Published online: 2019-09-25

Original Article

Comparative evaluation of antimicrobial efficacy of an alternative natural agent for disinfection of toothbrushes

R. Vignesh[1], C. Vishnu Rekha[1], Parisa Norouzi Baghkomeh[1], Sankar Annamalai[1], Ditto Sharmin[1]

Correspondence: Dr. R. Vignesh
Email: dhanam61@yahoo.co.in

[1]Department of Pediatric and Preventive Dentistry, Meenakshi Ammal Dental College and Hospital, Chennai, Tamil Nadu, India

ABSTRACT

Objective: The aim of this study was to assess the antimicrobial potency of aqueous extract of *Psidium guajava* leaves in two different concentrations as a toothbrush disinfectant against three oral bacterial species. **Materials and Methods:** Aqueous extracts of *P. guajava* leaves were prepared at 20% and 30% concentrations and 0.2% chlorhexidine was used as control. The toothbrushes were equally divided into 9 groups with 10 toothbrushes per disinfectant, which were contaminated with *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis*. Microbial culture was done after 5 min and 3 h of decontamination. **Results:** Group Ia and Ib showed that the presence of *E. faecalis* was observed in 8 (40%) of 20 toothbrushes. Group IIa and IIb showed a significant reduction in colony forming unit/toothbrush during 3 h evaluation. Group IIIa and IIIb showed nil growth during 3 h evaluation. Nil growth was observed with the control group for all three organisms. Statistically significant values were obtained for 5 min ($P < 0.001$) and 3 h ($P < 0.001$) disinfection period against *L. acidophilus* at two different concentrations. **Conclusion:** Aqueous extracts of guava leaves can be used as an alternative organic product for disinfection of toothbrushes.

Key words: Guava, toothbrush, *Streptococcus mutans*

INTRODUCTION

Oral diseases can be controlled through proper oral hygiene by reducing the microbial load.[1] Although toothbrushes are free from microorganisms after the manufacturing process, they become contaminated on regular usage.[2,3] Although rinsing with tap water is the common disinfecting protocol, it resulted in continued high levels of contamination.[4] *Psidium guajava* has high level of antibacterial activity[5] and preferred to use as a mouth rinse by Kraivaphan *et al*. and Esimone *et al*.[6,7] However, there is minimal information on its use as toothbrush disinfectant. This study was aimed to assess the antimicrobial potency of aqueous extract of *P. guajava* leaves in two different concentrations as a toothbrush disinfectant against three oral bacterial species.

Access this article online

Quick Response Code:

Website: www.eurjdent.com

MATERIALS AND METHODS

Study products

One hundred Colgate® junior toothbrushes with standardized dimensions, bristles, and color were obtained. They were taken from their original packages and were autoclaved before its use. The

How to cite this article: Vignesh R, Rekha CV, Baghkomeh PN, Annamalai S, Sharmin D. Comparative evaluation of antimicrobial efficacy of an alternative natural agent for disinfection of toothbrushes. Eur J Dent 2017;11:111-6.

DOI: 10.4103/ejd.ejd_196_16

brushes were then kept in nutrient broth covering the bristles and incubated for 24 h at 37°C to check for sterility. Ten toothbrushes with turbid broth suggesting contamination were discarded from the study.

Test organisms

Streptococcus mutans (Microbial Type Culture Collection [MTCC] 890), *Lactobacillus acidophilus* (MTCC 495), and *Enterococcus faecalis* (MTCC 439) were obtained from (MTCC) gene bank, Chandigarh, India. The revival of bacteria was done using nutrient broth kept in an incubator at 37°C for 24 h. Standardized suspensions of these strains were made at 0.5 McFarland scale and 30 toothbrushes per organism were immersed into the suspensions and incubated at 37°C for 10 min.

Preparation of extract

The plant specimen (leaves of *P. guajava*) was collected from botanical garden in Anna Nagar, Chennai. The leaves were cleaned and shade dried for 1 week at room temperature. Then, the leaves were weighed and stored in air tight bottles. The dried leaves were grounded to powder form and were sieved. Aqueous extracts were prepared in the Central Research Laboratory, Meenakshi Ammal Dental College and Hospital, Chennai, India. The procedure of aqueous decoction followed to prepare the extracts was based on a previous study by Pai *et al.*[8] Decoctions were prepared by boiling 10 g of the powder in 100 ml sterile distilled water over a low flame for 15 min. The flasks were then plugged and allowed to cool for 45 min. After cooling, the contents were filtered with Whatman's filter paper. Each extract was then diluted with sterile water to obtain two different concentrations of 20% and 30% and stored in refrigerator until use. 0.2% chlorhexidine gluconate was taken as control.

