



RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF ANTIPLAQUE AND ANTIGINGIVITIS EFFICACY OF
COFFEA CANEPHOREA AND CHLORHEXIDINE

^{1,*}Anshul and ²Kumari Amrita

¹Private Practitioner, Lucknow, Uttar Pradesh, India

²Intern, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh, India

ARTICLE INFO

Article History:

Received 19th July, 2017

Received in revised form

10th August, 2017

Accepted 17th September, 2017

Published online 31st October, 2017

Key words:

Black coffee mouthwash,
Chlorhexidine mouthwash,
Gingivitis,
Gingival index,
Quigley Hein plaque index.

ABSTRACT

Aim: To compare the efficacy of black coffee mouthwash and chlorhexidine gluconate mouthwash in prevention of gingivitis and plaque formation.

Materials and methods: A total of 30 randomly selected subjects visiting the private clinic, were considered for the study. The gingival index (GI) by Loe and Silness was recorded which was followed by Turesky-Gilmore-Glickman modification of Quigley Hein plaque index (TQHPI) at 0 and 15 days. Individuals who gave an informed consent, subjects in the age group of 25 to 35 years with having fair and poor gingival index scores, were included in the study.

Results: Results showed statistically significant reduction ($p < 0.05$) in mean plaque index (PI) with chlorhexidine gluconate mouthwash when compared with black coffee mouthwash. No significant difference in mean gingival index (GI) was seen when chlorhexidine mouthwash was compared with Coffea canephora extract.

Conclusion: From the above observations, it can be concluded that chlorhexidine gluconate as well as black coffee mouthwash can be effectively used as an adjunct to mechanical plaque control methods in prevention of plaque and gingivitis. chlorhexidine gluconate has been found to be more effective when antiplaque property was considered.

Copyright©2017, Anshul and Kumari Amrita. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Anshul and Kumari Amrita, 2017. "A comparative evaluation of antiplaque and antigingivitis efficacy of Coffea canephora and Chlorhexidine", International Journal of Current Research, 9, (10), 59231-59235.

INTRODUCTION

Dental plaque accumulation is the pre requisite for the development of any dental problem be it carries or simple gingivitis or periodontitis (Loe H, 1965), hence its removal on a daily routine is one of the major factor in the preventive measure. Periodontal diseases being one of the most frequent oral diseases, affecting more than 90% of the population, regardless of age, sex (Salgado *et al.*, 2006). So, daily and effective supragingival plaque control using tooth brushing and dental floss is necessary to arrest its progression to periodontitis. Although mechanical plaque control methods have the potential to maintain adequate levels of oral hygiene, studies have shown that such methods are not being employed accurately. Therefore, several chemotherapeutic agents such as triclosan, essential oils, and chlorhexidine have been developed to control bacterial plaque. Among these, chlorhexidine has been the gold standard since ages due to its profound antibacterial and antiplaque activity. However several side effects are also associated with its use like staining of teeth and restorations, unpalatable taste with taste alteration have stimulated the search for new alternatives.

Coffee has also demonstrated significant antibacterial properties (Toda *et al.*, 1989) against the cariogenic bacteria *Streptococcus mutans* and *Streptococcus mitis* and has also been found to be effective against the periodontal pathogens *P. gingivalis* and *P. intermedia*, as well as *Candida albicans* (Fardiaz, 1995). Most laboratory preparations of coffee and its extract are not similar to the coffee preparations used commercially or at home. Many coffee producers often blend up to 30% of chicory with coffee, which cuts down on the caffeine content and may have other health benefits (Sharma *et al.*, 2014). The aim of this study was to compare the efficacy of commercially available coffee extract against gold standard 0.2% chlorhexidine. To the best of our knowledge, till date no such study has been carried out.

