

EFFECT OF NEEM EXTRACT ON TOOTH DISCOLOURATION AND MORPHOLOGY

Running title: The effect of neem extract on tooth colour and its morphology.

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ABSTRACT

Background: Neem extract is one of the major traditional medicines of India. It also helps to inhibit the growth of microbes in our oral cavity. Its value as a traditional plant has been passed from generation to generation and the usage of neem sticks as toothbrushes was very common.

Aim: The aim of the present study is to study the effect of neem extract on tooth discoloration and morphology.

Materials and methods: Aqueous extract of neem was prepared using neem powder. Extracted teeth were collected and immersed into the neem extract (n=6) and distilled water (n=6). Tooth measurements were taken daily for three consecutive days using a vernier caliper and photographs of the teeth were also taken to note the changes in the colour of the tooth.

Results: There was no significant change in the tooth measurements and the discoloration of teeth was not evident.

Conclusion: Neem has no harmful effect on teeth. It can be used in toothpastes and mouthwashes for effective microbial inhibition in our oral cavity.

KEYWORDS: Discoloration, innovative technology, neem extract, dental caries, tooth morphology, novel method

INTRODUCTION

Neem is a very popular evergreen tree in many parts of the subcontinent. It is a traditional medicine used by our ancestors for curing and preventing many ailments and also for maintaining a healthy gum. It is now popularly used among the ayurvedic people for the treatment of many diseases (1). They are also used in the treatment of many cancers. They contain glycosides which are an active complex substance which contains carbon, hydrogen and oxygen. They help in the contractile functions of the heart (2,3). Neem shows antibacterial, antifungal, and antiprotozoal effects (3). Neem contains many chemical substances such as glycosides, flavonoids, steroids, anthraquinone, tannic acid etc. These antibiotics are actually the defence mechanisms used against various pathogens (4). Neem commonly known as Margosa, belongs to the *Meliaceae* family, *Meloidea* subfamily. It's a tropical plant with immense potential. Almost every part of this tree is used in one way or the other. It's used to control respiratory disorders, prevention of cavities, intestinal helminthiasis, to control leprosy and also constipation which are controlled using bark and leaf extract. Cultivation of neem is also easy as it is resistant to various climatic and topographical changes (5). Neem extract against some specific bacteria causes infections and disease in human beings. When used continuously it was found that many microbes have developed resistance to antibiotics. The concentration of the neem extract was one of the main parameters which determines the effect of neem on the microbes (5,6). Neem also has its own value in the field of medicine, agriculture and is well known for its pesticide activity (7). The microbes present in our oral cavity release acid during degradation of food particles. These acids are responsible for dental caries as it erodes a layer of the teeth which eventually leads to the formation of dental caries. Studies showed that neem has an inhibiting effect on the growth of these microbes thereby reducing the extent of caries (8).

Tooth discoloration is an esthetic problem which causes yellowing and dull colour of tooth. There are many factors which affect the colour of our teeth from natural to iatrogenic factors. Tooth discoloration due to iatrogenic factors can be reduced by using non staining endodontic treatments (9). Our team has extensive knowledge and research experience that has translated into high quality publications (10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28),(29)

This study aims to find out the effect of neem extract on tooth morphology and tooth discoloration.

MATERIALS AND METHOD

The study was conducted in Saveetha dental college in the Biochemistry department. Aqueous extract of neem was prepared using neem powder. Neem extract was prepared using neem powder and distilled water. Extracted human teeth were collected from the department of the oral maxillofacial surgery. Care was taken to avoid carious teeth and only non carious teeth which were extracted due to periodontal reasons was taken for the study. The teeth were collected and kept in a sterile environment. Now the extracted teeth were washed and one set of teeth (n=6) were placed in distilled water and the others were (n=6) placed in the neem extract. Tooth measurements i.e. buccal length, palatal length, buccal width, palatal width, mesial and distal width were recorded using vernier caliper for monitoring the morphological changes at an interval of 24 hours for 3 days. Photographs were also taken to note colour differences in the teeth.

RESULTS

Prior to placing the teeth in the neem extract the following measurements of the tooth were taken using a vernier caliper.

Measurements of the same tooth placed in neem extract and distilled water were taken for two consecutive days. There was no change in the measurements of teeth for both the samples. Each day a picture was taken for comparison of the colour of teeth. While comparing we found no discolouration of teeth.

