ANTIMICROBIAL EFFECTIVENESS OF ROOT CANAL IRRIGANT FROM MANGOSTEEN PERICARP EXTRACTS WITH PAPAIN AND PROPOLIS EXTRACTS WITH PAPAIN ON MIXTURE OF STREPTOCOCCUS GORDONII AND ENTEROCOCCUS FAECALIS

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Abstract

The purpose of this study was to evaluate antimicrobial effectiveness of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain on mixture of *Streptococcus gordonii* and *Enterococcus faecalis*. Root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain which obtain the concentration of 50 mg/ml as the working solution. The antimicrobial activities of root canal irrigant on mixture of *Streptococcus gordonii* and *Enterococcus faecalis* were tested by the agar well diffusion method, using 2.5% sodium hypochlorite as a positive control group and 50% dimethylsulfoxide and mixture of 50% dimethylsulfoxide with papain as a negative control group. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by using the broth dilution method. The results demonstrated that root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain was effective against mixture of *Streptococcus gordonii* and *Enterococcus faecalis* with the inhibition zone size 11.25±0.66 and 10.42±0.72 mm, respectively. Sodium hypochlorite showed the inhibition zone 14.83±0.36 mm. Inhibition zone of dimethylsulfoxide and mixture of dimethylsulfoxide with papain was not found. MIC of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain were 25 mg/ml and MBC of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain were 50 mg/ml for mixture of *Streptococcus gordonii* and *Enterococcus faecalis*.

Keywords: minimum inhibitory concentration, minimal bactericidal concentration, *Streptococcus gordonii*, *Enterococcus faecalis*, mangosteen pericarp extracts, propolis extracts, papain.

Introduction

Root canal treatment is currently treated by mechanical debridement followed by chemical disinfection. Irrigants are used during the endodontic treatment to flush out loose debris, lubricate the dentinal walls, dissolve organic matter in the canal, and provide antimicrobial activity (Siqueira, Rôças et al. 2002). Sodium hypochlorite (NaOCl), at concentrations between 0.5–6%, is the most popular irrigating solution due to its antimicrobial activity and its ability to dissolve necrotic tissue (Siqueira Jr, Machado et al. 1997). Sodium hypochlorite dissolves proteins and forming chloramines residues on the remaining peptide fragments, thus not only aiding in debridement but also contributing to antimicrobial action. Furthermore, it inactivates the sulfhydryl groups of bacterial enzymes by forming hypochlorous acid(Çalişkan, Türkün et al. 1994). However, it is highly irritating to periapical tissues especially at high concentrations (Becking 1991; Ercan, Özekinci et al. 2004).

The major cause of endodontic failure is the survival of microorganisms in the root-filled teeth. Numerous authors have identified *Enterococcus faecalis* as the predominant microorganism found in root-treated canals displaying persistent periapical disease (Hancock, Sigurdsson et al. 2001). The difficulty in eliminating *Enterococcus faecalis* from the root canal is due to its ability to adapt to environmental changes while retaining its pathogenicity (Stuart, Schwartz et al. 2006). Previous studies report a prevalence of *Enterococcus faecalis* ranging from 24–77% in teeth with failed endodontic treatment (Molander, Reit et al. 1998; Sundqvist, Figdor et al. 1998; Peciuliene, Reynaud et al. 2001; Pinheiro, Gomes et al. 2003).

Streptococcus gordonii are pioneer bacteria that initiate the formation of biofilms on tooth surfaces known as dental plaque. These ubiquitous initial colonizers constitute a majority of the cultivable bacteria found in dental plaque (Nyvad and Kilian 1990). Additionally, *Streptococcus gordonii* mixed with *Enterococcus faecalis* to be exchanged genetic resistance to antibiotics in root canals of teeth (Sedgley, Lee et al. 2008).

