gourd fibre brushes the first one gave a wear index of 1.08 and the second, a WI of 0.38. Of the two nylon brushes, one gave 0.25 and the other gave 0.04. The least wearing of bristles among the four types used, was seen in the Bamboo bristles of which one gave a WI of 0.13 and the other gave 0.04

Discussion

Plants and their products have been used extensively, even in the past, to maintain oral hygiene and improve dental health [45]. In some countries where brushing with toothbrushes is uncommon, brushing with chewing sticks has been followed [46].

Some studies have postulated that antimicrobial substances inherent in plants and plant extracts may provide protection against caries causing bacteria [47]. The various benefits of using natural toothbrushes are, they act as antibacterial agents, have anti-inflammatory qualities, abrasive qualities, and significant plaque inhibiting properties. Apart from these, increased salivation, aiding healthy teeth and bone development, removal of stains, countering halitosis, and plaque and caries prevention, are few other benefits of using them [48].

Besides, previous studies have stated that natural toothbrushes are comparable to synthetic ones in providing dental protection, and are more ecological in their life-cycle, being of a lower cost [49]. The disadvantages reported, however, include that they may be hard on the soft tissues in the mouth and may become easily contaminated, hence requiring frequent replacement [50].

In the present study, the novel use of natural fibres including *Luffa acutangula* (Ridge Gourd), *Cocos nucifera* (Coconut), and *Bambusa vulgaris* (Bamboo), have been incorporated to assess their ability to remove biofilm on tooth surfaces. Coconut fibres are commonly used in the Indian household as a tooth cleaning agent, on account of its antimicrobial properties. Previous researches have illustrated its antimicrobial property through extracts derived from the fibrous husk [51]. The ridge gourd, is another common vegetable with its fibrous peels used traditionally as a scrubber. Previous studies have recorded its fibres as well, to possess several essential and non-essential amino acids with high antioxidative properties. Besides, the presence of a varied number of flavonols and phenolic acids which are known to exhibit several therapeutic benefits, indicates their potential to be used for the alleviation of many disorders [52]. Bamboo brushes are yet another natural fibre brushes, currently popular for their biodegradable nature, preferred by those opting against plastics [53].

To assess the biofilm removal capability of the brushes, the plaque on the teeth sample surfaces were scored based on the Turesky modification of the Quigley and Hein plaque index. The index takes into account the entire buccal and lingual surface and grades the plaque over the surface on a scale of 0 to 5 (from 0=no plaque or debris to 5=plaque covering more than ⅔ of the tooth surface) [54]. Based on this, it was observed from the study results that, because of uniform growth of biofilm obtained from in vitro culture on the tooth surface, the pre-brushing mean plaque score on staining with erythrosine dye was 5. Post-brushing, for Ridge Gourd fibres, there was a decrease in the mean plaque score to 4. In the case of the Coconut fibres, the mean plaque score decreased to 2. For Bamboo brushes as well, the mean plaque score decreased to 2. However, for the nylon brushes, the plaque score post brushing was 0 indicating that there is only a minimal amount of plaque accumulation on the tooth surface. Hence, as far as the plaque removal efficacy is concerned, nylon brushes were the most effective, followed by Bamboo and Coconut fibres which are comparable in their efficacy. Ridge Gourd fibres are least efficacious in plaque removal. This has been illustrated in Fig 7.

In the present study, the wear indices of each toothbrush after brushing was calculated. This was done using the formula given by Rawles et al. The silhouette of the brushes from both the side and end views were measured. As viewed from the side, the free end and the fixed end of the bristles were measured separately, and the latter was subtracted from the former. The same was done on the end view. The

values of both views after subtraction were added and the total sum was divided by twice the length of the longest bristle of the brush.
After calculating the wear indices for each of the brushes, it was observed that the samples made of Ridge Gourd showed the highest wear with 1.06 and 0.38 as the wear indices for the two brushes respectively, in the group. The nylon brushes, next gave wear indices of 0.25 and 0.04 each. This was followed by Coconut fibres that gave wear indices 0.21 and 0.16. The Bamboo brushes gave the least wear indices of 0.13 and 0.04. These have been graphically represented in Fig 8.

From these results it can be inferred that in terms of efficacy of plaque removal, the nylon brushes were most efficacious. However, among the natural brushes, Bamboo and Coconut brushes were equally efficacious while Ridge Gourd was the least efficacious. In terms of resistance to wear, Bamboo bristles are even more effective than the commercial nylon brushes. The Coconut and Ridge gourd fibre brushes were progressively less resistant to wear.

