ORIGINAL ARTICLE

THE FFECTS OF A DENTIFRICE CONTAINING PROPOLIS ON MUTANS STREPTOCOCCI: A CLINICO-MICROBIOLOGICAL STUDY

Mohsin S[1], Manohar B[1], Rajesh S[1], Asif Y[2]

ABSTRACT

BACKGROUND: Propolis is a natural resinous mixture produced by honeybees, which exhibits anti-microbial, anti-inflammatory, cytostatic and cariostatic properties. The aim of the study was to evaluate the anti-bacterial efficacy of a propolis based dentifrice on Mutans Streptococci colonizing the oral cavity of young patients using Dentocult® SM strip mutans test.

METHODS: Screening of 367 male subjects within the age group of 7-12 years was carried out. A total of 30 children were included in the study. They were instructed to use a Propolis dentifrice (Probee,™ Quasi-Medical Products, Seoul Propolis) daily for three minutes over a period of four weeks. Plaque and salivary samples were collected at baseline, 1st week, 3rd week and 4th week and were analyzed for Mutans Streptococci count using Dentocult® SM strip Mutans kit (Orion Diagnostica Oy, Finland). Student paired t-test and Friedman test were used for statistical analysis.

RESULTS: It was unveiled that mean Mutans streptococci count at 1st week and 4th week, showed significant reduction (p≤0.0001), compared to baseline scores. Using Friedman's test, statistically significant difference was found between baseline and 1st week, 3rd week and 4th week follow up (P < 0.001).

CONCLUSION: Propolis dentifrice reduces in-vivo microbial load in microenvironments especially against Mutans streptococci in the oral cavity of young patients. Thus, it's potential to be inculcated and used as an alternative measure to prevent dental caries can be considered and further investigation involving greater number of participants is recommended.

KEYWORDS: Propolis, dental plaque, dentifrice, antimicrobial, streptococci.

DOI: **http://dx.doi.org/10.4314/ejhs.v25i1.3**

INTRODUCTION

Dental caries is the most widespread disease affecting humans particularly during childhood. Few microorganisms found in the oral cavity are able to adhere to the teeth. The specific cariogenic microbiota consists of *Mutans Streptococci*, *Lactobacillus* and some *Actinomyces* species. However, during the initial phase of caries disease, *Mutans Streptococci* is the most frequently associated (1) and the most cariogenic microorganism among the oral streptococci (2). There is a positive correlation between the number of *Mutans Streptococci* in dental plaque and the occurrence of dental caries (3, 4). *Mutans Streptococci* can colonize the tooth surface and initiate plaque formation through the synthesis of extracellular polysaccharides, mainly water-insoluble glucan from sucrose by using glucosyltransferase (GTFs) (5, 6). GTFs aid in adhesive interactions with *Mutans Streptococci* and are essential in the expression of virulence by these microorganisms. The glucans synthesized by GTFs not only promote the accumulation of cariogenic streptococci on the tooth surface, but also contribute significantly to the bulk of dental plaque (7). The GTFs secreted by *S. mutans* bind

[1]Department of Pedodontics and Preventive Dentistry, Jaipur Dental College, Jaipur, Rajasthan, India
[2]Department of Public Health Dentistry, Jaipur Dental College, Jaipur, Rajasthan, India
Corresponding Author: Asif Yousuf, Email: asify11@gmail.com

avidly to the pellicle formed on the tooth surface and to bacterial surfaces and are enzymatically active, when they are exposed to sucrose, glucans are formed *in situ* within minutes (8, 9, 10, 11).

