Al-Rafidain Dental Journal
rden.mosuljournals.com

Analysis of the Role of CPP-ACPF Paste in Combination with Aqueous Extracts of Propolis to Improve Enamel Roughness after Demineralization Challenge

Mayada Kashmoola[1]*, Aisha A. Qasim [2]

[1] Ministry of Health, Ninawa Health Directorate.
[2] Department of Pedo. Otho. Preventive Dentistry, College of Dentistry, University of Mosul

Article information

Received: November 5, 2020
Accepted: December 5, 2020
Available online: March 9,2022

Keywords:
Enamel demineralization
Propolis
CPP-ACPF paste
Surface Roughness

***Correspondence**:
Mayada Kashmoola

E-mail:
maiadakashmoola75
@gmail.com

Abstract

Aims: This study aims to evaluate the combined efficacy of aqueous extracts of propolis and Fluoridated Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP.ACPF) paste or Minimal Intervention (MI) paste plus on improving enamel surface roughness after demineralization challenge. **Materials and methods**: A total of (75) posterior wisdom teeth were used in the study. Enamel blocks were prepared and divided into five groups randomly, the teeth in all groups were subjected to demineralization cycle and then treated with: Sinjar's aqueous extract of propolis (AEP)-MI paste plus cream n. (15), Sulaymaniah's AEP-MI paste plus cream n. (15), Duhok's AEP-MI paste plus cream group n. (15), control positive group of MI paste plus alone n. (15), and control negative group of artificial saliva alone n. (15). The Roughness of enamel blocks was measured using a profilometer machine at baseline, after demineralization cycle and finally after the treatment protocol.**Results**: There were statistically high significant differences among study groups after the demineralization cycle and there was an increase in surface roughness in all groups after demineralization, but the least elevation in surface roughness belonged to a mixture of Sulaymaniah's aqueous extract of propolis with MI past plus followed by Duhok's aqueous extract of propolis with MI past plus group after treatment protocol. Statistically, there were high significant reductions in surface roughness in all groups after the remineralization except in an artificial saliva group. **Conclusions**: Combination CPP-ACPF Paste with Aqueous Extracts of Propolis reducing enamel's roughness and increasing resistance to demineralization.

الخلاصة

الأهداف: تهدف الدراسة إلى تقييم الفعالية المجمعة للمستخلصات المائية للعكبر ومعجون Casein Phosphopeptide– Amorphous Calcium Phosphate (CPP-ACPF) المفلور في تحسين خشونة سطح المينا بعد تحدي إزالة المعادن. **المواد وطرائق العمل**: تم استخدام إجمالي (٧٥) ضرس عقل خلفي في الدراسة. حُضرت كتل المينا وقسمت إلى خمس مجموعات عشوائياً ، تعرضت الأسنان في كل المجموعات لدورة نزع المعادن ثم عولجت بـ: مجموعة كريم المستخلص المائي لعكبر سنجار بالإضافة إلى معجون MI ((Minimal Intervention المفلور أو (15) (CPP-ACPFعينه(, مجموعة كريم المستخلص المائي لعكبر السليمانية بالإضافة إلى معجون MI المفلور(١٥عينه(,مجموعة كريم المستخلص المائي لعكبر دهوك بالإضافة إلى معجون MI المفلور(١٥عينه(, مجموعة السيطرة الإيجابية من معجون MI المفلور وحده (١٥عينه(, ومجموعة السيطرة السلبية من اللعاب الاصطناعي لوحده (١٥عينه(. تم قياس خشونة كتل المينا باستخدام آلة مقياس التشكيل الجانبي عند خط الأساس ، بعد دورة إزالة المعادن وأخيراً بعد بروتوكول العلاج. **النتائج**: كانت هناك فروق ذات دلالة إحصائية عالية بين مجموعات الدراسة بعد دورة إزالة المعادن وكان هناك زيادة في خشونة السطح في جميع المجموعات بعد النزع ، ولكن أقل ارتفاع في خشونة السطح ينتمي إلى خليط من مستخلص دنج السليمانية المائي مع MI past plus يليه مجموعة خليط مستخلص دنج دهوك المائي مع MI past plusبعد بروتوكول العلاج. إحصائياً كان هناك انخفاض معنوي كبير في خشونة السطح في جميع المجموعات بعد إعادة التمعدن ما عدا مجموعة اللعاب الاصطناعي. **الاستنتاجات**: مزيج CPP.ACP المفلورمع المستخلصات المائية من العكبر يقلل من خشونة المينا ويزيد من المقاومة لدورة نزع المعادن.