MATERIALS AND METHODS

The study was conducted at private clinic. An ADA Type III clinical examination was done (Sharma *et al.*, 2014). Total 30 subjects both male and female with moderate gingivitis were selected from age group 25 to 35 years who visited the OPD and gave an informed consent, Patients with systemic disorders, subjects under antimicrobial therapy, smokers, and

*Corresponding author: Anshul

Private Practitioner, Lucknow, Uttar Pradesh, India.

pregnant women were excluded from the study. The subjects were randomly distributed into the following 2 groups:

- Group 1 (n = 15): Subjects were instructed to use 10 ml of 0.2% chlorhexidine mouthwash (HEXIDINE by ICPA) twice daily for 14 days
- Group 2 (n = 15): Subjects were instructed to use 10 ml of *Coffea canephora* extract mouthwash twice daily for 14 days.

The study was carried by a single operator, the gingival index (GI) by Loe and Silness was recorded which was followed by Turesky-Gilmore- Glickman modification of Quigley-Hein plaque index (TQHPI). 'Plaksee' disclosing solution containing erythrosine was used to disclose plaque before recording. Recording of indices was done on 0 and 15 days and all records were maintained on a record chart. Oral hygiene and mouthwash usage instructions were given. The subjects of group 1 were asked to rise with 10 ml of mouthwash in 1:1 dilution with water and group 2 were asked to rinse using 10 ml of *C. Canephora* mouthwash twice a day after brushing. Compliance was checked with the help of are minder sheet to be filled by the subject daily after using the mouthwash. These compliance sheets were checked by the investigator during subsequent examinations. Subjects, whose compliance was less, were reinforced with oral hygiene instructions during subsequent examinations. All the mouthwashes were provided to the study subjects free of cost during the entire duration of the study by the investigator.

Preparation of black coffee (*Coffea canephora*) mouth wash

Powder of roasted *Coffea canephora* beans was obtained commercially. Aqueous extracts of coffee were obtained by brewing procedure. Preparation of 20% extract was done by percolating 100ml of pre-boiling (95°C) sterile water through 20 g of ground coffee. A filter paper was used to filter the extracts. After preparation of 20% aqueous extract of coffee, further dilution was done using sterile water to obtain the concentrations

Data were collected at baseline, and 15 days utilizing the indices, i.e.

- Plaque index by Turskey Gilmore Glickman modification of Quigley Hein plaque index
- Gingival index by Loe and Silness

Statistical analysis

Descriptive analysis was done and Student *t* test was used for inter-group and intra-group comparison. *P* value was adjusted at <0.05.

RESULTS

On comparing the plaque index and gingival index chlorhexidine mouthwash was superior in all aspects to black coffee mouthwash as percentage of reduction in score was higher in chlorhexidine mouthwash group than black coffee mouth wash group. On analyzing Plaque index between *Coffea canephora* and chlorhexidine mouthwash revealed that significantly more reduction in plaque score was seen in

chlorhexidine group as compared with *Coffea canephora* mouthwash [$P < 0.05$; [Table 1]. Mean \pm SD for *Coffea canephora* mouthwash group has decreased from 3.816 ± 0.5361 at baseline to and 2.882 ± 0.6284 at 15 days with % reduction of 28.11% respectively ($P < 0.05$). Significantly greater reduction was seen in chlorhexidine group, i.e., from 3.882 ± 0.4291 at baseline and 2.667 ± 0.6360 at 15 days with % reduction of 31.31%, respectively ($P < 0.05$). On analyzing the gingival index, it was seen that both the groups led to significant reduction in gingival scores ($P < 0.05$). Mean decrease in *Coffea canephora* group was from 1.644 ± 0.1670 at baseline to 1.181 ± 0.1854 at 15 days with % reduction of 35.82% ($P < 0.05$). Significantly more reduction was seen in chlorhexidine group, i.e., from 1.641 ± 0.1761 at baseline to 0.991 ± 0.2393 at 15 days with % reduction of 39.61%, ($P < 0.05$). Hence, chlorhexidine mouthwash was a more effective antigingivitis agent as compared with black coffee mouthwash [Table 2].