The quandary with the use Antibiotics as antimicrobial agents is because of the potential of resistance to them. Therefore, many studies have attempted to identify antimicrobial agents from natural extracts (12), and researchers are currently focusing on the natural substances which offer as alternatives for the control of caries in terms of antimicrobial response and lower associated risks. One such antimicrobial agent is Propolis or bee glue, which is a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from parts of plants, buds and exudates (12). Etymologically, the word *Propolis is* derived from the Greek *pro* (for 'in front of', 'at the entrance to') and *polis* (for 'community' or 'city'), meaning that this natural product contributes to hive defence (12). It has been widely used as an antimicrobial agent in traditional medicine worldwide (13). The precise composition of Propolis varies with the geographic origin ranging from amino acids, minerals, ethanol, Vitamins A, B complex, E and the highly active mixture of compounds known as bioflavonoids (13). Compounds found in Propolis affect the growth and glucosyltransferase activity of *Mutans Streptococci.* Of the various components of Propolis, tt-farnesol is the most effective antibacterial agent, while apigenin is a potent inhibitor of glucosyltransferase (14). It is known that Propolis exhibits several biological activities such as anti-microbial, anti-inflammatory, anesthetic, cytostatic and cariostatic properties. Its antibacterial effect (15, 16) on both isolated oral streptococci and salivary bacterial counts (17) have been demonstrated. Propolis has an effect on the cytoplasmic membrane and has an inhibitory effect on the bacterial motility and enzymatic activity. It has bacteriostatic activity at low concentrations and can be bactericidal at high concentrations (12). It breaks down bacterial cell wall, cytoplasm and prevents bacterial cell division.

Based on its effects of use in the field of dentistry, the objective of this study was designed to evaluate the anti-bacterial efficacy of a Propolis based dentifrice on Mutans streptococci colonizing the oral cavity of young children by using Dentocult® SM Strips test.

METHODS AND MATERIAL

Before the study was conducted, ethical approval was acquired from the Institutional Research Review Board of Jaipur Dental College. All procedures were performed according to the ethical principles established under the Declaration of Helsinki. After permission was obtained from three local Islamic Schools/Madrassas of Jaipur, Rajasthan, screening of 367 male children (within the age group of 7-12 years) was carried out and a total of 30 subjects fulfilling the inclusion criteria were selected. Informed consent was obtained from parents of all the children. Inclusion criteria for participation included subjects in their mixed dentition period, DMFT or dmft score more than 4, systemically healthy patients, absence of any fixed or removable orthodontic appliances or a prosthesis, no history of oral prophylaxis for at least three months prior to the study and the subjects who gave the informed consent. Unwilling children and subjects who gave a history of any anti-microbial oral hygiene procedure several hours before sample collection and subjects with a history of antibiotic therapy within three months were excluded.

After selection of the children, oral prophylaxis of all the selected subjects was done using an Ultrasonic scaler. Then, the subjects were instructed to abstain from any oral hygiene measures for the next 24 hours. On the next day, each child was provided with a 50gm pack of Propolis dentifrice (Probee, ™ Quasi-Medical Products, Seoul Propolis). The method of brushing and the quantity of dentifrice to be dispensed was demonstrated after distributing the dentifrices. Subjects were instructed to brush once daily in the morning for three minutes for a period of four weeks. During the entire study period, participants were advised to abstain from using any mouthwash or any other dentifrice. All the subjects were provided with a daily reminder card on which they were supposed to put a tick mark after using the dentifrice for that particular day to ensure regular usage of the dentifrice by the participants.

Plaque Sample collection: After 24 hrs of Oral prophylaxis, baseline samples were collected.

Culture vials were taken at room temperature one hour prior to the sample collection. Bacitracin discs were placed in the culture vials 15 minutes before the sample collection and the vials were shaken thoroughly. Plaque samples were collected with a sterile probe tip from four specific sites (18) including the buccal surface of right maxillary 1st molar, labial surface of maxillary incisor, lingual surface of mandibular Incisor and lingual surface of the left mandibular 1st molar. Collected plaque samples were evenly and thoroughly distributed on square tip plaque strip (Dentocult SM strip Mutans kit, Orion Diagnostica Oy, Finland) (19, 20) using sterile cotton buds.