DOI: 10.33899/rdenj.2022.173020

INTRODUCTION

Tooth enamel is one of the four major tissues that make up the human's tooth structure [(1)]. It is the most mineralized tissue of the body is enamel which forms a very hard, thin, translucent layer of calcified tissue that covers the entire anatomic crown of the tooth [(2)].

Main process as caries occurs at the outer layer of enamel which is principally in contact with oral environment [(3)]. The first visible sign of tooth caries, the white-spot lesion, has been defined as "subsurface enamel porosity from carious demineralization [(4)]. This subsurface porosity is caused by an imbalance between the dynamic biological processes of de- and remineralization [(5)].

The principles of minimally invasive dentistry clearly dictate the need for clinically effective measures to remineralize early enamel caries lesions [(6)]. The CPP-ACP nanocomplexes are derived from bovine milk protein, casein, and calcium and phosphate, preventing demineralization and enhancing remineralization [(7)]. The combination of CPP–ACP with fluoride caused localization of calcium and phosphate ions with fluoride ions at the enamel surface [(8)].

The use of natural products by human beings as a therapeutic alternative resembles ancient times [(9)] as propolis, which is a gummy and balsamic substance, is a honeybee product [(10)] with beneficial properties like antimicrobial, antiviral, antifungal, and anti-inflammatory [(11)].

Roughness is a fundamental property of teeth, which affects the attachment of exogenous materials to enamel surfaces and caries progression [(12)]. Thus, the surface roughness is the irregularity of enamel surface characteristics due to the demineralization process [(13)]. Enamel surface roughness assessment is a useful means of evaluating the stage and activeness of carious lesions [(14)].

The aim of the current study is to analyze the synergistic effect of aqueous extracts of propolis on roughness improvement power of fluoridated CPP-ACP paste after demineralization challenge.

MATERIALS AND METHODS

Collection and Extraction of Propolis

The unrefined propolis (Apis mellifera) was obtained from three different regions in the north of Iraq. The first type was from Sinjar's mountains/Ninawa, the second type was from Duhok's mountains and the third one was from Sulaymaniah's mountains. For aqueous extract preparation, Krell method [(15)] was followed. The extract was clear from any impurities, dark and viscous and its odor is also distinguishable for each type [(16)].

Formulations of the Fluoridated CPP–ACP Propolis Extracts Tooth Coating Complex

Both ingredients were combined into a cream formulation made from a base of starch and other suitable binding material (glycerin). The fluoridated CPP-ACP paste and distilled water were adjusted at a specific ratio and mixed with the base solution, then propolis extracts 30% were added after complete dissolving in ethanol, shake well via vortex tube stirrer then placed in the lyophilizer till complete evaporation of the solvent and homogenous cream was obtained for the three types of propolis as shown in Figure (1).

Figure (1): Formulation & Application of Tooth Coating Cream, **(A)** Crude Propolis, **(B)** Aqueous Extract of Propolis, **(C)** MI Plus Paste, **(D)** AEP-MI Plus Paste Complex After Mixing, **(E)** AEP-MI Plus Paste Complex After Freeze Drying, **(E)** Application of Different Tooth Coating Creams**.**

Sample Collection

The sample in this study consisted of (75) human permanent third molars extracted for impaction reasons. After extraction, the teeth were cleaned with tap water and examined with 10X magnifying lens, the selection of the teeth followed specific criteria; the teeth must be sound, free from enamel defects, decay, stain, cracks, hypoplasia, and fluorosis and unaltered by extraction procedure. The teeth were stored in 0.1% thymol solution at 4C^{o} [17] to avoid dehydration and prevent bacterial growth until their use within three months.

Preparation of Enamel Blocks

Sound extracted third molars were cleansed accurately before using, they were scrubbed with non-fluoridated pumice and white rubber prophylactic cup using a low speed hand piece, wiped free of soft tissue debris and sanitized in tap water then the crowns separated from the roots via a diamond disc bur in the high speed hand piece cooled with water, after that the crowns were mounted in

cylindrical plastic tubes (16mm diameter×14mm depth) with cold cure acrylic resin with the outer buccal enamel surface exposed. The buccal surface of each sample was polished t using 240, 400, 600, and 1200grit silicon carbide abrasive papers under flooding water to obtain standardized flat enamel surface [18]. Then smoothed by using the universal polisher machine. Each specimen was then coated under the digital stereomicroscope (X 40) with two layers of an acid - resistant nail varnish, leaving 3×3mm^2 window on the middle third of the enamel surface to define the experimental area [19, 20] as shown in Figure (2).

