INTRODUCTION
Dental plaque is defined as a highly specific variable structural entity formed by sequential colonization of microorganisms on the tooth surface, epithelium, and restorations. It is also defined as soft deposits that form the biofilm adhering to the tooth surface or other hard surfaces in the oral cavity including removable and fixed restorations. Dental plaque is researched extensively and proved to be the paramount factor in the initiation and progression of gingival and periodontal disease.

Dental plaque accumulation is a prerequisite for the development of gingivitis [1]. Gingivitis may progress to periodontitis in susceptible individuals. If prevention of gingivitis is successful, progression to periodontitis can be delayed or prevented. Since both gingivitis and periodontitis are plaque-associated oral conditions, the removal of dental plaque will inhibit their occurrence and progression.

Potential removal of plaque by means of toothbrush remains the most widely accepted method of disease prevention. Continuation of effective personal oral hygiene regimens requires a well-motivated patient who does oral hygiene practices in a proper fashion for a sufficient duration of time and with adequate frequency. Chemical plaque control is considered as an adjunct to mechanical oral hygiene practices, and these agents are used in the form of mouth rinse.

Phytotherapy has been practiced in India since ages. There are many herbal agents used in toothpaste which serve as excellent antiplaque agents, namely triphala, meswak, tulsi, and aloe vera. Studies are also done using Garcinia kola stem wood extract in toothpaste which has shown very good antibacterial property and can be used for personal oral hygiene [2].

Ganoderma lucidum is a Basidiomycetes fungus belonging to the family Polyporaceae [4]. It has been used for more than thousand years for its medicinal properties in Traditional Chinese Medicine. It has many ranges of nutritional and health benefits, was assessed for its oxidative properties in the healing of oral submucous fibrosis and leukoplakia. Radhika et al. assessed its antiplaque efficacy when used in mouthwash.

MATERIALS AND METHODS
The present work was an in vitro study. Pooled saliva was collected in a sterile container from the volunteers after taking the consent. The culture plate with 12 (3 × 4) wells was chosen. Pooled saliva of 20 mL was added to each well using the micropipette and was kept in the incubator at 37°C for 72 h. After 72 h, saliva was removed without touching the walls or the base of the wells. Each row was treated either with slurry prepared with Ganoderma/herbal/Colgate total toothpaste or herbal/chlorhexidine mouthwash/distilled water. One row of wells was kept as a control using erythrosine dye. After 30 s, all the wells were rinsed with distilled water. Erythrosine dye was added to all the wells, kept for 30 s, and rinsed with distilled water. The tissue culture plate was kept in the ELx800MS machine (ELISA reader) which was set at 540 nm, and the readings were obtained.

Financial support: The present work was supported by Manipal Academy of Higher Education, Manipal, Karnataka, India.

Acknowledgements: The authors are grateful to the principal of Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, India, for providing all the necessary facilities for the study.

Keywords: Ganoderma lucidum, Plaque, Mouthwash, Toothpaste.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). DOI: http://dx.doi.org/10.22159/ajpcr.2018v11i11.27465
72 h, the saliva in each well was removed using a pipette leaving behind the plaque on the walls and base of the wells (Fig. 2).

*G. lucidum* and Himalaya Herbal Complete Care toothpaste were mixed in distilled waters separately to make a smooth slurry, and chlorhexidine 0.2% mouthwash, Himalaya Hiora mouthwash, and distilled water were used for analysis.

For the first row of the tissue culture plate (4 wells), two drops of disclosing agent (erythrosine) were added in each well. After 30 s of adding the disclosing agent, it was pipetted out and rinsed with 20 mL of distilled water using micropipette. This well was taken as a control. The second and third row, *Ganoderma* toothpaste slurry and Himalaya Herbal Complete Care toothpaste slurry, were added and kept for 30 s and rinsed with 20 mL of distilled water using micropipette.

In fourth, fifth, and sixth row, chlorhexidine mouthwash, herbal mouthwash, and distilled water were added and were kept for 30 s and rinsed with distilled water. Two drops of disclosing agent were added in all the wells from second to sixth row and were kept for 30 s and rinsed with 20 mL of distilled water using micropipette.

After the analysis, using various agents, 20 mL of distilled water was added in all the wells of tissue culture plates and kept in the ELx800MS machine (ELISA Reader) (Fig. 3) for the analysis.