Table 1. Plaque index of black coffee and Chlorhexidine group at baseline, and 15th day

Group	Time	Mean \pm SD	% Reduction	P value
Black coffee	0 Day	3.816 \pm 0.5361		
	15 Day	2.882 \pm 0.6284	28.11	< 0.05
Chlorhexidine gluconate	0 Day	3.882 \pm 0.4291		
	15 Day	2.667 \pm 0.6360	31.31	< 0.05

Table 2. Gingival index of black coffee and Chlorhexidine group at baseline, and 15th day

Group	Time	Mean \pm SD	% Reduction	P value
Black coffee	0 Day	1.644 \pm 0.1670		
	15 Day	1.181 \pm 0.1854	35.82	< 0.05
Chlorhexidine gluconate	0 Day	1.641 \pm 0.1761		
	15 Day	0.991 \pm 0.2393	39.61	< 0.05

Table 3. Contents of cinnamic acid derivatives (phenolic compounds) and caffeine in *Coffea canephora* extract at 20 %

Chemical compounds	Mean \pm SD
Cinnamic acid derivatives	3650.78 \pm 74.0
3-CQA (Cafeoylquinic Acids)	808.6 \pm 16.8
5-CQA	1342.2 \pm 52.7
3-FQA (Feruloylquinic Acids)	136.6 \pm 7.0
4-CQA	863.4 \pm 22.3
5-FQA	78.7 \pm 6.5
4-FQA	181.08 \pm 7.4
3,4-diCQA (dicafeoylquinic Acids)	125.30 \pm 4.4
3,5-diCQA	42.75 \pm 0.4
4,5-diCQA	72.14 \pm 1.1
CA (Caffeic Acids)	87.33 \pm 35.0
Caffeine	2110 \pm 0.4

Table 4. Mineral contents of *Coffea canephora* extract at 20% and BHI medium

Minerals	<i>Coffea canephora</i> extract 20% (mean \pm SD)
Zinc	0.52 \pm 0.03
Stontium	1.11 \pm 0.05
Silicon	6.66 \pm 0
Sulphur	314.66 \pm 9.6
Phosphorus	491.67 \pm 22.77
Sodium	26.66 \pm 1.67
Manganese	1.94 \pm 0.03
Maganesium	589.69 \pm 18.32
Potassium	10173.58 \pm 182
Iron	3.22 \pm 0.15
Copper	0.46 \pm 0.02
Calcium	216.71 \pm 11.93
Fluoride	0.018 \pm 0

DISCUSSION

Dental plaque is a complex, specific but highly variable structural entity resulting from colonization of microorganisms embedded in a gelatinous extracellular matrix on tooth surfaces, restorations and other parts of oral cavity (Gupta *et al.*, 2015). Available antiplaque agents are based on the use of broad-spectrum antimicrobial agents such as chlorhexidine, quaternary ammonium compounds and antibiotics. Chlorhexidine is the leading antiplaque agent till date, because of its many ideal properties, and its efficacy has been proven by many studies. Chlorhexidine acts by damaging the cell membrane of prokaryotes and by disrupting the cytoplasmic constituents (Gupta *et al.*, 2014). These synthetic antimicrobial agents have resulted in considerable side effects, antimicrobial resistance and the emergence of previously uncommon infections owing to their improper usage (Li and Xu, 2008). Though around 6000 plants in India are used in herbal medicines, the use of plant extracts with medicinal properties represents a concrete alternative for the treatment of different disease. This includes the use of natural product as antimicrobial agents; (Oshima *et al.*, 2000; Alviano *et al.*, 2008; Zou *et al.*, 2008) little research has been conducted on efficacy, safety, and properties of herbal products. Among all the natural products with antimicrobial properties, the use of coffee extract, it could not be disapproved, since this beverage is part of the habitual diet for many people, except for those who are very sensitive to caffeine. There is no doubt that coffee is the most consumed beverage in the world after mineral water. In addition to its pleasant flavor, it has been considered as a potential functional food for its biopharmacological properties demonstrated in clinical and epidemiological researches (Oshima *et al.*, 2003; Johnston *et al.*, 2003).