Disinfection and microbial analysis

Ten toothbrushes were used under each test organism for each disinfectant [Figure 1]. The toothbrushes were immersed into test tubes containing the disinfectant solutions covering up to the bristle head and kept in incubator for 5 min at 37°C. An aliquot was taken using a pipette and streaked using nichrome loop over a brain–heart infusion agar plate for culturing and placed in an incubator at 37°C for 24 h. The same procedure was repeated after 3 h disinfection period. After 24 h incubation, the agar plates were subjected to colony counting procedure using the stereo microscope under reflected light and the number of colony forming unit per toothbrush (cfu/toothbrush) was recorded by a blinded examiner.

Statistical analysis

Kruskal–Wallis test was done to compare the cfu/toothbrush using the disinfectants for all the test microorganisms. Bonferroni-corrected Mann–Whitney test was done for pairwise comparison and Wilcoxon signed-rank test was done to compare between time points. A $P < 0.05$ is considered as statistically significant.

RESULTS

In Group Ia and Ib, during the first (after 5 min) and second (after 3 h) evaluation, the presence of *E. faecalis* was observed in eight (40%) of 20 toothbrushes with number of colonies ranging from 10 to 100. In Group IIa and IIb, during the first evaluation, the presence of *L. acidophilus* was observed in all toothbrushes with number of colonies ranging from 50,000 to >100,000; however, in second evaluation, the number of cfu reduced significantly. In Group IIIa and IIIb, during the first evaluation, nil growth of *S. mutans* was observed in 16 (80%) of 20 toothbrushes and all 20 toothbrushes (100%) showed nil growth by the end of second evaluation. In Group Ic, IIc, and IIIc where 0.2% chlorhexidine was used, nil growth of all microorganisms was observed by the end of both first and second evaluation [Figure 2-4].

On comparing the concentration of the disinfectants in all the groups, statistically significant values were obtained for 5 min ($P < 0.001$) and 3 h ($P < 0.001$) disinfection period against *L. acidophilus*. Group IIb showed a significant reduction in microbial load than Group IIa. Group Ib and IIIb showed a considerable reduction of microbial load than Group Ia and IIIa, but they were not statistically significant at both time intervals ($P > 0.001$) [Table 1].

When compared between the disinfection period, there was statistically significant reduction of *L. acidophilus*

Table 1: Kruskal-Wallis test to compare between concentration levels

Variable	Concentration level	*n*	Mean rank	*P*
L. acidophilus (5 min)	20%	10	22.70	<0.001
	30%	10	18.30	
	Control	10	5.50	
L. acidophilus (3 h)	20%	10	23.40	<0.001
	30%	10	17.60	
	Control	10	5.50	