The ELx800MS was set at 540 nm as the absorbency range of disclosing agent (erythrosine) is 525–530 nm. The readings were obtained by the printer which was connected to ELx800MS machine. The results were analyzed using SPSS 20 software and tabulated using one-way analysis of variance (ANOVA) followed by post hoc Tukey’s test.

**RESULTS**

Comparison of multiple agents was performed using ANOVA followed by post hoc Tukey’s test, and p value was kept at 0.005. Overall, there was a significant difference in the mean score between the control (Group 1), Group 6, and other Groups (p=0.000). Post hoc analysis showed that Group 1 had a higher mean score than Groups 2–5. Similarly Group 6 had higher mean score than Groups 2–5. This implies that there was less amount of plaque in Groups 2–5. Group 2 showed better results than Groups 4 and 5 (p=0.001 and 0.005). Similarly, Group 3 showed better results than Group 4 (p=0.003). There was no significant difference between Groups 2 and 5. There was no significant difference between Groups 2 and 3 (p=0.965) and between Groups 4 and 5 (p=0.916). (Table 1)

**DISCUSSION**

Plaque is characteristically observed on the gingival third of the tooth surface [10]. A common method for detecting plaque is by the use of a disclosing agent. They are available as tablets, lozenges, or wafers, which contain dye or other coloring agents. The various available disclosing agents are erythrosine (PLAKSEE), two tone dye (alpha plaque), PLAKLITE, skinners iodine, mercurum-chrome solution (0.5%), Bismark brown (Easlick disclosing solution), and malachite green [11].

In the present study, erythrosine was used as a disclosing agent. Erythrosine has a single wavelength, and this can be easily measured using ELISA reader or any colorimetric analysis. Erythrosine is an extremely colored molecule which absorbs light nearly 500 nm and emits longer wavelength. The Lamda max (λ max) of erythrosine was 525 nm, as UV spectrum of erythrosine showed its maximum absorbance at 529 nm by Ramakrishna SP 2007 [12]. Other studies by Tinsley and Chadwich in 1997 said that λ max of erythrosine to be 530 nm [13]. Based on the previous studies, wavelength used in the present study was 540 nm.

*G. lucidum* showed better results than chlorhexidine and Hiora mouthwash. However, there was no statistical difference between the two toothpastes. Supragingival plaque mainly consists of Gram-positive bacteria. *G. lucidum* being a mushroom extract acts mainly on the Gram-positive organisms. Kosanić and Ranković, in 2011, said that Gram-positive bacteria are more susceptible to various mushroom extracts than Gram-negative bacteria, due to the absence of lipoproteins in the cell wall [14]. Turkoglu et al. demonstrated that phenols present in mushroom extracts are the major carriers of antibacterial activity [15]. Karaman et al. reported the inhibitory activity of *Ganoderma applanatum* and *G. lucidum* and chloroform extracts against *Staphylococcus aureus*
other free to interact with bacteria attempting to colonize the tooth surface [21]. It is diacitic at pH levels above 3.5. It prevents plaque accumulation, and hence, it is an antiplaque and anti gingivitis agent [22] and reduces the adherence of Porphyromonas gingivalis to epithelial cells [23]. It can be bacteriostatic or bactericidal depending on the dose. The process of plaque prevention would, therefore, occur at the tooth surface itself by tooth bound chlorhexidine [24,25].

**CONCLUSION**

*G. lucidum* toothpaste has shown good results and can be considered as an effective antiplaque agent. Mechanical plaque control always remains the primary method to maintain oral health. The use of chemical plaque control adjunct to mechanical plaque control aids in improving the oral health of the individual.

**AUTHORS’ CONTRIBUTION**

Dr. Prabhjeet Singh: Protocol preparation, ethical submissions, conducting the study, and review of the final manuscript. Dr. Meena Anand Kukkamalla: Selection of the topic, protocol preparation, ethical submissions, conducting the study, data analysis, drafting the manuscript, and review of the final manuscript. Dr. Jesil Mathew: Providing resource material and review manuscript.

**CONFLICTS OF INTEREST**

There is no conflict of interest in the present study. The study was self-funded.