Figure (2): Preparation of Enamel Blocks, **(A)** Cylindrical Plastic Tubes, **(B)** Separation Of The Crowns From The Roots, **(C)** Crowns After Cutting, **(D)** Cold Cure Acrylic Mold &Varnish Application**.**

MATERIALS AND METHODS

Approval of study was from the Scientific Research Committee / Department of Pedo. Ortho. Preventive Dentistry / College of Dentistry / University of Mosul. Commercially available topical cream with bioavailable calcium and phosphate (GC America, Recaldent, Alsip, USA), which contains 10% by weight of CPP−ACP in addition to Sodium fluoride 0.20%, (MI Paste Plus) was used in remineralization of one group of the samples in addition to previously formulated fluoridated CPP–ACP-propolis aqueous extracts tooth coating complex of three types (Sinjar, Sulaymaniah and Duhok) for remineralization of other groups in the study.

Design of Study and Methods of Application

The total number of teeth samples in the study was (75) samples, randomly divided into five groups, (15) samples in each group as follows:

Group 1: control negative group (N. =15), after immersion of the teeth samples in demineralization solution, they were immersed in artificial saliva only that it is changed daily for 14 days.

Group 2: control positive group (N. =15), after immersion of the samples in demineralization solution, the enamel

surfaces were coated by a fine brush with a thin layer of MI paste plus and left for 30minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

Group 3: Sinjar's AEP-MI plus paste complex group (N. =15), after immersion of the samples in demineralization solution, the enamel surfaces were coated by a fine brush with a thin layer of Sinjar's AEP-MI plus paste complex and left for 30minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

Group 4: Sulaymaniah's AEP-MI plus paste complex group (N. =15), after immersion of the samples in demineralization solution, the enamel surfaces were coated by a fine brush with a thin layer of Sulaymaniah's AEP-MI plus paste complex and left for 30minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

Group 5: Duhok's AEP-MI plus paste complex group (N. =15), after immersion of the samples in demineralization solution, the enamel surfaces were coated by a fine brush with a thin layer of Duhok's AEP-MI plus paste complex and left for 30minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

Demineralization Procedure

Before application of treatment protocol, each group was individually suspended in demineralizing solution for 5 days at temperature of 37ºC to create artificial caries like lesions. The demineralizing solution contained 2.2 mmol/L$CaCl_2$, 2.2mmol/L NaH_2PO_4 and 50 mmol/L acetic acid adjusted to pH 4.5 with NaOH at 37ºC. The pH values of the demineralization solution were checked every day using a pH meter and the solution was changed every day [19].

Surface Roughness Measurement

In order to evaluate the changes in the surface texture of the teeth, the enamel surface roughness was chosen. The procedure includes measurement of surface roughness for sound enamel at baseline, before and after demineralization-cycling regime and after treatment regime in each tested group. Such evaluation was conducted by a profilometer machine with a magnification of x50 as shown in figure (3).It is the maximum of all peak-to-valley values [21].and is measured in micrometers (μm) [22].Each specimen's surface was placed parallel to the horizontal plane and perpendicular to the tip of the profilometer which measured the roughness of the specimen along a 3-mm path. The distance between the peaks and valleys of the sampled line was gauged in the y direction. The cut-off value for surface roughness was 0.8 mm [23]. All readings

were carried out by the same examiner using the same calibrated machine.

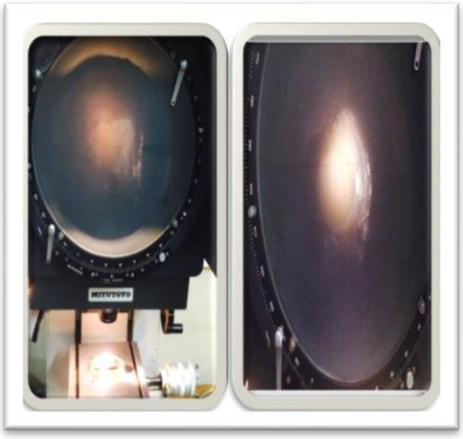

Figure (3): Surface Roughness Measurement Using Profilometer Machine with Magnification Of X50.