L. acidophilus: *Lactobacillus acidophilus*

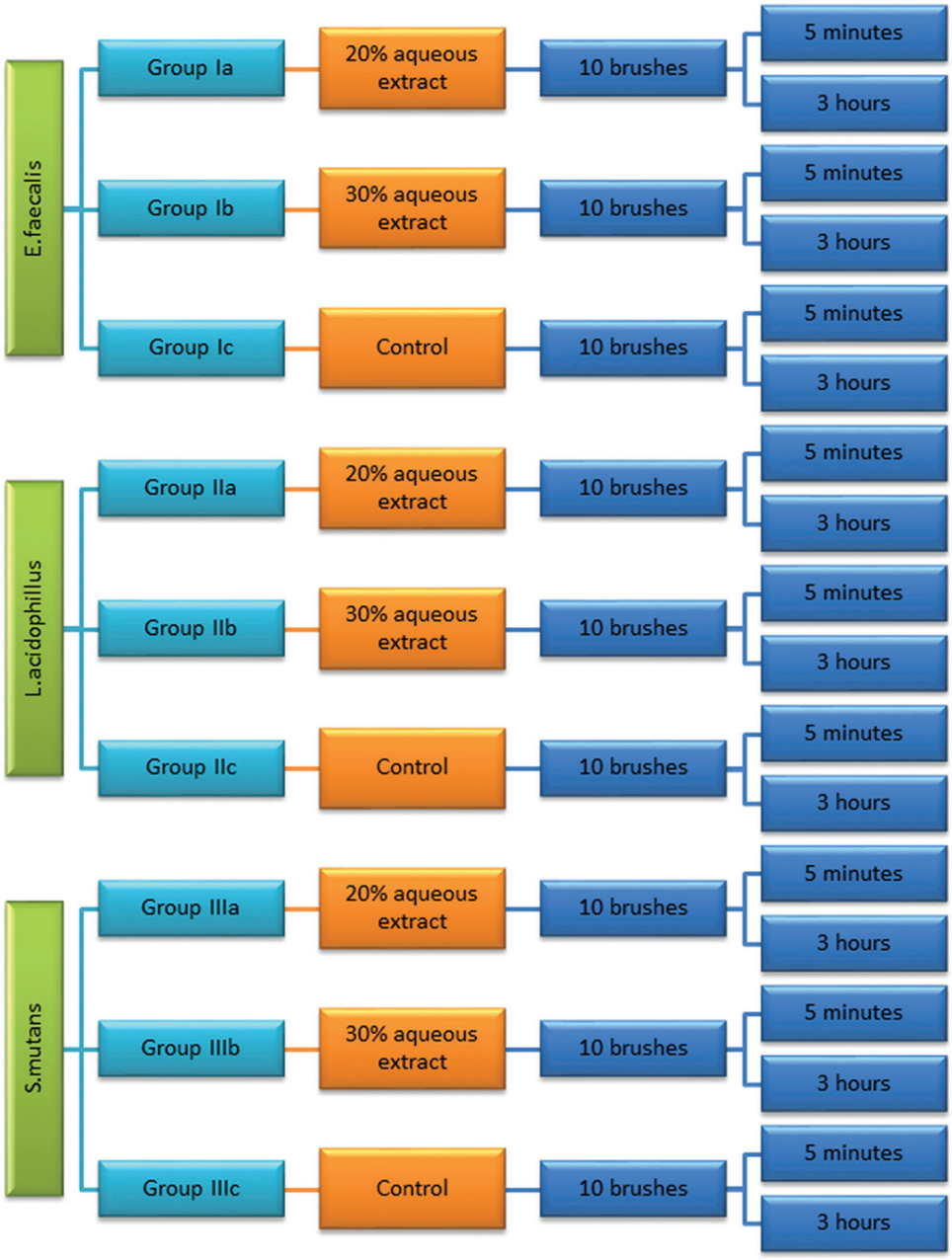

Figure 1: Flow chart explaining the distribution of groups

from 5 min to 3 h for both Group IIa (P = 0.027) and Group IIb (P = 0.011) while other groups did not show statistically significant values [Table 2].

On pairwise comparison, both Group IIa and Group IIb gave a statistically significant reduction of *L. acidophilus* similar to Group IIc (0.2% chlorhexidine) [Table 3].

DISCUSSION

In the present *in vitro* study, there was nil growth of the three test organisms in the group of toothbrushes disinfected for 5 min and 3 h in Group Ic, IIc, IIIc which were disinfected with 0.2% chlorhexidine gluconate. This result shows that the methodology is correct and it can be used as a positive control in *in vitro* tests to evaluate the efficacy of different disinfection protocols. We accept with Moshrefi that chlorhexidine solution is the "gold standard" antimicrobial, compared to other agents used for dental biofilm control.[9]

Although various studies evaluated the efficacy of antimicrobial activity of guava leaves against common oral pathogens,[10,11] the present study evaluated its antibacterial efficacy in toothbrushes. The necessity for taking aqueous extracts for the study is because it