Saliva Sample Collection: Before salivary samples were collected, the subjects were asked to chew Paraffin tablets for one minute and then spit the remaining saliva. Saliva samples were collected by pressing the round tip salivary strips against the dorsal surface of tongue and removed with gently closed lips. After collection of both saliva and plaque samples, both the strips were placed in the labeled culture vials, attached back-to-back to the cap and the vial was then recapped. It was then incubated at 37˚C for 48 hours. After the incubation, the colony counts of Mutans Streptococci were interpreted using Model chart (Figure 1) provided by the manufacturer, and scores were given from 0-3 after comparing the incubated strips with model chart.

Similarly, plaque and saliva samples were obtained. The same procedure was repeated on 1st week, 3rd week and 4th week follow up for analysis of Mutans Streptococci colony counts.

Statistical Procedures: Statistical analysis was done by applying Student paired-t test and Friedman test. The collected data was compiled systematically and was analyzed using MedCalc v12.2.1.0. For all tests, a p-value of 0.05 or less was used for statistical significance.

RESULTS

A total of 30 male subjects between 7 to 12 years were included in the study. They were instructed to use the dentifrice once daily over a period of four weeks and were analyzed for Mutans Streptococci count at baseline, 1st week, 3rd week and 4th weeks.

Table 1 displays the differences in the Streptococcus Mutans count following use of a dentifrice containing Propolis using Dentocult (SM) strips at weekly intervals. At the 1st week, the results showed statistically significant reduction in the mean value of Mutans Streptococci between baseline and 1st week (p= 0.000). After four weeks, the mean value, when compared to the baseline, showed a statistically significant reduction. (p= 0.000).

Table 1: Effects of a Dentifrice containing Propolis on Mutans Streptococci using Dentocult (SM) strips

Units	Day of Examination	N	Mean	Std. Deviation	'T'	p*
CFU/mL	Base Line	30	2.3333	.47946	6.595	**≤0.0001**
	1st Week	30	1.7333	.44978		
CFU/mL	Base Line	30	2.3333	.47946	NA†	-
	3rd Week	30	1.3333	.47946		
CFU/mL	Base Line	30	2.3333	.47946	19.039	**≤0.0001**
	4th Week	30	.6667	.47946		

**Student paired t test*

†*The correlation and t cannot be computed because the standard error of the difference is 0.*

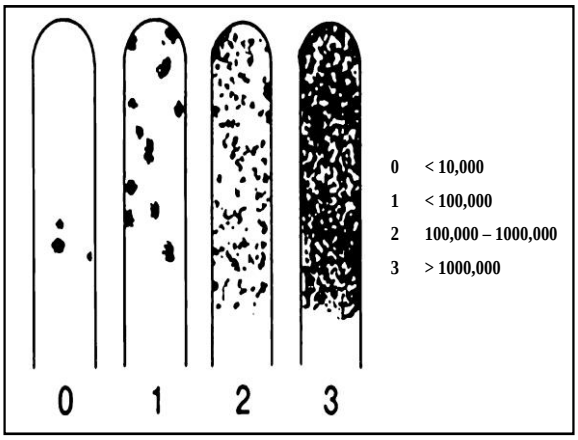


Figure 1: *Dentocult SM Strip Mutans Model Chart.*

The reduction of *Streptococcus mutans count* at weekly intervals from baseline till 4th week is shown in Figure 2. A steep decline in the Streptococcus Mutans Count was observed after baseline which continues till the 4th week follow up.