Mangosteen (*Garcinia mangostana* L.) is a tropical evergreen tree and is one of the most famous fruits in Thailand. The pericarp of Mangosteen have been widely used for the treatment of diarrhea, skin infection and chronic wounds (Mahabusarakam, Wiriyachitra et al. 1987). Extracts from its pericarp have been demonstrated to possess the antimicrobial activity against a wide variety of microorganisms (Mahabusarakum, Phongpaichit et al. 1983; Sundaram, Gopalakrishnan et al. 1983; Mahabusarakam, Wiriyachitra et al. 1986; Nguyen and Marquis 2011). Previous studies have shown that the extracts from various parts contain varieties of secondary metabolites such as prenylated and oxygenated xanthones. Xanthones is a secondary metabolite found in some higher plant that involves mangosteen (Peres, Nagem et al. 2000). Xanthones could be isolated from peel, whole fruit, bark, and leaves of mangosteen. Several studies also show that extracted xanthones from mangosteen have remarkable biological activities such as antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal, and antiviral activities (Suksamrarn, Komutiban et al. 2006; Pedraza-Chaverri, Cárdenas-Rodríguez et al. 2008).

Propolis (bee glue) is a resinous substance produced by honeybees. The bees mix exudates collected from plants with waxes and glandular secretions to synthesize the substance which is used for the construction and adaptation of their hives (Bankova, de Castro et al. 2000). Bees use propolis as a 'chemical weapon' against pathogenic microorganisms. Humans as a remedy in flok medicine for its health properties as early in Egypt 3000 BC (El Hady and Hegazi 2000). Antimicrobial activity of propolis has been reported by various investigators (Grange and Davey 1990; Park, Koo et al. 1998; Sforcin, Fernandes et al. 2000). It can cure minor ulcers in the mouth, angina, as well as skin infections, therapy of mastitis caused by microorganisms resistant to antibiotics (Oksuz, Duran et al. 2005). Biological properties and chemical compositions of propolis may vary according to different plant sources that bees could visit, collecting time and geographic locations (Bankova, de Castro et al. 2000; Boyanova, Gergova et al. 2005; Gonsales, Orsi et al. 2006). Chemical analysis revealed that propolis contains more than 200 constitutes. Among them are phenolic compounds predominant including flavonoids as a major component (Boyanova, Gergova et al. 2005).

Papain, latex of the leaves and fruit of the green adult papaya, is a proteolytic enzyme. It has bactericide, bacteriostatic and anti-inflammatory characteristics (Osato, Santiago et al. 1993; Mandelbaum, Di Santis et al. 2003). Similarly to the human pepsin, papain acts as a debridant anti-inflammatory agent which does not damage the healthy tissue and accelerates the cicatricial process. Papain is indicated in all phases of the cicatricial process; dry or exudative wounds, colonized or infected, with or without areas of necrosis. Papain promotes chemical debridement, granulation and epithelialization, which hastens the phases of cicatrization, and stimulation of the tensile strength of the scars(Mandelbaum, Di Santis et al. 2003). Papain acts only in infected tissues because infected tissues lack a plasmatic anti protease called a1-anti-trypisin. The a1-antitrypisin is only present in sound tissues and it inhibits protein digestion. The absence of the a1-anti-trypisin in infected tissues allows papain to break the partially degraded collagen molecule (Flindt 1978; Flindt 1979).

The purpose of this study was to evaluate antimicrobial effectiveness of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain on mixture of *Streptococcus gordonii* and *Enterococcus faecalis*.

Materials and methods

Sample preparation

A series of experiments were carried out at oral biology research unit, Faculty of Dentistry, KhonKaen University, Thailand. Root canal irrigant was made from mangosteen pericarp extracts (Club nature, USA) with papain (Across organic, Basil) and propolis extracts (Wealthy health, Australia) with papain. The concentration of papain is 1.5 times of the volume of the solution. Mangosteen pericarp extracts with papain and propolis extracts with papain were dissolved in 50% DMSO to obtain the concentration of 50 mg/ml. The samples were kept in dark bottles with no exposure to bright light, at 4^{o}C, extracts were filtered and ready to use for the study.

Bacterial inoculation

Streptococcus gordonii (DMST 20560) and *Enterococcus feacalis* (DMST 4736) used in this study was obtained from National Institute of Health, Department of Medical Sciences, Ministry of Public Health of Thailand. The bacterial sample was thawed and grown for 24 hours on a solid culture medium (Mitis salivarious agar) at 37 ^{o}C under aerobic conditions. Ten bacterial colonies were placed in 25ml of nutrient broth and incubated for an additional at 37 ^{o}C for 24 hours under aerobic conditions. The purity of the strain was confirmed by Gram's stain. The initial turbidity of optical density (OD) = 0.1 at absorbance of 600 nm by DU 730 Spectrophotometer (Beckman Coulter, USA) was adopted test. *Streptococcus gordonii* and *Enterococcus faecalis* were mixed together for the susceptibility test.