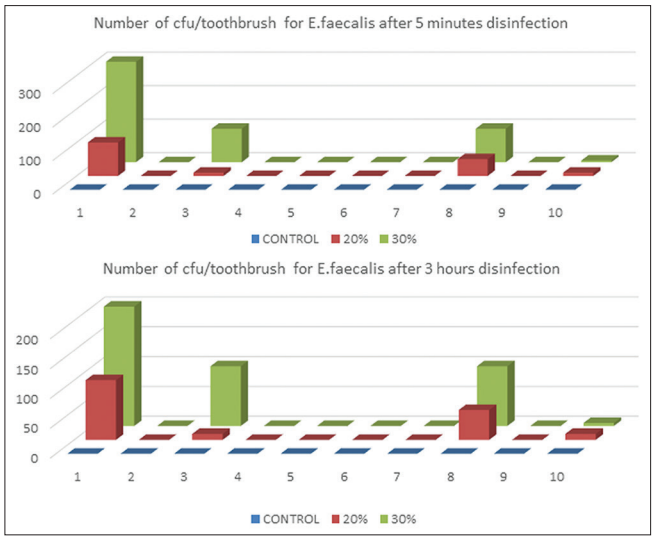

Figure 2: Difference in colony forming unit/toothbrush for *Enterococcus faecalis* at 20% and 30% concentration against control at 5 min and 3 h disinfection period

Table 2: Wilcoxon signed-ranks test to compare between time points

Concentration level	Variables	*P*
IIa	*L. acidophilus* (3 h and 5 min)	0.027
IIb	*L. acidophilus* (3 h and 5 min)	0.011

L. acidophilus: *Lactobacillus acidophilus*

Table 3: Bonferroni adjusted Mann-Whitney test for pairwise comparison

Pair	*P*	
	***L. acidophilus* (5 min)**	***L. acidophilus* (3 h)**
IIa versus IIb	0.755	0.370
IIa versus IIc	<0.001	<0.001
IIb versus IIc	0.003	0.004

L. acidophilus: *Lactobacillus acidophilus*

was aimed to reach the public community for the ease in preparation and as a way to recycle the dried-out leaves of guava.

The selection of microorganisms used in the present study was based on their involvement in development and progression of dental caries. *S. mutans* is the most common microorganism which has its role in the initiation of human dental caries[12] and *L. acidophilus* is responsible for progression of carious lesion rather than in initiation of the disease.[12,13] *E. faecalis* is the most common species that has been implicated in endodontic infections.[14] Unfortunately, many of these organisms remain on the toothbrush and can reinfect our teeth, that is, every subsequent brushing introduces new bacteria into the oral cavity.[1]

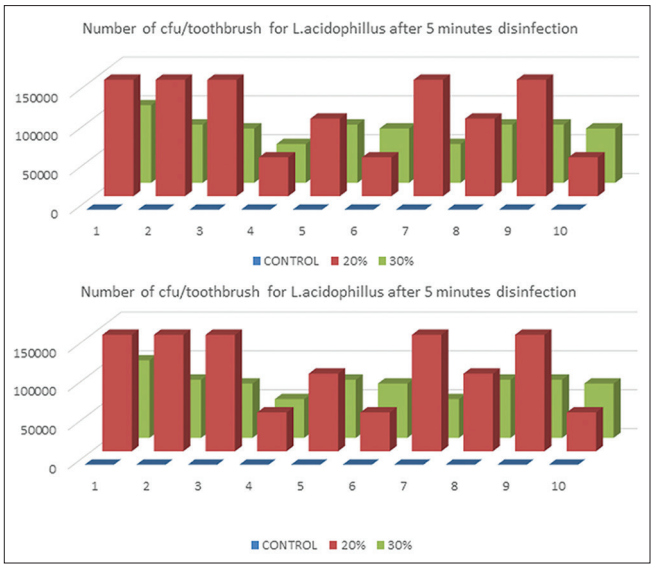

Figure 3: Difference in colony forming unit/toothbrush for *Lactobacillus acidophilus* at 20% and 30% concentration against control at 5 min and 3 h disinfection period

P. guajava (guava) is proven for its antimicrobial, antiparasitic, antitussive, hepatoprotective, antioxidant, antigenotoxic, antimutagenic, antiallergic, anticancer, and antihyperglycemic effects in medical field.[15] In the field of dentistry, its antiplaque, antimicrobial, and anti-inflammatory properties are proven by various authors.[16,17]

Our present study shows a strong antibacterial activity of aqueous extracts of guava leaves in two different concentrations. Mehta *et al.*[10] reported that as the concentration of the aqueous extract was increased, that is, from 5% to 20%, there was an increase in the zone of inhibition. This was the reason to keep the concentrations of disinfectants prepared in the study at 20% and 30%. Our present study showed an increase in antibacterial efficacy against *L. acidophilus* at both the disinfection periods when the concentration of the disinfectant was at 30% with a statistically significant result ($P < 0.05$) and the 20% and 30% concentration had a statistically significant reduction when compared with the control ($P < 0.01$ for 20% and $P < 0.05$ for 30%).