Clinical Relevance

Scientific rationale for the study: The present study was conducted to identify natural materials for use as cost effective and biodegradable alternatives for toothbrushes considering their inherent antimicrobial and mechanical cleansing properties.

Principle findings: Among the natural brushes, Bamboo and Coconut brushes were equally efficacious in plaque removal and the Bamboo bristles were most wear resistant.

Practical Implications: The use of these brushes in-vivo will require further studies to process and soften these bristles considering the durability and inherent coarseness of the fibres, so that the medicinal value and the mechanical cleansing of these materials can be harnessed together.

Authorship

S.A.R. and S.M. conceived the ideas; data collection was done by S.A.R., J.S. and S.A. and S.G.A.S.; the data analysis and writing were done by S.A.R. and S.M.

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Conflict of Interest

The authors declare none

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Figures

Figure 1. Mounting of tooth samples on acrylic blocks

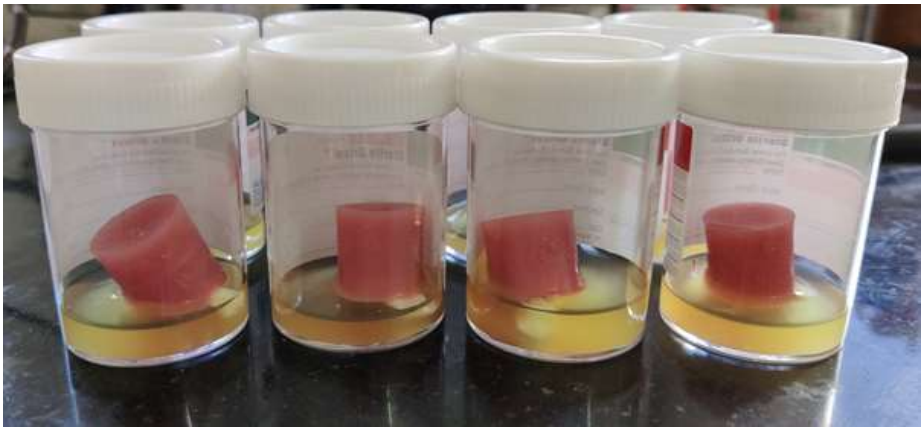

Figure 2. Tooth samples placed in S. mutans broth for allowing biofilm growth over the tooth surfaces

Figure 3. Different types of brushes used in order from left to right, Bamboo bristles, Ridge Gourd bristles, Coconut bristles and Nylon bristles

Figure 4. Biofilm over the tooth surface after immersion in S.mutans broth, pre-brushing, identified by staining with the plaque disclosing agent

Figure 5. Brushing of tooth samples on the Tooth Brush Simulator with different types of brushes