Previous studies have been performed to evaluate the antimicrobial effect of several different types of coffee extracts *in vitro*, (Sharma) on dental biofilm (Antonio *et al.*, 2011; Antonio *et al.*, 2010) and on caries development (Sharma *et al.*, 2014; Antonio *et al.*, 2011). The main compounds responsible for such activity in roasted coffee extracts are chlorogenic acids, caffeic acid and caffeine. Other minor compounds described in literature are trigonelline, α -dycarbonyl compounds and protocatechuic acid (Antonio *et al.*, 2010; Daglia *et al.*, 2007; Almeida *et al.*, 2006). Caffeine modulates both innate and adaptive immune responses, it can suppress human "neutrophil and monocyte chemotaxis, and also suppress production of the pro-inflammatory cytokine tumor necrosis factor (TNF)- α ." Caffeine was found to suppress human lymphocyte function as indicated by reduced T-cell proliferation and impaired production of Th1 (interleukin [IL]-2 and interferon [IFN]- γ), Th2 (IL-4, IL-5) and Th3 (IL-10) cytokines." Horrigan *et al.* suggested that "at least some of the immunomodulatory actions of caffeine are mediated via inhibition of cyclic adenosine monophosphate (cAMP) phosphodiesterase, and consequential increase in intracellular cAMP concentrations." They also noted that many of caffeine's immunomodulatory effects occur at concentrations that are relevant to normal human consumption (Horrigan *et al.*, 2006). There has been much research done on the effects of caffeine and coffee consumption in animals and humans, with evidence of both beneficial and detrimental effects. For example, caffeine has been found to reduce inflammation and tissue damage in various animal studies, including alcohol induced hepatic injury in mice (Lv *et al.*,

2010) and myocardial ischemia in rats (Li *et al.*, 2011). In contrast, in an animal model of periodontitis, high doses of caffeine have been shown to increase alveolar bone loss in ligature-induced periodontitis in rats. However the dosage given was the human equivalent of consuming 16 cups of coffee per day (Bezerra *et al.*, 2008). Sufficient scientific literature supports that the nature of the coffee extract may influence its antimicrobial efficacy. Factors influencing includes species of the coffee beans, degree of roasting of coffee beans, decaffeination, composition and blend of coffee and last but not the least, the concentration of the extract/solution. Of the two widely used species of coffee, i.e. Arabica and Canephora/Robusta, the latter has been shown to have better antimicrobial efficacy, at least against the cariogenic bacteria *S. mutans* (Antonio *et al.*, 2010). Green or non-roasted coffee appears to have the highest antimicrobial activity. Furthermore, degree of roasting is inversely proportional to its antimicrobial efficacy (Antonio *et al.*, 2010). This is attributed to the deposition of CGAs, the main active compound in coffee, during roasting (Fardiaz, 1995). Decaffeination also lowers the efficacy of coffee as an antibacterial agent (Antonio *et al.*, 2010).

In several studies various concentrations of coffee extract have been tested. One of the commonly used formulations is that described by Antonio *et al.*, 2012 to achieve a 20% stock solution (20 g/100 ml) by percolating 100 ml of distilled water through 20 g of coffee powder and filtering the extracts. Other concentrations that have been used range from 100 μ g/ml down to 0.2 μ g/ml (Bharath *et al.*, 2015). The latter study has also shown that coffee at very low concentrations of 0.2 μ g/ml shows antibacterial properties against *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* but shows activity against *F. nucleatum* only at a higher concentration of 3.125 μ g/ml. Overall, it would appear that an increase in the concentration of coffee extract significantly increases the antibacterial activity of coffee. The phenolic compounds, caffeine and mineral concentrations in the coffee canaephora aqueous extract at 20% are presented in the Tables 3 and 4 respectively (Antonio *et al.*, 2010). The current study has used a commercially available ground and medium roasted blend of coffee canaephora. The results have shown that this type of black coffee mouthwash also possesses antimicrobial properties against the periodontal pathogens, *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*, though not as robust as that of the gold standard 0.2% chlorhexidine. This can be anticipated as other studies showing equal efficacy (Mehta *et al.*, 2014) of 20% coffee extract to 0.2% chlorhexidine have used pure green coffee extracts without roasting and measures to ensure highest antimicrobial efficacy. Perhaps the most convincing evidence to date on the clinical effectiveness of coffee on periodontal disease comes from a longitudinal study by Ng *et al.*, (2014) in which they observed a small, but statistically significant reduction in the number of teeth with periodontal bone loss. Moreover, it has also been concluded that though the benefits of coffee towards the periodontium may be currently unclear, another important finding is that its consumption is in no way harmful towards periodontal health (Ng *et al.*, 2014; Duarte *et al.*, 2015). Coffee consumption has also been explored widely in the medical field and various studies have observed benefits such as reduced risk for mortality, (Freedman *et al.*, 2012) type II diabetes, (Ding *et al.*, 2014) and stroke in women (Lopez-Garcia *et al.*, 2009). Therefore, based on the findings of this study, we believe that further clinical trials of short- and long-