Figure 1: Buccal length

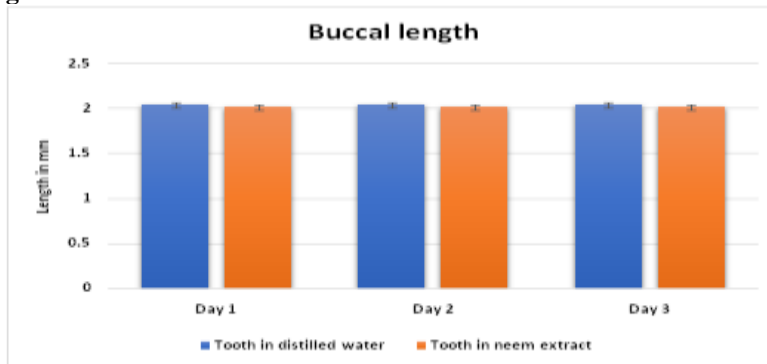


Figure 1: The above graph shows the buccal length measurements of the tooth immersed in neem extract and distilled water. X axis represents the number of days the teeth are immersed and Y axis represents the measurements of buccal length of the teeth immersed in the neem extract and distilled water. Each bar represents a mean \pm SEM of six independent observations. The mean value of buccal length of the tooth immersed in neem is 2.1 cm and that of tooth immersed in distilled water is also 2.1 cm. There observed no significant change in the buccal length measurements of the tooth during the three days.

Figure 2: Palatal length

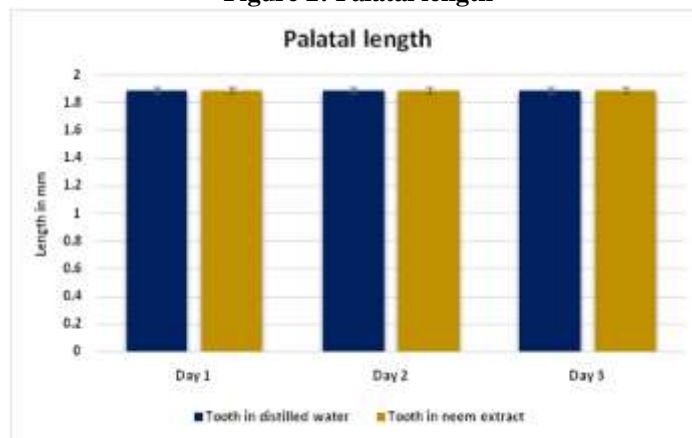


Figure 2: The above graph shows the buccal length measurements of the tooth immersed in neem extract and distilled water. X axis represents the number of days the teeth are immersed and Y axis represents the measurements of palatal length of the teeth immersed in the neem extract and distilled water. Each bar represents a mean \pm SEM of six independent observations. The mean value palatal length of the tooth immersed in neem is 1.95 cm and that of tooth immersed in distilled water is also 1.95 cm. No significant change was observed in the buccal length measurements of the tooth during the three days.

Figure 3: Buccal width

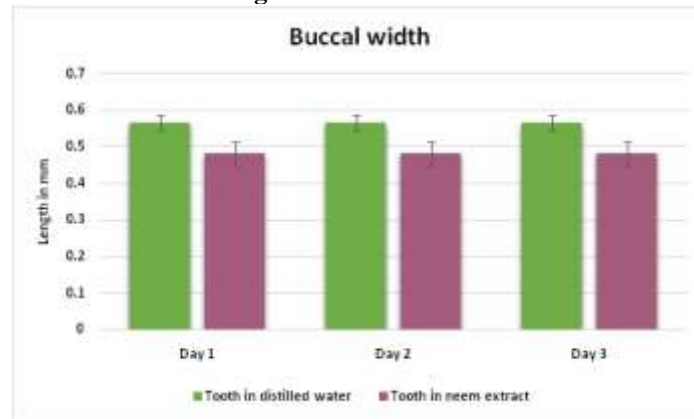


Figure3:The above graph shows the buccal length measurements of the tooth immersed in neem extract and distilled water .X axis represents the number of days the teeth are immersed and Y axis represents the measurements of buccal width of the teeth immersed in the neem extract and distilled water. Each bar represents a mean \pm SEM of six independent observation.The mean value buccal width of the tooth immersed in neem is 0.5 cm and that of tooth immersed in distilled water is 0.6 cm .No significant change was observed in the buccal width measurements of the tooth during the three days.