RESULTS

The data were analyzed using SPSS program (version 19). Table (1) delineates one way analysis of variance (ANOVA) test for comparison of mean roughness values between the groups at baseline, after the demineralization cycle and after treatment scheme. Results showed that there was highly significant difference at $p \leq 0.01$ of mean roughness values among tested groups in the three stages of the study.

Table (1): Analysis of Variance (ANOVA) Test of Mean Roughness Values for Comparison Between Aqueous Extracts for Sinjar, Sulaymaniah & Duhok's Propolis Groups & Controls at Every Stage in The Study

Time	Source of variance	Sum of Squares	DF	Mean Square	F	Sig.
Baseline data	Between Groups	1.009	4	.252	20.367	.000*
	Within Groups	.867	70	.012*		
	Total	1.877	74			
After demineralization	Between Groups	.081	4	.020	3.441	.013
	Within Groups	.410	70	.006*		
	Total	.490	74			
After treatment	Between Groups	8.040	4	2.010	341.197	.000*
	Within Groups	.412	70	.006*		
	Total	8.452	74			

***Highly significant difference at $p \leq 0.01$.**

Table (2) formulates means, number, standard deviation and Duncan's multiple range tests of roughness mean values of the enamel blocks of the tested groups. Statistically, the results of the mean roughness values were significantly different for the groups at all stages but after treatment, the least elevation in

surface roughness mean value belonged to mixture of Sulaymaniah's aqueous extract of propolis with MI past plus followed by Duhok's aqueous extract of propolis with MI past plus group then Sinjar's AEP-MI plus paste complex group_followed by control positive group (MI plus paste alone) while the highest value of roughness was found in control negative group that was preserved in artificial saliva only. It is obvious that all of the remineralizing treatment pastes decreased the surface roughness values above the baseline means except for artificial saliva.

Table (2): Mean Values, Standard Deviation and Duncan's Multiple Range Test of Surface Roughness for Comparison Between Aqueous Extracts for Sinjar, Sulaymaniah & Duhok's Propolis Groups & Controls In The Three Stages Of Experiment.

Groups	Variables	Baseline data	After demineralization	After treatment
Control -	Mean	.831 a	1.966 ab	1.273 a
	N	15	15	15
	Std. Deviation	.04627	.08025	.09612
Control +	Mean	.791 a	2.006 ab	.623 b
	N	15	15	15
	Std. Deviation	.09877	.05499	.06747
Sinjar aqueous	Mean	.664 b	1.975 ab	.560 b
	N	15	15	15
	Std. Deviation	.13500	.07150	.10747
Sulaymaniah aqueous	Mean	.632 b	1.929 b	.355 c
	N	15	15	15
	Std. Deviation	.17013	.12009	.03583
Duhok aqueous	Mean	.508 c	2.023 a	.422 c
	N	15	15	15
	Std. Deviation	.05375	.01676	.05321

***Duncan's Multiple Range Tests: Means with different letters are statically significant vertically (within the same column).**
***Group with letter (a) has the highest value of roughness.**

DISCUSSION

Statistical analysis revealed the mean roughness values of enamel specimens in all groups were increased compared with the baseline values after demineralization cycle, next, roughness of all groups decreased after treatment protocol compared to the surface roughness values measured after demineralization. Differences in the decreasing value of enamel surface roughness of each group occurred due to differences in mechanism and effectiveness of remineralized materials used. The effectiveness of the remineralization material is influenced by numerous factors such as the degree of acidity, the concentration and solubility of the remineralization material, complex forming reaction, temperature, and position of the balance point and the

chemical formulation of the remineralization material [24].

The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) substances cause increases the density of the hydroxyapatite crystals and also the density of the enamel prism. This increase in enamel prism density can result in decrease surface roughness [25].

Propolis displayed a slight ability to prevent demineralization, though, they were better than the other groups. This shows that those natural products release several remineralizing agents, however with slight impact on inhibition of demineralization [26]. Propolis also decreased the accumulation of dental plaque and its insoluble external polysaccharide content [27]. It is a non-toxic material and its antimicrobial activity is due to the presence of flavonoids and terpenoids [28].

The existing study showed that the mean roughness values for groups with different letters are significantly different from each other at $p \leq 0.05$. Also, showed that there was highly significant difference at $p \leq 0.01$ of mean roughness values among tested groups in the three stages.