The disinfection period was kept at 5 min to evaluate the rapid antimicrobial activity of the extract and 3 h to evaluate any reduction in the antimicrobial activity as the disinfectant is a natural product and applicable for molecular deterioration. Our present study showed a statistically significant reduction of *L. acidophilus* from 5 min to 3 h at both 20% ($P < 0.027$ and 30% ($P < 0.011$) concentrations.

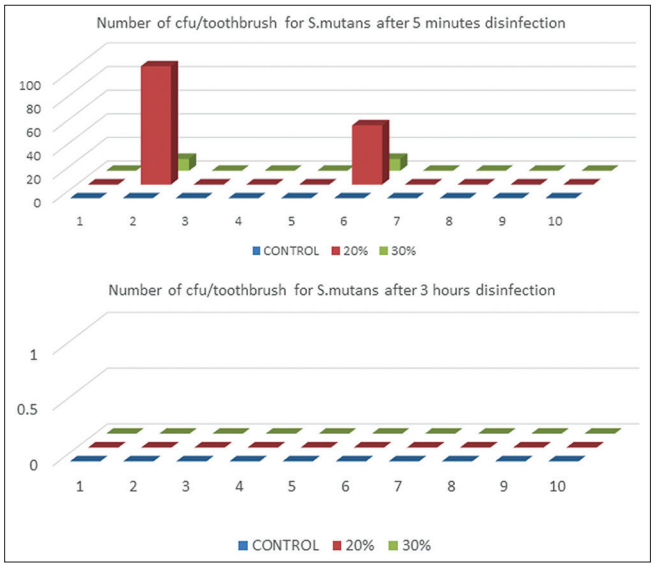

Figure 4: Difference in colony forming unit/toothbrush for *Streptococcus mutans* at 20% and 30% concentration against control at 5 min and 3 h disinfection period

The antibacterial activity of guava leaves is attributed to the presence of terpenes,[11] flavonoids,[5,11] and tannins.[18] Barbalho *et al*. in 2011 identified eight flavonoids from guava fresh leaves out of which Guaijaverin has high potential to inhibit the growth of the *S. mutans*[19] and the most highly abundant flavonoid present is quercetin.[20] The antibacterial action of quercetin is probably due to the disruption of membrane and inactivation of extracellular proteins by forming irreversible complexes and prevents adhesive glucan formation disrupting the initial adhesion[15,21] Guaijaverin binds to the cell surface proteins thereby decreasing the hydrophobicity.[15] These flavonoids also interact with microbial membrane proteins, enzymes, and lipids, thereby altering cell permeability and permitting the loss of protons, ions, and macromolecules.[21] Tannins can form hydrogen bonds with the protein contained in bacterial cells leading to a conformational change in the protein molecule causing denaturation of proteins.[18] Terpenoids are membrane-active in nature causing sublethal injury of bacterial cell membranes thereby altering their permeability and affect the ability to adequately osmoregulate or exclude toxic materials.[22]

CONCLUSION

From the results of the current study, it could be concluded that:

a. Aqueous extracts of guava leaves can be used as a natural disinfectant for disinfecting contaminated toothbrushes as effective as 0.2% chlorhexidine
b. Since the preparation of the aqueous extract is very simple, it could be used as an alternative organic product for disinfection of toothbrushes in households.