Figure 4: Palatal width

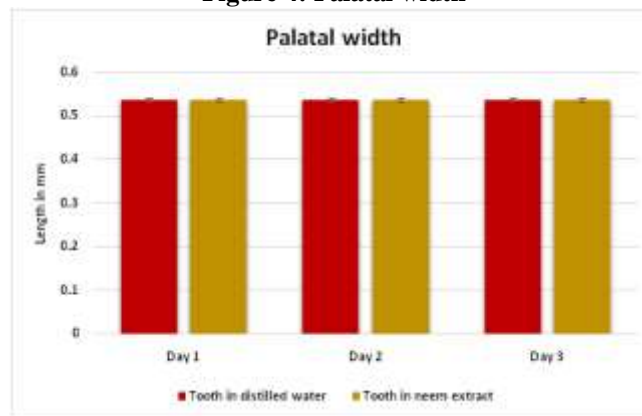


Figure4:The above graph shows the buccal length measurements of the tooth immersed in neem extract and distilled water .X axis represents the number of days the teeth are immersed and Y axis represents the measurements of palatal width of the teeth immersed in the neem extract and distilled water. Each bar represents a mean \pm SEM of six independent observation .The mean value of palatal width of the tooth immersed in neem is 0.55 cm and that of tooth immersed in distilled water is 0.55 cm .No significant change was observed in the palatal width measurements of the tooth during the three days

Figure 5: Mesial width

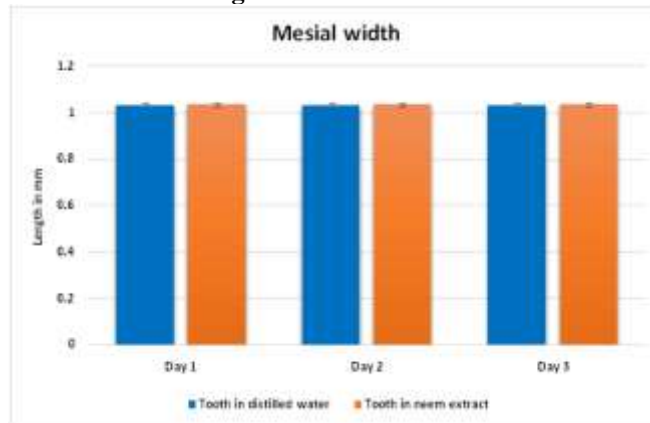


Figure 5: The above graph shows the buccal length measurements of the tooth immersed in neem extract and distilled water. X axis represents the number of days the teeth are immersed and Y axis represents the measurements of mesial width of the teeth immersed in the neem extract and distilled water. Each bar represents a mean \pm SEM of six independent observation. The mean value of mesial width of the tooth immersed in neem is 1.1 cm and that of tooth immersed in distilled water is 1.1 cm. No significant change was observed in the mesial width measurements of the tooth during the three days.

Figure 6: Distal width

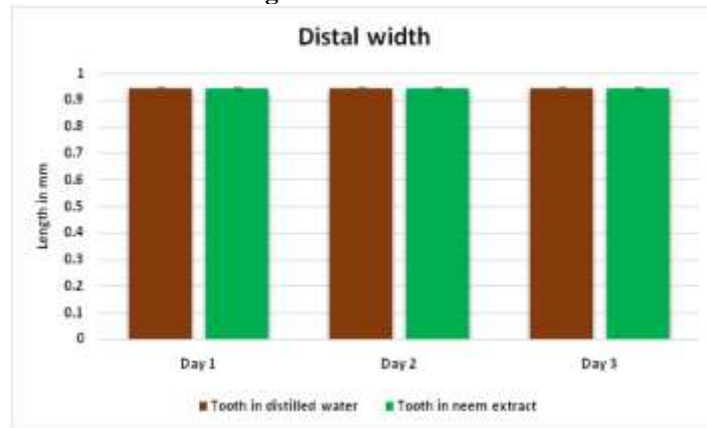


Figure 6: The above graph shows the buccal length measurements of the tooth immersed in neem extract and distilled water. X axis represents the number of days the teeth is immersed and Y axis represents the measurements of distal width of the teeth immersed in the neem extract and distilled water. Each bar represents a mean \pm SEM of six independent observation. The mean value of mesial width of the tooth immersed in neem is 0.95 cm and that of tooth immersed in distilled water is also 0.95 cm. No significant change was observed in the distal width measurements of the tooth during the three days.