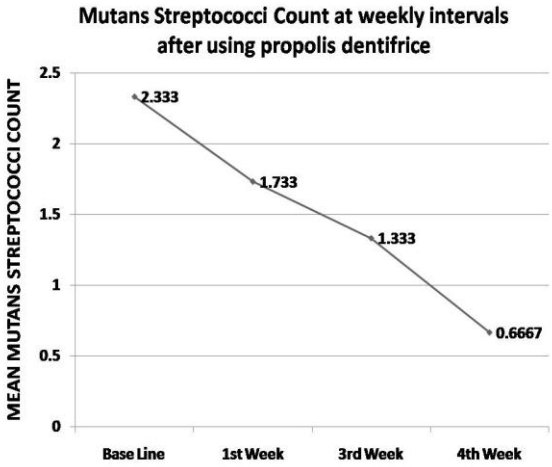


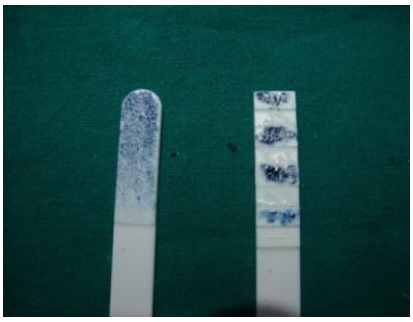
Figure 2: *Mutans Streptococci count at weekly intervals after using Propolis dentifrice*.

Applying Friedman test, it was observed that statistically significant reduction in the mean Mutans Streptococci count between baseline and 1st week, 3rd week and 4th week follow up (χ^2 sub R= 78.138; $p < 0.001$) as presented in Table 2 and Figure 3.

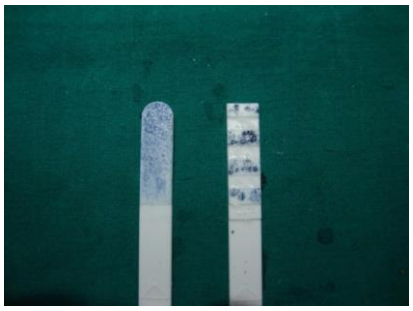
Table 2: Mutans Streptococci count during subsequent weekly intervals

Day of Examination	N	Mean	Standard Deviation	Median	Minimum	Maximum	Chi-Square Sub R	p*
Base Line	30	2.333	0.4795	2	2	3	78.138	<0.001
1st Week	30	1.733	0.4498	2	1	2		
3rd Week	30	1.333	0.4795	1	1	2		
4th Week	30	0.6667	0.4795	1	0	1		

**Friedman Test*

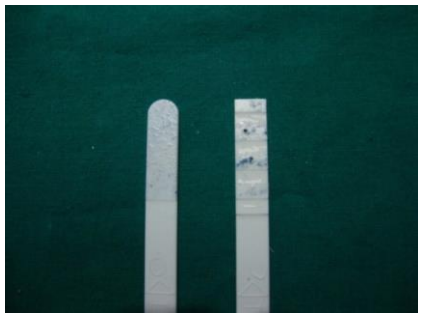


Baseline (Score-3)



1st Week (Score -2)

3rd Week (Score -1)



4th Week (Score -0)

Figure 3: *Mutans Streptococci (CFU/mL) in Plaque and Saliva at baseline, 1st week, 3rd week & 4th week interval based on Dentocult SM strip Mutans model chart.*

DISCUSSION

Control of the bacterial biofilm on teeth is indispensable for the maintenance of oral health. The application of natural agents with antimicrobial activities on dental surfaces promotes a reduction in biofilm formation. In addition, these agents can hinder bacterial colonization, growth and metabolism (21). Many pharmaceutical properties including antibacterial, antifungal, antiviral, antiprotozoan, anti-inflammatory, antioxidant, hepatoprotective, immune-stimulating, antitumor, cytostatic and anticaries activities have been reported for Propolis (22). Propolis contains flavones, flavonols, flavonones and isoflavones, which contribute to its antimicrobial activity. There is a wide variability in the quantity and quality of flavonoids present among different types of Propolis dependent on the region from which it is obtained (23).