Among all extracts types investigated, aqueous extract of propolis (AEP) had the lowest number of active substances and weakest biological effects. The solubility of propolis compounds increased by the addition of co-solvent Pg (Polyethylene glycol) which also enhance penetration of active substances into cells however has no effect on the biocompatibility of the product. As a result, such type of extract could be suggested to be used not only for biological research but as well for design of pharmaceutical products [29]

Sometimes, the compounds in propolis have been considered to arise from three sources: plant exudates gathered by honey-bees, substances secreted from bee metabolism and substances that are introduced during the elaboration of propolis [30].

Honeybees gather propolis from practically any abundant plant source in the neighborhood of the hive. The chemical profile of propolis that is made by the same species is not always the same although different species of honeybee prefer different plants [31]. For that reason, the alternative chemical composition of propolis depends on the bees' preferences of botanical sources and the species and varieties of bees. Moreover, the method would not recognize the plant neither less if it had the same chemical constituents as those from propolis. The other is that the chemical constituents may differ with each part of the plant or even the growth of the plant or season of sampling [32.]

The chemical composition of propolis differs greatly depending on, plant vegetation, geographical location, type of bees, season and time of collections, and the concentration and nature of the solvents used for the extraction [33] so the chemical composition

of propolis is unsteady and varies according to hive, season and region [10]. Daugsch *et al.* in 2008 [31] concluded that the botanical origin and its abundance are necessary for the production of the type of propolis when the bees' gathered resins from various plants to yield propolis.

CONCLUSION

Combination CPP-ACPF Paste with Aqueous Extracts of Propolis from the three regions reducing enamel's roughness obviously and all have superior results to MI paste Plus alone.

Limitations of the study:

The chief limitation of the current study is that it is an *in vitro* study in which the demineralization cycle was achieved by using chemical products and did not occur due to the presence of Streptococcus mutans bacteria and it is acid byproducts. Also, surface roughness *in vitro* may be different when compared to the natural dynamic conditions in the oral cavity *in vivo*.

REFERENCES

1. Towle I, Dove ER, Irish JD, Groote ID. Severe Plane-Form Enamel Hypoplasia in a Dentition from Roman Britain. *Dental Anthropology*. 2017; 30(1):16-24.
2. Jacobson A. Oral development and histology. 3rd edition. New York: Thieme Medical Publishers, Inc. 2002.
3. Argenta RMO, Tabchoury CPM and Cury JA. A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. *Pesqui Odontol Bras*. 2003; 17 (3):241-246.
4. Summitt JB, Robbins JW, Hilton TJ, and Schwartz RS. Fundamentals of Operative Dentistry: A Contemporary Approach. Hanover Park, IL, Quintessence Publishing, 2006.
5. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. *J Dent Res*. 2004; 83 (Spec N): 35-38.
6. Philip N. State of the Art Enamel Remineralization Systems: The Next Frontier in Caries Management. *Caries Res.* 2019; 53:284–295.
7. Hegde MN, Moany A. Remineralization of enamel subsurface lesions with casein phosphopeptide-amorphous calcium phosphate: A quantitative energy dispersive X-ray analysis using scanning electron microscopy: An in vitro study. *Year*. 2012; 15(1):61-67.
8. Cochrane NJ, Saranathan S, Cai F, Cross KJ and Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilized solutions of calcium, phosphate and fluoride. *Caries Res*. 2008; 42:88-97.
9. Valadas LAR, Gurgel MF, Mororó JM, Fonseca SGC, Fonteles CSR, Carvalho CBM, et al. Dose-response

evaluation of a copaiba-containing varnish against streptococcus mutans in vivo. *Saudi Pharmaceutical Journal*. 2018; 27(3):363-367.