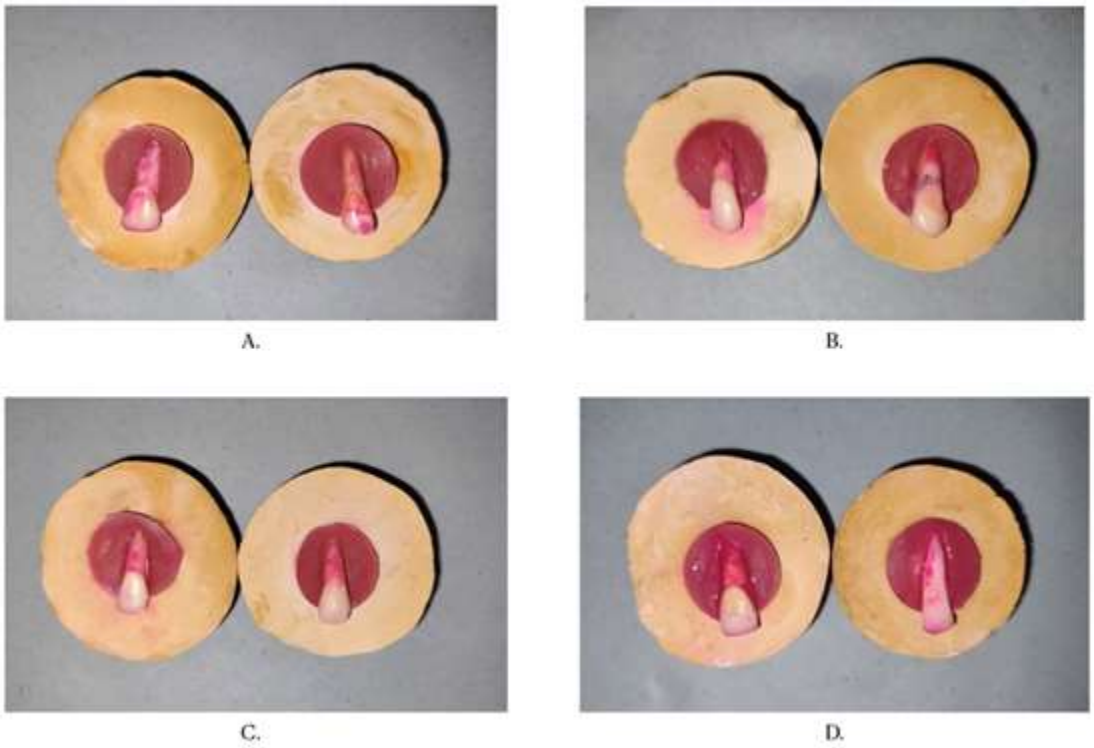

Figure 6. Biofilm remaining over the tooth surface post brushing with A. Ridge Gourd bristles, B. Bamboo bristles, C. Nylon bristles, D. Coconut bristles, identified by staining with the plaque disclosing agent,

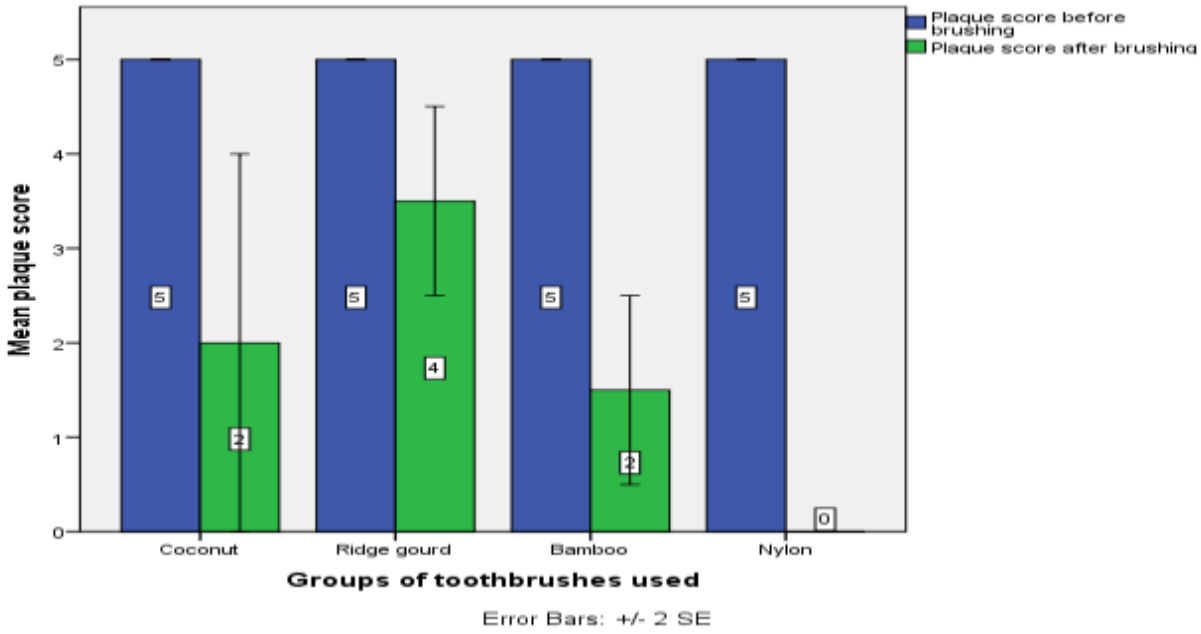

Figure 7. The Graph is representative of the mean plaque scores calculated pre and post brushing of the tooth surface on the toothbrush simulator. The X axis represents the different groups of toothbrushes

used in the study. The Y axis is representative of the mean plaque scores calculated for each group before (blue colour) and after brushing (green colour). It is inferred from the graph that the mean plaque score before brushing for all the groups is 5. Post brushing, the mean plaque scores showed maximum reduction with nylon bristles (post brushing mean plaque score is 0), followed by bamboo and coconut fibres among the natural bristles (post brushing mean plaque score is 2).