Susceptibility test methods

Susceptibility tests were performed by the Agar well diffusion method of Bauer *et al.* (Bauer, Kirby et al. 1999) with Mitis salivarious agar. The antimicrobial activities of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain on mixture of *Streptococcus gordonii* and *Enterococcus faecalis* were performed in the culture medium. Using 2.5% sodium hypochlorite as a positive control group and 50% DMSO and mixture of 50% DMSO with papain as negative control group. Zones of inhibition were measured after 48 h of incubation at 37 ^{o}C. MIC was determined by a broth microdilution method (Standards 1993). Serial two-fold dilution of the test substances was mixed with nutrient broth in microtiter plates. MIC was recorded as the lowest concentration that had no visible turbidity. MBC was determined by transferring of MIC test tubes and streak on mitis salivarious agar. The plates were incubated for 24 hours. MBC was recorded for each of bacterial strains as a lowest concentration of test substance that had bactericidal activity. MIC and MBC experiments were repeated three times.

Results

Root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain was effective against mixture of *Streptococcus gordonii* and *Enterococcus faecalis* with the inhibition zone size 11.25±0.66 mm and 10.42±0.72 mm, respectively. Sodium hypochlorite showed the inhibition zone size 14.83±0.36 mm. Inhibition zone of dimethylsulfoxide and mixture of dimethylsulfoxide with papain was not found (Figure1). MIC of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain were 25 mg/ml and MBC of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain were 50 mg/ml (Table1).

Table 1 MIC and MBC of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain

Type of root canal irrigants	MIC (mg/ml)	MBC (mg/ml)
Mangosteen pericarp extracts with papain	25	50
Propolis extracts with papain	25	50
2.5% sodium hypochlorite	3.13	3.13
50% dimethylsulfoxide	250	ND
Mixture of 50% dimethylsulfoxide with papain	250	ND

ND = Not Detected

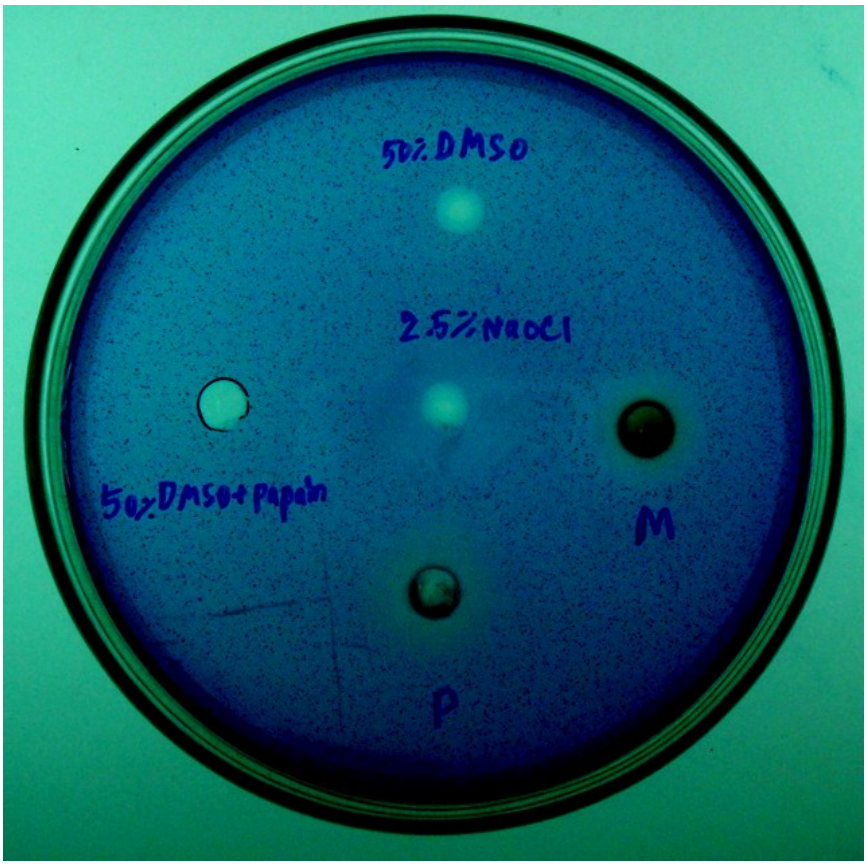

Figure1. Inhibition zone on mixture of *Streptococcus gordonii* and *Enterococcus faecalis* by Agar well diffusion method (M = mangosteen pericarp extracts with papain , P = propolis extracts with papain)