Figure 7: Tooth colour before placing in neem extract

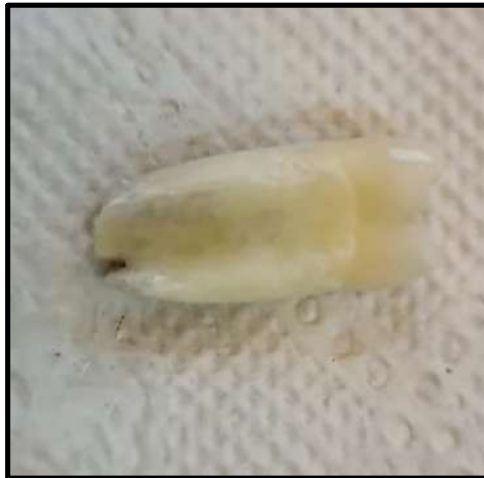


Figure 8: Tooth colour after 3 days exposed to neem extract



Figure 9: Tooth colour before placing in distilled water



Figure 10: Tooth colour after 3 days exposed to distilled water

DISCUSSION

Previous literature found that neem extract reduced the ability of some streptococci to colonize on the tooth surface, thereby reducing the possibility of cavities. These microbes release acids during the degradation of leftover food particles in the oral cavity. These acids were the main factor of dental caries (7,30). A previous study on mango and neem extract showed that the mixture of mango and neem extract was more effective in inhibiting the streptococcus mutans than the individual extracts. The mixture of 50 % mango and neem extract completely inhibited the growth of the microbes (7,30,31). Dental gel containing neem extract was used as a method to reduce the dental caries and it was found that it has significantly reduced the plaque index and bacterial count, which proves its anti-adhesive mechanism (7,30-32). From our study it was evident that there are no significant changes in the buccal width, mesial width, distal width, palatal width, palatal length and buccal length among the teeth immersed in neem extract in all the 3 days (Figure 1-6). There also observed no changes in the colouration of the tooth before and after the exposure of neem extract and distilled water (Figure 7-10). Hence the results showed that neem is harmless to the teeth as there were no changes in tooth morphology and colour of the teeth.

Limitations of the study

Small sample size and less duration are the limitations of the present study.

Future scope

In the future the study can be expanded by increasing the sample size, duration of experiment and inclusion of other characteristics and chemical composition of neem extract to study the effect of neem in detail.

CONCLUSION

The present study revealed the Neem extract does not show any detrimental changes in the morphology of teeth. Thus neem extract has no harmful effect on tooth morphology and colouration. Neem can be used in toothpastes and brushes to maintain a healthier oral cavity.

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Author Contribution :

Indugayathrie VT - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

Vishnu Priya V- contributed in study design, guiding the research work, manuscript correction.

Kavitha S, Gayathrie R, Abhirami Arthanari - study design, statistical analysis, manuscript proofreading and correction.

Conflict of Interest :

The authors hereby declare that there is no conflict of interest in this study.