Acknowledgments

We would like to thank Mr. Arun from People's Diagnostic Laboratory for helping us in the microbiological analysis in the current study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Karibasappa GN, Nagesh L, Sujatha BK. Assessment of microbial contamination of toothbrush head: An *in vitro* study. Indian J Dent Res 2011;22:2-5.
2. Nelson-Filho P, da Silva LA, Ds Silva RA, da Silva LL, Ferreira PD, Ito IY. Efficacy of microwaves and chlorhexidine on the disinfection of pacifiers and toothbrushes: An *in vitro* study. Pediatr Dent 2011;33:10-3.
3. Nagaraj A, Acharya S, Jeph V, Biswas G. Toothbrush contamination a review of Indian literature. Int J Dent Clin 2014;6:20-1.
4. Frazelle MR, Munro CL. Toothbrush contamination: A review of the literature. Nurs Res Pract 2012;2012:420630.
5. Garode AM, Waghode SM. Antibacterial activity of *Psidium guajava* Linn (Guava) leaves extracts on bacterial pathogens. Int J Bioassays 2014;3:1794-6.
6. Kraivaphan V, Boonyamanound L, Amornchat C, Trirantana T, Kraivaphan P. The effect of a mouthrinse containing *Psidium guajava* Leaf extract on gingivitis. J Dent Assoc Thai 1991;41:323-8.
7. Esimone CO, Nworu CS, Ekong US, Iroha IR, Okolin CS. A case for the use of herbal extracts in oral hygiene: The efficacy of *Psidium guajava*-based mouthwash formulations. Res J Appl Sci 2007;2:1143-7.
8. MB Pai, GM Prashant, KS Murlikrishna, KM Shivakumar, GN Chandu. Antifungal efficacy of Punica granatum, Acacia nilotica, Cuminum cyminum and Foeniculum vulgare on Candida albicans: An in vitro study. Indian J Dent Res 2010;21:334-36.
9. Moshrefi A. Chlorhexidine. J West Soc Periodontol Periodontal Abstr 2002;50:5-9.
10. Mehta VV, Rajesh G, Rao A, Shenoy R, B.H.M. Pai. Antimicrobial efficacy of *Punica granatum* mesocarp, *Nelumbo nucifera* Leaf, *Psidium guajava* Leaf and *Coffea Canephora* extract on common oral pathogens: An *in-vitro* study. J Clin Diagn Res 2014;8:ZC65-8.
11. Braga T, Dores R, Ramos C, Evangelista F, Tinoco L, Varotti F, *et al*. Antioxidant, antibacterial and antitumor activity of ethanolic extract of the *Psidium guajava* Leaves. Am J Plant Sci 2014;5:3492-500.
12. Krishnakumar R, Singh S, Subba Reddy VV. Comparison of levels of mutans streptococci and lactobacilli in children with nursing bottle caries, rampant caries, healthy children with 3-5 dmft/DMFT and healthy caries free children. J Indian Soc Pedod Prev Dent 2002;20:1-5.
13. Ravindran S, Chaudhary M, Gawande M. Enumeration of Salivary Streptococci and Lactobacilli in children with differing caries experiences in a rural Indian population. ISRN Plast Surg 2013;2013:1-6.
14. Cogulu D, Uzel A, Oncag O, Aksoy SC, Eronat C. Detection of *Enterococcus faecalis* in necrotic teeth root canals by culture and polymerase chain reaction methods. Eur J Dent 2007;1:216-21.
15. Ravi K, Divyashree P. *Psidium guajava*: A review on its potential as an adjunct in treating periodontal disease. Pharmacogn Rev 2014;8:96-100.
16. Fathilah AR. *Piper betle* L. and *Psidium guajava* L. in oral health maintenance. J Med Plant Res 2011;5:156-63.
17. Prabu GR, Gnanamani A, Sadulla S. Guaijaverin – A plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. J Appl

Microbiol 2006;101:487-95.
18. Mailoa MN, Mahendradatta M, Laga A, Djide N. Antimicrobial activities of tannins extract from guava leaves (*Psidium guajava* L) on pathogens microbial. Int J Sci Technol Res 2014;3:236-41.
19. Barbalho SM, Farinazzi-Machado FM, de Alvares Goulart R, Brunnati AC, Otoboni AM, *et al*. *Psidium guajava* (Guava): A plant of multipurpose medicinal applications. Med Aromat Plants 2012;1:104.
20. Rattanachaikunsopon P, Phumkhachorn P. Contents and antibacterial activity of flavonoids extracted from leaves of *Psidium guajava*. J Med Plants Res 2010;4:393-6.
21. Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A. Plant polyphenols and their anti-cariogenic properties: A review. Molecules 2011;16:1486-507.
22. Jasmine R, Selvakumar BN, Daisy P. Investigating the mechanism of action of terpenoids and the effect of interfering substances on an Indian medicinal plant extract demonstrating antibacterial activity. Int J Pharm Stud Res 2011;2:19-24