Discussion

The results of this experiment showed that root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain was very effective in killing the tested microorganisms. These findings are in agreement with those of others(Mahabusarakum,

Phongpaichit et al. 1983; Sundaram, Gopalakrishnan et al. 1983; Mahabusarakam, Wiriyachitra et al. 1986; Iinuma, Tosa et al. 1996; Suksamrarn, Suwannapoch et al. 2003; Bruschi, Lara et al. 2006; Kitti Torrungruang, Piraporn Vichienroj et al. 2007; Ophori, Eriagbonye et al. 2010)

Enterococcus faecalis is among the few facultative anaerobic microorganisms, often associated with persistent apical periodontitis, was chosen for the test organism in this study because it was difficult to eliminate from root canals(Haapasalo and Ørstavik 1987). *Streptococcus gordonii* was chosen as the test organism because it can exchange genetic resistance to antibiotics when mixture with *Enterococcus faecalis* in root canals of teeth (Sedgley, Lee et al. 2008).

The extracts from mangosteen pericarp have been known for its broad-spectrum antibacterial activity against several Gram-positive and Gram-negative bacteria, especially those associated with skin infection, diarrhea, tuberculosis or acne (Mahabusarakum, Phongpaichit et al. 1983; Sundaram, Gopalakrishnan et al. 1983; Mahabusarakam, Wiriyachitra et al. 1986; Iinuma, Tosa et al. 1996; Suksamrarn, Suwannapoch et al. 2003). Among xanthone derivatives from mangosteen extracts, α-mangostin have been known to exert the most potent antimicrobial activity (Mahabusarakum, Phongpaichit et al. 1983; Sundaram, Gopalakrishnan et al. 1983; Mahabusarakam, Wiriyachitra et al. 1986; Iinuma, Tosa et al. 1996; Suksamrarn, Suwannapoch et al. 2003; Chomnawang, Surassmo et al. 2005). Kitti *et al*. (Kitti Torrungruang, Piraporn Vichienroj et al. 2007) showed the antibacterial activity of mangosteen pericarp extracts against cariogenic *Streptococcus mutans* .

The antimicrobial activity of propolis against *Enterococcus faecalis* have been reported(Bruschi, Lara et al. 2006) but *Streptococcus gordonii* have not been reported. The result in Table 1 showed the antimicrobial activity of propolis to mixture of *Streptococcus gordonii* and *Enterococcus feacalis*. It has been reported that the antimicrobial activity of propolis is as a result of the high content of flavonoids present (Ophori, Eriagbonye et al. 2010). However, this activity varies according to geographical regions and pH of the culture medium (Meresta and Meresta 1980; Glinski and Meresta 1993). The presence of flavonoids and derivatives of caffeic acid were associated with the bactericidal activity (Bosio, Avanzini et al. 2000). The mechanism of antibacterial action of propolis has been mentioned only in a few publications. Takalsi-Klkuni and Schilcher (Takaisi-Kikuni and Schilcher 1994) shown through electron microscopy and micro-calorimetric assays that propolis extracts interferes with the division of *Streptococcus* through the formation of pseudomulticellular forms, cytoplasm disorganization, inhibition of protein synthesis leading to lysis of the bacteria.

Papain is the main ingredient of Papacarie, a gel used for chemomechanical dental caries removal. Besides the advantage of avoiding the use of rotary cutting tools, it does not interfere in the bond strength of restorative materials to dentin(Lopes, Mascarini et al. 2007). Papain was chosen for the test in this study because it facilitates the cleaning necrotic tissues and does not damage the sound tissues.

Conclusion

Root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain was effective against mixture of *Streptococcus gordonii* and *Enterococcus faecalis,* with the inhibition zone size 11.25±0.66 mm. and 10.42±0.72 mm. respectively. Both extracts have MIC value at 25 mg/ml and MBC value at 50 mg/ml. Suggestion for further study is needed to verify its efficiency for its laboratory and clinical use in infected root canals.