A study (24) conducted in 2011 found that Propolis extract with the highest flavonoid concentration presented the highest antimicrobial activity. Another study (7) revealed that type-6 or flavonoid-free Propolis remarkably reduced GTF activity and inhibited Mutans streptococci growth and adherence. Propolis affects cytoplasmic membrane, inhibits bacterial motility and enzyme activity, exhibits bacteriostatic activity against different bacterial genera and can be bactericidal in high concentrations. The antibacterial efficacy of Propolis increases at higher temperature (37˚C) and at acidic pH (pH 5.0) (12). As an anti-inflammatory agent, Propolis is shown to inhibit synthesis of prostaglandins, stimulate cellular immunity and augment healing effects on epithelial tissues. Additionally, Propolis contains elements, such as iron and zinc that are important for the synthesis of collagen [25].

In the present study, we have evaluated the anti-bacterial efficacy of a Propolis based dentifrice on Mutans Streptococci colonizing the oral cavity of young patients using *Dentocult SM strip Mutans kit (Orion diagnostic, Finland).* Dentocult SM is regarded as the paramount test for the diagnosis of the presence of caries and its prognosis with a high statistical significance, and its reliability has been proved in various studies

(18, 19, 20, 26). The advantages of this test are that, being a chair side test, it assures greater patient compliance especially for young subjects. It also requires minimal armamentarium, is less time-consuming and facilitates sample collection (26). The sensitivity, specificity and accuracy of Dentocult SM were found to be better than those of conventional methods (27). Dentocult-SM Strip Mutans test in children was a good indicator and accurate procedure for Mutans streptococci count infection (19). Few studies (18, 26) also estimated the Mutans streptococci count using Dentocult SM Strips. *A study conducted* in 2010 (28) found strong antimicrobial activity of Ethanol extract of Propolis (EEP) against Mutans streptococci. It was also established that EEP exerted bacteriostatic and bactericidal effects against *Mutans streptococci*, respectively, at concentrations of 1.875 and 3.75 µg/mL or more. They stated that organisms were most susceptible to EEP at acidic pH followed by neutral and alkaline pH (5). In another study (23), Propolis mouthrinse was found to have an effective antimicrobial action against Mutans Streptococci.

The present study revealed a significant reduction of Mutans streptococci count at weekly intervals as compared to the baseline values, after using Propolis dentifrice. At 1st week, statistically significant reduction in Mutans streptococci count was noted as compared to baseline mean value. In a study, Propolis showed bacteriostatic effect on Mutans streptococci at intervals of three hours, six hours, 12 hours and 24 hours (13). This shows that Propolis can act quickly, and the findings are in agreement with our study where we have achieved the results at 1st week interval suggesting that Propolis can be a vital anti-caries agent. The results were also similar to a study conducted in 2011 (24) where geo-Propolis was found to be having a significant antimicrobial action against Mutans streptococci at intervals of 1, 2 , 3 & 4 hours respectively with significant inhibitory activity against Mutans streptococci biofilms. Our observations were also in accordance with another study (1), where a statistically significant reduction in bacterial concentration in 81% of the samples, after the use of Propolis extract, between baseline and 1st week was observed. They stated that Propolis extract possesses in vivo antimicrobial activity against Mutans Streptococci present in the oral cavity and might be used as an anticaries agent. This can be attributed to the antibacterial activity of Propolis and its role in inhibition of cell adherence and inhibition of water insoluble glucan formation (1).

In the present study, at the 3rd and 4th weeks, the results showed significant reduction in Streptococcus mutans count as compared to baseline value. A double-blind parallel, controlled clinical trial (29) evaluated the superiority of using Propolis-containing toothpaste after sucrose challenge on the recovery of dental plaque pH. The Propolis toothpaste showed statistically significant inhibiting effect for Mutans streptococci at the end of one week and four weeks. The findings were consistent with the findings of our study, both at 1st week and 4th week intervals.