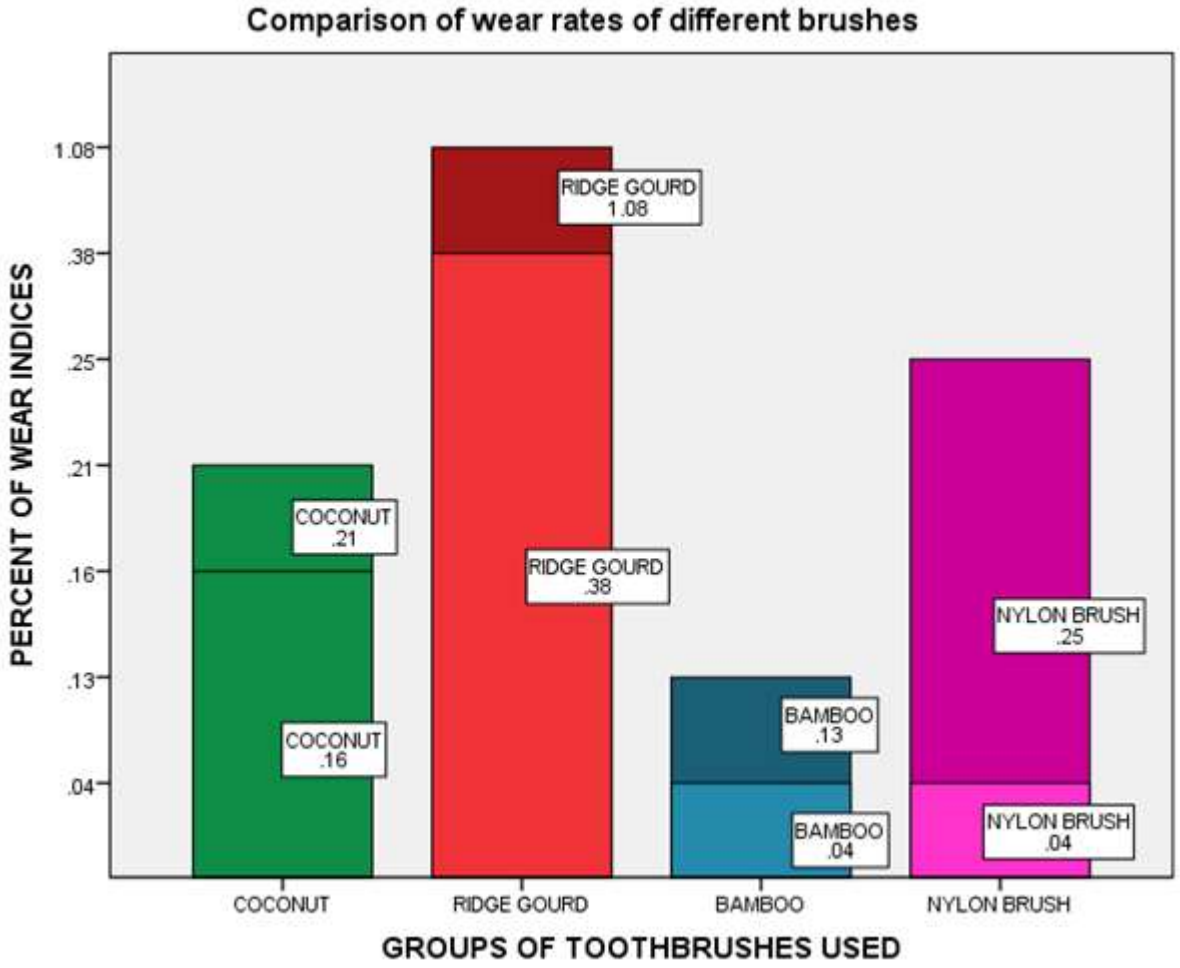

Figure 8. The graph represents the comparative wear of the individual groups of brushes after their use on the tooth brush simulator over the tooth surfaces. The wear index (WI) has been calculated based on the formula given by Rawles et al. The X axis represents the different groups of brushes used. The Y axis is indicative of the percent WI calculated post brushing. The coconut fibre brushes (represented by the green shades) which gave a WI of 0.21 and 0.16. The two ridge gourd fibre brushes (depicted by the red shades) gave a wear index of 1.08 and 0.38 respectively. The two Nylon brushes (depicted by the pink shades) gave wear indices of 0.25 and 0.04. The least wearing of bristles was seen in the bamboo bristles which gave wear indices 0.13 and 0.04 (represented by the blue shades).