term durations hold promise for assessing the clinical efficacy of coffee in the field of periodontics. There are, however, a few drawbacks to the present study. The stock solution of 20% was used based on a study by Antonio *et al.*, 2011 but could have been modified or increased to see if increased concentrations would increase the antibacterial activity or it would achieve a 'ceiling effect'.

Conclusion

In the absence of vigilant oral care, plaque and tartar will build up, resulting in gingivitis and possibly progressing to periodontitis. So, various herbal products have been tried and have shown promising results with minimal side effects. Also, their additional effect on inflammatory pathways and antioxidant potential make them eligible to be used as effective antigingivitis agents. Based on our present findings, we can conclude that commercially available coffee extract does indeed possess antimicrobial activity. However, more clinical trials are required to address its ideal concentration and clinical efficacy in future clinical trials of short and long duration, along with its action on the periodontal biofilm.

REFERENCES

- Almeida AA, Farah A, Silva DA, Nunan EA, Glória MB. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. *J Agric Food Chem.* 2006; 54(23): 8738-43
- Alviano WS, Alviano DS, Diniz CG, Antonioli AR, Alviano CS, Farias LM, Carvalho MA, Souza MM, Bolognese AM. In vitro antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. *Arch Oral Biol.* 2008;53(6): 545-52
- Antonio AG, Iorio NL, Farah A, Netto dos Santos KR, Maia LC. Effect of Coffeacanephora aqueous extract on microbial counts in ex vivo oral biofilms: A case study. *Planta Med.* 2012;78: 755-60
- Antonio AG, Iorio NL, Pierro VS, Candreva MS, Farah A, dos Santos KR, *et al.* Inhibitory properties of Coffeacanephora extract against oral bacteria and its effect on demineralisation of deciduous teeth. *Arch Oral Biol.* 2011;56: 556-64
- Antonio AG, Moraes RS, Perrone D, Maia LC, Santos KR, Iório NL, *et al.* Species, roasting degree and decaffeination influence the antibacterial activity of coffee against *Streptococcus mutans*. *Food Chem.* 2010;118: 782-8
- Bezerra JP, da Silva LR, de AlvarengaLemos VA, Duarte PM, Bastos MF. Administration of high doses of caffeine increases alveolar bone loss in ligature-induced periodontitis in rats. *J Periodontol.* 2008;79: 2356-60
- Bharath N, Sowmya NK, Mehta DS. Determination of antibacterial activity of green coffee bean extract on periodontogenic bacteria like porphyromonasgingivalis, Prevotellaintermedia, Fusobacteriumnucleatum and Aggregatibacteractinomycetemcomitans: An in vitro study. *ContempClin Dent.* 2015;6: 166-9
- Bharath N, Sowmya NK, Mehta DS. Determination of antibacterial activity of green coffee bean extract on periodontogenic bacteria like Porphyromonasgingivalis, Prevotellaintermedia, Fusobacteriumnucleatum and Aggregatibacteractinomycetemcomitans: An in vitro study. *ContempClin Dent.* 2015;6: 166-9
- Daglia M, Papetti A, Grisoli P, Aceti C, Spini V, Dacarro C, Gazzani G. Isolation, identification, and quantification of roasted coffee antibacterial compounds. *J Agric Food Chem.* 2007;55(25): 10208-13
- Ding M, Bhupathiraju SN, Chen M, van Dam RM, Hu FB. Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: A systematic review and a dose-response meta-analysis. *Diabetes Care.* 2014;37: 569-86