A significant reduction in the number of colonies in the samples was the result of the effect of the Propolis extract on bacterial growth which has also been proven by earlier studies (1, 5, 7, 13, 23, 24, 28). The antimicrobial activity of Propolis appears to be multifactorial. Some distinct chemical groups (Apigenin and tt-farnesol) present in Propolis are effective inhibitors of Glucosyltransferases (30). The antibacterial activity of another important component, Flavonoids, is well established.

The results reinforced the exceptional role of Propolis dentifrice in reducing the microbial load throughout the human oral cavity in children. Thus, it can be concluded that Propolis dentifrice possesses an in-vivo antimicrobial activity against Mutans streptococci. Based on these findings, the use of these dentifrices, as a caries preventive measure is advocated. Further studies have to be conducted with a larger number of participants to extrapolate the findings of this study.

ACKNOWLEDGEMENTS

We are grateful to Dr. Rajeev Yadav, Associate Professor, Department of Preventive and Social Medicine, S.M.S Medical College, Jaipur, for extending his suggestions throughout our study.

REFERENCES

1. Silvana Alves de Carvalho Duailibe, Azizedite Guedes Goncalves, Fernando Jorge Mendes Ahid. Effect of a Propolis extract on Streptococcus Mutans counts in vivo. *J Appl Oral Sci*, 2007; 15 (5): 420-3.

2. Loesche WJ. Role of Streptococcus mutans in human dental decay. *Microbiology Reviews,* 1986; 50: 353–80.
3. De Stoppelaar JD, Van Houte J, Backer Dirks O. The relationship between extracellular polysaccharide- producing streptococci and smooth surface caries in 13-year-old children. *Caries Res,* 1969; 3: 190-9.
4. Ikeda T, Sandham HJ, Bradley EL. Changes in Streptococcus mutans and lactobacilli in plaque in relation to the initiation of dental caries in Negro children. *Arch Oral Biol,* 1973; 18: 555-66.
5. Hsin-Yi-Yi Yang, Wei-Li Ho, Cheng-Ming Chang, Cheng-Chun. Antibacterial Activity of Propolis Ethanol Extract Against Streptococcus Mutans as influenced by concentration, temperature, pH and cell age. *Journal of Food and Drug Analysis,* 2007; 15 (1): 75-81.
6. Koo H, Rosalen PL, Cury JA et al. Effect of a new variety of Apis mellifera Propolis on mutans streptococci. *Curr Microbiol,* 2000; 41: 192-6.
7. Simone Duarte, Hyun Koo, William Henry Bowen et al. Effect of a Novel Type of Propolis and its Chemical Fractions on Glucosyltransferases and on Growth and Adherence of Mutans Streptococci. *Biol. Pharm. Bull,* 2003; 26 (4): 527-31.
8. Hannig C, Ruggeri A, Al-Khayer B et al. Electron microscopic detection and activity of glucosyltransferase B, C, and D in the in situ formed pellicle. *Arch Oral Biol,* 2008; 53:1003–10.
9. McCabe RM, Donkersloot JA. Adherence of Veillonella species mediated by extracellular glucosyltransferase from Streptococcus salivarius. *Infect Immun,* 1977; 18: 726–34.
10. Schilling KM, Bowen WH. The activity of glucosyltransferase adsorbed onto saliva-coated hydroxyapatite. *J Dent Res,*. 1988; 67: 2–8.
11. Vacca-Smith AM, Bowen WH. Binding properties of streptococcal glucosyltransferases for hydroxyapatite, saliva-coated hydroxyapatite, and bacterial surfaces. *Arch Oral Biol,* 1998; 3: 103–10.
12. Fokt H, Pereira A, Ferreira AM et al. How do bees prevent hive infections? The antimicrobial properties of Propolis; Current research, technology & education topics in applied microbiology & microbial biotechnology. 2010; 1: 481-93.
13. Min Jung Kim, Chun Sung Kim, Byung-Hoon Kim et al. Antimicrobial Effect of Korean Propolis against the Mutans Streptococci isolated from Korean. *The Journal of Microbiology,* 2011; 49 (1): 161-4.
14. Barira Islam, Shahper N. Khan, Asad U. Khan. Dental caries: From infection to prevention. *Med Sci Monit,* 2007; 13(11): RA196- 203.
15. Steinberg D, Kaine G, Gedalia I. Antibacterial effect of Propolis and honey on oral bacteria. *Am J Dent,* 1996; 9: 236-9.
16. Ikeno K, Ikeno T, Miyazawa C. Effect of Propolis on dental caries in rats. *Caries Res,* 1991; 25: 347-51.
17. Koo H, Cury JA, Rosaleu PL et al. Effects of Propolis from two different regions of Brazil on oral microorganisms. *J Dent Res,* 1998; 77: 1157.
18. Asokan S, Rathan J, Muthu M.S et al. Effect of oil pulling on Streptococcus mutans count in plaque and saliva using Dentocult SM Strip mutans test: A randomized, controlled, triple-blind Study. *J Indian Soc Pedod Prevent Dent,* 2008; 26 (1): 12-7.
19. Fieniliakkinen K, Jokela J. A simple method for monitoring mutans streptococci in young children. *Eur J Oral Sci,* 1995; 103; 61-2.
20. El-Ncideef MAI, Kalfas S, Edwarchson S, Ericson D. Influence of transient salivary flora on assessment of mutans streptococci level by the "Strip mutans" method. *Seand J Dent Res,* 1992; 100: 149-5.
21. Soley Arslan, Sibel Silici, Duygu Percin et al. Antimicrobial activity of poplar Propolis on Mutans Streptococci and caries development in rats. *Turk J Biol,* 2012; 36: 65-73.
22. Yaghoubi SMJ, Ghorbani GR, Soleimanian Zad S et al. Antimicrobial activity of Iranian Propolis and its chemical composition. *DARU,* 2007; 15 (1): 45-8.
23. Neeraj Malhotra, Sugandhi P. Rao, Shashirashmi Acharya et al. Comparative In Vitro Evaluation of Efficacy of Mouthrinses against Streptococcus mutans, Lactobacilli and Candida albicans. *Oral Health Prev Dent,* 2011; 9: 261-68.
24. Silvana A Liberio, Antônio Luís A Pereira, Richard P Dutra et al. Antimicrobial activity against oral pathogens and