10. Toreti VC, Sato HH, Pastore GM and Park YK. Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-Based Complementary and Alternative Medicine*. 2013; 2013:1-13.
11. Khurshid Z, Naseem M, Zafar MS, Najeeb S and Zohaib S. Propolis: A natural biomaterial for dental and oral health care. *J Dent Res Dent Clin Dent Prospect*. 2017; 11(4): 265 -274.
12. Patil N, Choudhari S, Kulkarni S, and Joshi SR. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. *Journal of Conservative Dentistry*. 2013; 16(2): 116-120.
13. Cross SE, Kreth J, Wali RP, Sullivan R, Shi W and Gimzewski JK. Evaluation of bacteria-induced enamel demineralization using optical profilometry. *J Dent Mat*. 2009; 25(13): 1618-1626.
14. Piątek-Jakubek K, Nowak J, Bołtacz-Rzepkowska E. Influence of infiltration technique and selected demineralization methods on the roughness of demineralized enamel: An in vitro study. *Adv Clin Exp Med*. 2017; 26(8): 1179−1188.
15. Krell R.A. Value-added products from beekeeping FAO Agricultural Services Bulletin. 1996; NO.124 Rome, Italy.
16. Bankova V, Bertelli D, Borba R, Conti BJ, Cunha IBS , Daner C *et al*. Standard methods for Apis mellifera propolis research. Extraction of propolis. *Journal of Apicultural Research*. 2019; 58(2):1-49.
17. Amoras DR, Corona SA, Rodrigues AL and Serra MC. Effect of beverages on bovine dental enamel subjected to erosive challenge with hydrochloric acid. *Braz Dent J*. 2012; 23(4):367-372.
18. Rirattanapong, P, Vongsavan, K, and Tepvichaisillapakul M. Effect of Five Different Dental Products on Surface Hardness of Enamel Exposed to Chlorinated Water In Vitro. *Southeast Asian Journal of Tropical Medicine and Public Health*. 2011; 42(5):1293-1298.
19. Ambarkova V, Gorseta K, Jankulovska M, Glavina D and Skrinjaric. Effect of the fluoride gels and varnishes comparing to CPP-ACP complex on human enamel demineralization /remineralization. *Acta stomatol Croat*. 2013; 47(2):99-110.
20. Shetty S, Hegde M and Bopanna T. Enamel remineralization assessment after treatment with three different remineralizing agents using surface microhardness: An in vitro study.

Journal of Conservative Dentistry. 2014; 17(1):49–52.

21. Zygo Corporation (2013). Surface texture parameters. Laurel Brook Road Middlefield, CT 06455, USA. P: 1-19.
22. Worschech CC, Rodrigues JA, Marcondes Martins LR and Bovi Ambrosano GM. In vitro evaluation of human dental enamel surface roughness bleached with 35% carbamide peroxide and submitted to abrasive dentifrice brushing. *Pesqui Odontol Bras*. 2003; 17(4):342-8.
23. Taher NM, Alkhamis HA and Dowaidi SM. The influence of resin infiltration system on enamel microhardness and surface roughness: An in vitro study. *Saudi Dent J*. 2012; 24(2): 79–84.
24. Amaral CM, Mesng M, Correa DS, Silva EM. Sodium fluoride and casein phosphopeptide- amorphous calcium phosphate cream plus sodium fluoride efficacy in preventing enamel erosion in a simulated oral environment study model. *Indian J Dent Res*. 2015; 24(4): 464 – 469.
25. Divyapriya GK, Yavagal PC and Veeresh DJ. Review article casein phosphopeptide–amorphous calcium phosphate in dentistry: an update. *Int J Oral Health Sci*. 2016; 6: 18–25.
26. Wassela MO and Khattabb MA. Antibacterial activity against Streptococcus mutans and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. *J Adv Res*. 2017; 8(4): 387–392.
27. Koo H, Pearson SK, Scott-Anne K, Abranches J, Cury JA, Rosalen PL et al. Effects of apigenin and ttfarnesol on glucosyltransferase activity, biofilm viability and caries development in rats. *Oral Microbiol Immunol*. 2002; 17(6):337-343.
28. Jeon JG, Rosalen PL, Falsetta ML and Koo H. Natural products in caries research: current (limited) knowledge, challenges and future perspective. *Caries Res*. 2011; 45:243–263.
29. Kubiliene L, Jekabsone A, Zilius M, Trumbeckaite S, Simanaviciute D, Gerbutaviciene R and Majiene D. Comparison of aqueous, polyethylene glycol-aqueous and ethanolic propolis extracts: antioxidant and mitochondria modulating properties. *BMC Complementary and Alternative Medicine*. 2018; 18:165.
30. Marcucci MC. Propolis: Chemical composition, biological properties and therapeutic activity. *Apidol*. 1995; 26: 83-99.
31. Daugsch A, Moraes CS, Fort P and Park YK. Brazilian red propolis chemical composition and botanical origin. *Evid Based Complement Alternat. Med.* 2008; 5: 435–441.
32. Leonhardt S, Zeilhofer S, Blüthgen N and Schmitt T. Stingless bees use

terpenes as olfactory cues to find resin sources. *Chem. Sens*. 2010; 35:603–611.

33. Teixeira EW, Message D, Negri G, Salatino A and Stringheta CP. Seasonal variation, chemical composition and antioxidant activity of Brazilian propolis samples. *J. Evidence-Based Complementary Altern Med*. 2010; 7:307-15