**REFERENCES**

1. Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. J Periodontol 1965;36:177-87.
2. Stephen YG, Francis A, Maecelt B, Vivian EA, Kofi A. *In-vitro* antimicrobial study of the efficacy of a tooth paste formulated *Garcinia kola* stem wood extract. Int J Pharm Pharm Sci 2010;2:98-101. 
3. Radhika M, Umashankar GK. Effectiveness of spirulina mouthwash on reduction of dental plaque and gingivitis: A clinical study. Int J Pharm Pharm Sci 2017;9:136-9.
4. Hibbert DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson CE, et al. A higher level phylogenetic classification of the Fungi. Mycol Res 2007;111:509-47.
5. Zhu M, Chang Q, Kong L, Wang FS, Li RC. Triterpene antioxidants from *Ganoderma lucidum*. Phytother Res 1999;13:529-31. 
6. Gao Y, Sh Z, Huang M, Xu A. Antibacterial and antiviral value of the genus *Ganoderma P. Karst.* Species (*Aphyllophoromycetidae*): A review. Int J Med Microbiol 2003;5:235-46.
7. Fang JN, Bao XF, Wang XS, Dong Q, Li XY. Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. Phytochemistry 2002;59:175-81.
8. Mau JL, Lin HC, Chen CC. Antioxidant properties of several medicinal mushrooms. J Agric Food Chem 2002;50:6072-7.
9. Nayak A, Nayak RN, Bhat K. Antifungal activity of a toothpaste containing *Ganoderma lucidum* against Candida albicans - *An in vitro* study. J. Int Oral Health 2010;2:51-7.
10. Newman MG, Takei H, Carranza FA. Clinical Periodontology. 9th ed. Philadelphia, PA, USA: WB Saunders; 2005. p. 98. 
11. Wilkins EM. Clinical Practice of Dental Hygienists. 5th ed. Philadelphia, PA: Lea and Febiger Co.; 1983. p. 405-8. 
12. Ramakrishnan SP, Lakshmi JB, Surya PR. Estimation of synthetic dye erythrosine in food stuff and formulation and effect of dye on the protein binding of drug in BSA. Pharm Lett 2011;3:361-73.
13. Tinsley D, Chadwick RG. The permeability of dental groves following exposure to certain dental materials. J Dent 1997;25:65-70.
14. Kosaníc M, Ranković B. Antioxidant and antimicrobial properties of some lichens and their constituents. J Med Food 2011;14:1624-30.
15. Turkoglu A, Duru ME, Mercan N, Kivrak I, Cezar K. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. Food Chem 2007;101:267-73.
16. Karaman M, Jovin E, Malbaša R, Matevuly M, Popović M. Medicinal and edible lichenous fungi as natural sources of antioxidative and antibacterial agents. Phytother Res 2010;24:1473-81.
17. Madhumitha M, Mahendra MC, Prabhad SP. Evaluation of the safety and efficacy of complete care herbal toothpaste in controlling dental
plaque, gingival bleeding and periodontal diseases. J Homeop Ayurv Med 2013;2:100-24.
18. Alali F, Lafi T. GC-MS analysis and bioactivity testing of volatile oil from the leaves of the toothbrush tree Salvadora persica L. Nat Prod Res 2003;17:189-94.
19. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis J Clin Periodontol 1988;15:488-98.
20. Ribeiro LG, Hashizume LN, Maltz M. The effect of different formulations of chlorhexidine in reducing level of mutans streptococci in the oral cavity. J Dent 2007;35:359-70.
21. Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. J Clin Periodontol 1988;15:415-24.
22. Corbet EF, Tam JO, Zee KY, Wong MC, Lo EC, Mombelli AW. Therapeutic effects of supervised chlorhexidine mouthrinses on untreated gingivitis. Oral Dis 1997;3:9-18.
23. Grenier D. Effect of chlorhexidine on the adherence properties of Porphyromonas gingivalis. J Clin Periodontol 1996;23:140-2.
24. Addy M. The use of antiseptics in periodontal therapy. In: Lindhe J, Karring T, Lang N, editors. Clinical Periodontology and Implant Dentistry. Oxford, UK: Blackwell Munksgaard; 2003. p. 476-81.
25. Jones CG. Chlorhexidine: Is it still the gold standard? Periodontol 2000 1997;15:55-62.