Immunomodulatory effects and toxicity of Geo Propolis produced by the stingless bee Melipona fasciculata Smith. *BMC Complementary and Alternative Medicine*, 2011; 11:108

25. Silvana A Libério, Antônio Luís A Pereira, Maria José A M Araújo et al. The potential use of Propolis as a cariostatic agent and its action on mutans group streptococci. *Journal of Ethnopharmacology*, 2009; 125: 1–9.
26. Jeevarathan J, Deepti A, Muthu M.S et al. Effect of fluoride varnish on Streptococcus mutans counts in plaque of caries free children using Dentocult SM strip mutans test: A randomized controlled triple blind study. *J Indian Soc Pedod Prev Den*, 2007; 25 (4):157-63.
27. Karjalainen S, Soderling E, Pienihakkinen K. Validation and inter-examiner agreement of mutans streptococci levels in plaque and Saliva of 10-year-old children using simple chair-side tests. *Acta Odontol Scand*, 2004; 62: 153-7.
28. Ophori EA, Eriagbonye BN, Ugbodaga P. Antimicrobial activity of Propolis against Streptococcus Mutans. *African Journal of Biotechnology*, 2010; 9 (31): 4966-9.
29. Y Deng. Experimental and Clinical Study of Anticarious activity of Propolis Toothpaste. Oral and Clinical medicine, Chongqing Medical University, China, 2007.
30. Koo H, Xiao J, Klein MI et al. Exopolysaccharides produced by Streptococcus mutans Glucosyltransferases Modulate the Establishment of microcolonies within multispecies biofilms. *Journal of Bacteriology*, 2010: 3024–32.