REFERENCE

1. Kaushik A, Kaushik M, Tanwar R. Ethnomedicine: Applications of Neem (*Azadirachta indica*) in dentistry [Internet]. Vol. 3, Dental Hypotheses. 2012. p. 112. Available from: <http://dx.doi.org/10.4103/2155-8213.103933>
2. Koul O, Wahab S. Neem Biotechnology — A Synthesis [Internet]. *Neem: Today and in the New Millennium*. p. 243–59. Available from: http://dx.doi.org/10.1007/1-4020-2596-3_12
3. Stark JD, Walter JF. Neem oil and neem oil components affect the efficacy of commercial neem insecticides [Internet]. Vol. 43, *Journal of Agricultural and Food Chemistry*. 1995. p. 507–12. Available from: <http://dx.doi.org/10.1021/jf00050a047>
4. Hellerer U, Jarayaman KS. Greens persuade Europe to revoke patent on neem tree... [Internet]. Vol. 405, *Nature*. 2000. p. 266–7. Available from: <http://dx.doi.org/10.1038/35012778>
5. Girish K. Synergistic effect of combinations of fungicides and bacterial extracts against *Phomopsis azadirachtae* causing die-back of neem [Internet]. Vol. 6, *African Journal of Microbiology Research*. 2012. Available from: <http://dx.doi.org/10.5897/ajmr11.1154>
6. Tewari DN. Monograph on Neem (*Azadirachta Indica* A. Juss.). 1992. 279 p.
7. Sharma V, Jakhar KK, Nehra V, Kumar S. Biochemical studies in experimentally *Escherichia coli* infected broiler chicken supplemented with neem (*Azadirachta indica*) leaf extract. *Vet World*. 2015 Nov;8(11):1340–5.
8. Hussain F, Khurshid MF, Masood R, Ibrahim W. Developing antimicrobial calcium alginate fibres from neem and papaya leaves extract. *J Wound Care*. 2017 Dec 2;26(12):778–83.
9. Thomson AD, Athanassiadis B, Kahler B, Walsh L. Tooth discoloration: Staining effects of various sealers and medicaments [Internet]. Vol. 38, *Australian Endodontic Journal*. 2012. p. 2–9. Available from: <http://dx.doi.org/10.1111/j.1747-4477.2011.00339.x>
10. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3297–305.
11. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constituents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating Nrf2 signaling in human epidermal keratinocytes (HaCaT cells). *J Photochem Photobiol B*. 2019 Aug;197:111518.
12. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, *Journal of Photochemistry and Photobiology B: Biology*. 2020. p. 111773. Available from: <http://dx.doi.org/10.1016/j.jphotobiol.2019.111773>
13. Babu S, Jayaraman S. An update on β -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed Pharmacother*. 2020 Nov;131:110702.
14. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from *Albizia lebbek* on HCT-116 colon cancer cell lines. *Artif Cells Nanomed Biotechnol*. 2020 Dec;48(1):1206–13.
15. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3577–84.
16. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomed*

- Pharmacother. 2018 Nov;107:1514–22.
17. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: *Terfezia clavaryi*, *Terfezia boudieri*, and *Tirmania nivea* [Internet]. *Food Reviews International*. 2021. p. 1–20. Available from: <http://dx.doi.org/10.1080/87559129.2021.1889581>
 18. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. *Environmental Nanotechnology, Monitoring & Management*. 2020 Dec 1;14:100333.
 19. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitro and in vivo studies. *Arabian Journal of Chemistry*. 2020 May 1;13(5):5501–16.
 20. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. *Crit Rev Food Sci Nutr*. 2020 Oct 27;1–8.
 21. Barma MD, Muthupandiyani I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol*. 2021 Jun;126:105132.
 22. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent*. 2021 Mar;31(2):285–6.
 23. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent*. 2021 May;31(3):436–41.
 24. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from *Scutellaria baicalensis* on human osteoblast-like MG-63 cells [Internet]. Vol. 119, *Materials Science and Engineering: C*. 2021. p. 111656. Available from: <http://dx.doi.org/10.1016/j.msec.2020.111656>
 25. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. *Environ Toxicol*. 2021 Jun;36(6):1011–20.
 26. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). *Saudi J Biol Sci*. 2021 Jul;28(7):3633–40.
 27. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. *Saudi J Biol Sci*. 2021 Jul;28(7):3641–9.
 28. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15, *Journal of Dental Sciences*. 2020. p. 562–3. Available from: <http://dx.doi.org/10.1016/j.jds.2020.02.009>
 29. Theertha M, Sanju S, Priya VV, Jain P, Varma PK, Mony U. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis. *Cell Mol Immunol*. 2020 Oct;17(10):1114–6.
 30. Wolinsky LE, Mania S, Nachnani S, Ling S. The Inhibiting Effect of Aqueous *Azadirachta indica* (Neem) Extract Upon Bacterial Properties Influencing in vitro Plaque Formation [Internet]. Vol. 75, *Journal of Dental Research*. 1996. p. 816–22. Available from: <http://dx.doi.org/10.1177/00220345960750021301>
 31. Chandu GN, Murulikrishna KS, Shafiulla MD, Prashant GM. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus sanguis*: An in vitro study [Internet]. Vol. 18, *Indian Journal of Dental Research*. 2007. p. 148. Available from: <http://dx.doi.org/10.4103/0970-9290.35822>
 32. Polaquini SRB, Svidzinski TIE, Kimmelmeier C, Gasparetto A. Effect of aqueous extract from *Neem* (*Azadirachta indica* A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by *Candida albicans* [Internet]. Vol. 51, *Archives of Oral Biology*. 2006. p. 482–90. Available from: <http://dx.doi.org/10.1016/j.archoralbio.2005.11.007>