References:

1. Bankova, V. S., S. L. de Castro, et al. (2000). "Propolis: recent advances in chemistry and plant origin." Apidologie 31(1): 3-16.
2. Bauer, A., W. Kirby, et al. (1999). "1966 Antibiotic Susceptibility Testing by a Standardized Single Disk Method." Microbiology: a centenary perspective: 40.
3. Becking, A. G. (1991). "Complications in the use of sodium hypochlorite during endodontic treatment:: Report of three cases." Oral Surgery, Oral Medicine, Oral Pathology 71(3): 346-348.
4. Bosio, K., C. Avanzini, et al. (2000). "In vitro activity of propolis against Streptococcus pyogenes." Letters in applied microbiology 31(2): 174-177.
5. Boyanova, L., G. Gergova, et al. (2005). "Activity of Bulgarian propolis against 94 Helicobacter pylori strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods." Journal of medical microbiology 54(5): 481-483.
6. Bruschi, M., E. Lara, et al. (2006). "Preparation and Antimicrobial Activityof Gelatin Microparticles Containing Propolis Against Oral Pathogens." Drug development and industrial pharmacy 32(2): 229-238.
7. Çalişkan, M., M. Türkün, et al. (1994). "Allergy to sodium hypochlorite during root canal therapy: acase report." International Endodontic Journal 27(3): 163-167.
8. Chomnawang, M. T., S. Surassmo, et al. (2005). "Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria." Journal of Ethnopharmacology 101(1): 330-333.
9. El Hady, F. K. A. and A. G. Hegazi (2000). "Egyptian propolis: 2. Chemical composition, antiviral and antimicrobial activities of East Nile Delta propolis." Extraction.
10. Ercan, E., T. Özekinci, et al. (2004). "Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: in vivo study." Journal of endodontics 30(2): 84-87.
11. Flindt, M. (1978). "Health and safety aspects of working with enzymes." Process biochemistry 13(8): 3-7.
12. Flindt, M. (1979). "Allergy to alpha-amylase and papain." Lancet 1(8131): 1407.
13. Glinski, Z. and T. Meresta (1993). "Trials to standardize an antibacterial activity of propolis." Medycyna Weterynaryjna 49: 27-27.
14. Gonsales, G., R. Orsi, et al. (2006). "Antibacterial activity of propolis collected in different regions of Brazil." Journal of Venomous Animals and Toxins including Tropical Diseases 12(2): 276-284.
15. Grange, J. and R. Davey (1990). "Antibacterial properties of propolis (bee glue)." Journal of the Royal Society of Medicine 83(3): 159.
16. Haapasalo, M. and D. Ørstavik (1987). "In vitro Infection and of Dentinal Tubules." Journal of Dental Research 66(8): 1375-1379.