- Duarte PM, Reis AF. Coffee consumption has no deleterious effects on periodontal health but its benefits are uncertain. *J Evid Based Dent Pract.* 2015;15: 77-9
- Fardiaz S. Antimicrobial activity of coffee (Coffearobusta) extract. *ASEA Food J.* 1995;10: 103-6
- Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. *N Engl J Med.* 2012;366: 1891-904
- Gupta D, Gupta RK, Bhaskar DJ, Gupta V. Comparative Evaluation of Terminaliachebula Extract Mouthwash and Chlorhexidine Mouthwash on Plaque and Gingival Inflammation - 4-week Randomized Control Trial. *Oral Health Prev Dent.* 2015;13(1): 5-12
- Gupta RK, Gupta D, BhaskarD,Yadav A, Obaid K, Mishra S. Preliminary antiplaque efficacy of aloe vera mouthwash on 4 day plaque re-growth model: Randomized control trial. *Ethiop J Health Sci.* 2014;24: 139-44
- Horrigan LA, Kelly JP, Connor TJ. Immunomodulatory effects of caffeine: Friend or Foe? *PharmacolTher.* 2006;111: 877-92
- Johnston KL, Clifford MN, Morgan LM. Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *Am J ClinNutr.* 2003;78: 728-33
- Li M, Xu Z. Quercetin in a lotus leaves extract may be responsible for antibacterial activity. *Arch Pharm Res.* 2008;31: 640-4
- Li XY, Xu L, Lin GS, *et al.* Protective effect of caffeine administration on myocardial ischemia/reperfusioninjuryin rats. *Shock.* 2011;36: 289-94
- Loe, H, Theilade E, Jensen SB. Experimental gingivitis in man. *J Periodontol* 1965; 36:177-87
- Lopez-Garcia E, Rodriguez-Artalejo F, Rexrode KM, Logroscino G, Hu FB, van Dam RM. Coffee consumption and risk of stroke in women. *Circulation.* 2009;119: 1116-23
- Lv X, Chen Z, Li J, *et al.* caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress. *Inflamm Res.* 2010;59: 635-45
- Meckelburg N, Pinto KC, Farah A, Iorio NL, Pierro VS, dos Santos KR, *et al.* Antibacterial effect of coffee: Calcium concentration in a culture containing teeth/biofilm exposed to CoffeaCanephora aqueous extract. *LettApplMicrobiol.* 2014;59: 342-7
- Mehta VV, Rajesh G, Rao A, Shenoy R, Pai M. Antimicrobial efficacy of Punicagranatum mesocarp, Nelumbonucifera leaf, Psidiumguajava leaf and Coffeacanephora extract on common oral pathogens: An invitro study. *J ClinDiagn Res.* 2014;8: ZC65-8
- Ng N, Kaye EK, Garcia RI. Coffee consumption and periodontal disease in males. *J Periodontol.* 2014;85: 1042-9
- Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumura M, Sobue S, Matsumoto M. Caries

- inhibitory activity of cacao bean husk extract in in-vitro and animal experiments. *Arch Oral Biol.* 2000; 45(8): 639-45
- Oshima T, Miyakawa Y, Watanabe T, Ohshima K. Effect of amphotericin B dilution with beverages on the survival of *Candida albicans* cells. *KansenshogakuZasshi.* 2003;77: 29-33
- Salgado AD, Maia JL, Pereira SL, de Lemos TL, Mota OM. Antiplaque and antigingivitis effects of a gel containing *Punicagranatum* Linn extract: a double-blind clinical study in humans. *J Appl Oral Sci.* 2006;14:162-6
- Sharma R, Reddy VK, Prashant G, Ojha V, Kumar NP. Antimicrobial and anti-adherence activity of various combinations of coffee-chicory solutions on *Streptococcus mutans*: An in-vitro study. *J Oral MaxillofacPathol.* 2014;18: 201-6
- Toda M, Okubo S, Hiyoshi R, Shimamura T. The bactericidal activity of tea and coffee. *LettApplMicrobiol.* 1989;8:123-5
- Zou L, Zhang L, Li J, Hao Y, Cheng L, Li W, Zhou X. Effect of *Gallachinensis* extract and chemical fractions on demineralization of bovine enamel in vitro. *J Dent.* 2008; 36(12): 999-1004