17. Hancock, H., A. Sigurdsson, et al. (2001). "Bacteria isolated after unsuccessful endodontic treatment in a North American population." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics 91(5): 579-586.
18. Iinuma, M., H. Tosa, et al. (1996). "Antibacterial activity of xanthones from guttiferaeous plants against methicillin‐resistant Staphylococcus aureus." Journal of pharmacy and pharmacology 48(8): 861-865.
19. Kitti Torrungruang, Piraporn Vichienroj, et al. (2007). "Antibacterial activity of mangosteen pericarp extract against cariogenic Streptococcus mutans." CU Dent J 30: 1-10.
20. Lopes, M. C., D. D. S. R. C. Mascarini, et al. (2007). "Effect of a papain-based gel for chemomechanical caries removal on dentin shear bond strength." group 1: 3.
21. Mahabusarakam, W., P. Wiriyachitra, et al. (1986). "Antimicrobial activities of Chemical Constituents from Garcinia mangostana Linn." J. Sci. Soc. Thailand, pp. 12: 239-242.
22. Mahabusarakam, W., P. Wiriyachitra, et al. (1987). "Chemical constituents of Garcinia mangostana." Journal of natural products 50(3): 474-478.
23. Mahabusarakum, W., S. Phongpaichit, et al. (1983). "Screening of antibacterial activity of chemicals from Garcinia mangostana." Songklanakarin Journal of Science and Technology 5: 337-339.
24. Mandelbaum, S. H., É. P. Di Santis, et al. (2003). "Cicatrização: conceitos atuais e recursos auxiliares-Parte I Cicatrization: current concepts and auxiliary resources-Part I." An Bras Dermatol 78(4): 393-410.
25. Meresta, L. and T. Meresta (1980). "Effect of pH on bactericidal activity of propolis." Bulletin of the Veterinary Institute in Pulawy 24(1-4): 21-25.
26. Molander, A., C. Reit, et al. (1998). "Microbiological status of root‐filled teeth with apical periodontitis." International Endodontic Journal 31(1): 1-7.
27. Nguyen, P. T. M. N. P. T. M. and R. E. M. R. E. Marquis (2011). "Antimicrobial actions of α-mangostin against oral streptococci." Canadian Journal of Microbiology 57(3): 217-225.
28. Nyvad, B. and M. Kilian (1990). "Comparison of the initial streptococcal microflora on dental enamel in caries-active and in caries-inactive individuals." Caries research 24(4): 267-272.
29. Oksuz, H., N. Duran, et al. (2005). "Effect of Propolis in the Treatment of Experimental Staphylococcus aureus Keratitis in Rabbits." Ophthalmic Research 37(6): 328-334.
30. Ophori, E., B. Eriagbonye, et al. (2010). "Antimicrobial activity of propolis against Streptococcus mutans." Afr. J. Biotechnol 9(31): 4966-4969.
31. Osato, J. A., L. A. Santiago, et al. (1993). "Antimicrobial and antioxidant activities of unripe papaya." Life sciences 53(17): 1383-1389.
32. Park, Y. K., M. H. Koo, et al. (1998). "Antimicrobial activity of propolis on oral microorganisms." Current microbiology 36(1): 24-28.
33. Peciuliene, V., A. Reynaud, et al. (2001). "Isolation of yeasts and enteric bacteria in root‐filled teeth with chronic apical periodontitis." International Endodontic Journal 34(6): 429-434.
34. Pedraza-Chaverri, J., N. Cárdenas-Rodríguez, et al. (2008). "Medicinal properties of mangosteen (Garcinia mangostana)." Food and chemical toxicology 46(10): 3227-3239.
35. Peres, V., T. J. Nagem, et al. (2000). "Tetraoxygenated naturally occurring xanthones." Phytochemistry 55(7): 683-710.

36. Pinheiro, E., B. Gomes, et al. (2003). "Microorganisms from canals of root ‐ filled teeth with periapical lesions." International Endodontic Journal 36(1): 1-11.
37. Sedgley, C. M., E. H. Lee, et al. (2008). "Antibiotic Resistance Gene Transfer between Streptococcus gordonii and Enterococcus faecalis in Root Canals of Teeth Ex Vivo." Journal of endodontics 34(5): 570-574.
38. Sforcin, J., A. Fernandes, et al. (2000). "Seasonal effect on Brazilian propolis antibacterial activity." Journal of Ethnopharmacology 73(1): 243-249.
39. Siqueira, J. F., I. N. Rôças, et al. (2002). "Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals." Journal of endodontics 28(3): 181-184.
40. Siqueira Jr, J., A. Machado, et al. (1997). "Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro." International Endodontic Journal 30(4): 279-282.
41. Standards, N. C. f. C. L. (1993). "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically." Wayne (PA)(^ s1).
42. Stuart, C. H., S. A. Schwartz, et al. (2006). " Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment." Journal of endodontics 32(2): 93-98.
43. Suksamrarn, S., O. Komutiban, et al. (2006). "Cytotoxic prenylated xanthones from the young fruit of Garcinia mangostana." Chemical and pharmaceutical bulletin 54(3): 301-305.
44. Suksamrarn, S., N. Suwannapoch, et al. (2003). "Antimycobacterial activity of prenylated xanthones from the fruits of Garcinia mangostana." Chemical and pharmaceutical bulletin 51(7): 857-859.
45. Sundaram, B., C. Gopalakrishnan, et al. (1983). "Antimicrobial activities of Garcinia mangostana." PLANT. MED. 48(1): 59-60.
46. Sundqvist, G., D. Figdor, et al. (1998). "Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 85(1): 86-93.
47. Takaisi-Kikuni, N. B. and H. Schilcher (1994). "Electron Microscope and Microcalorimetric Investigations of the Possible Mechanism of the Antibacterial Action of a Defined Propolis Provenance." Planta Medica-Journal of Medicinal Plant Research 60(3): 222-227.