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COMPARATIVE EVALUATION OF NEEM EXTRACT ,LEMON GRASS EXTRACT AND SODIUM HYPOCHLORITE AS AN IRRIGANT AGAINST ENTERCOCCUS FAECALIS ,STERTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS– AN INVITRO STUDY

Dental Science

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ABSTRACT

The aim of this study was to evaluate the antimicrobial activity of auxiliary chemical substances and natural extracts such as neem extract ,lemon grass oil and sodium hypochlorite on lactobacillus ,streptococcus mutans and Enterococcus faecalis inoculated in root canals

MATERIALS AND METHOD

Antibacterial efficacy of neem extract and citronella oil was evaluated using sodium hypochlorite and physiological saline as control on *Lactobacillus acidophilus, Streptococcus mutans* and *Enterococcus faecalis* by plate assay with well diffusion technique.A comparative bioactivity-guided fractionation of two medicinal plants and sodium hypochlorite was carried out using solvents with different concentrations in methanol. Cultures were spread over the surface of the plate, and wells were punched out in every plate. Each well was inoculated by each extract and incubated for 24 h at 37 0 C and the zone of inhibition around each well was recorded.. Data presented as mean and standard deviation values. One-way analysis of variance (ANOVA) was used for comparisons between more than two groups. Tukey's post-hoc test was used for pair-wise comparison between the groups when ANOVA test was significant.

RESULT: Sodium hypochlorite was taken as a positive control, for comparing the efficacy of the herbal extracts. Neem extract manifested similar antimicrobial efficacy like Sodium hypochlorite. Sodium hypochlorite showed good zone of inhibition against all selected microorganisms.

CONCLUSION: In the current study comparative analysis of the antibacterial efficacy of lemongrass oil and neem extract was made with Sodium hypochlorite and physiological saline as controls against Enterococcus faecalis, Lactobacillus acidophilus and Streptococcus mutans. Neem extract showed better results compared to the lemongrass oil. Hence neem extract can be used as root canal irrigant. Outcomes of the study indicated the efficacy of neem extract as better than the controls.

KEYWORDS

INTRODUCTION

Complete debridement and elimination of all microbial irritants, microorganisms and their toxins, is a fundamental prerequisite for successful endodontic therapy.[1] This goal cannot be achieved by mechanical instrumentation alone because of the complex root canal anatomy which provides an ideal environment for microorganisms to survive and continue their pathological process.[2].The chemo-mechanical preparation would give proper debridement and appropriate disinfection of the root canals.[3]

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Endodontic infections have a polymicrobial nature, with obligate anaerobic bacteria dominating in primary infections.

The main microorganisms that are isolated before a root canal treatment include Gram-negative anaerobic rods,Gram-positive anaerobic cocci, Gram-positive anaerobic and facultative rods, Lactobacillus species, and Gram-positive Streptococcus species(4)

Enterococcus faecalis (*E. faecalis*) is a Gram positive facultative anaerobic bacterium found in the human normal flora.(5,6) *E. faecalis* is usually present in persistent periradicular lesions after root canal in endodontics . It is one of the most frequent isolated microorganisms from endodontic infections. This is due to various survival and virulence factors; including its ability to compete with other microorganisms, invasion of dentinal tubules and resistance to nutritional deprivation.[7,8]Commonly seen in failed root canal

Sodium hypochlorite is the most common endodontic irrigant used because they destroy wide spectrum of microorganism. It has undesirable properties such as high toxicity, unpleasant taste, corrosive to instruments, inability to remove the inorganic portion of smear layer and reduction in elastic modulus and flexural strength of dentin.(9)

Because of the cytotoxic reactions of most of the commercial irrigants used and their inability to totally eliminate bacteria from root canals, biologic irrigants extracted from natural plants are used recently .[10].

Azadirachta indica (Neem) is the most commonly used traditional medicinal plant of India.. Neem leaf and its constituents such as limonoids have been demonstrated to exhibit anti-ioxidant, antifungal, and anticarcinogenic properties.The isoprenoid group of neem leaf and its constituents have demonstrated anti-inflammatory,immune-modulatory, antibacterial, antifungal properties(11,12)

Cymbopogon citratus (lemon grass) has shown to have favorable antibacterial, antifungal , anti-inflammatory, analgesic , anti-oxidant and cytoprotective activities .It also has been demonstrated that it had strong effects on gram positive and gram negative bacteria.it shows antimicrobial activity against standard and clinical strains of streptococci and is attributed to the presence of citral compounds (13)

AIM AND OBJECTIVE

The aim of the study is to evaluate the antimicrobial property of sodium hypochlorite, Neem extract and Lemon grass oil against the *Lactobacillus acidophilus, Streptococcus mutans* and *Enterococcus faecalis.*

MATERIALS AND METHOD OF STUDY

MATERIALS OF STUDY

PREPARATION

NEEM LEAF EXTRACT

Neem extract preparation: 5g of commercially available neem powder was taken and mixed with 20 ml of methanol. After overnight incubation, the extract was centrifuged and the supernatant was extracted. The extract was oven dried to remove the methanol content. Then the dried extract was dissolved in 2 ml of dimethyl sulfoxide. This mixture was used for the antimicrobial analysis

SODIUM HYPOCHLORITE

- 5.25% Sodium hypochlorite was used

LEMON GRASS OIL

commercially available lemongrass oil was used.

0.9% physiological Saline: Commercially available as control group

METHOD OF STUDY

Samples will be segregated into 4 groups

Group1-5.25%NaOCl

Group 2-lemon grass extract

Group3-neem extract

Group4-saline

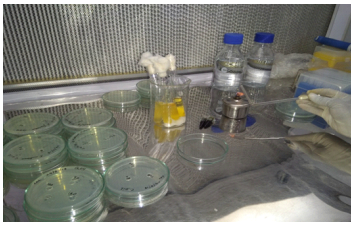
Procedure:

Primary culture preparation: Nutrient broth was prepared to inoculate L*actobacillus acidophilus* (clinical isolate) and *Enterococcus faecalis ATCC 29213*. Luria Bertani broth for *Streptococcus mutans* (clinical isolate) and incubated at 37° C for 24hrs.

Nutrient agar plates were prepared and 4 wells were made for antimicrobial assay.Pre-cultured microbial samples were spread into the plate before adding the antibacterial agents.50 µl, 100 µl and 150 µl of antimicrobial agents were added in their respective wells and the plates were incubated for 24hrs at 37℃ After 24 hrs incubation, the zone of inhibition of each sample was determined.The study was performed 3 times and mean zone of inhibition was recorded in millimetre

Commercially available neem powder

Lemon grass used to take extract



Preparation of culture plates and inoculation

RESULT

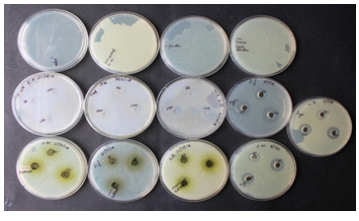


Fig 1: Zone of inhibition of *Enterococcus faecalis, Lactobacillus acidophilus* and *Streptococcus mutans* against neem extract, citronella oil and sodium hypochlorite.

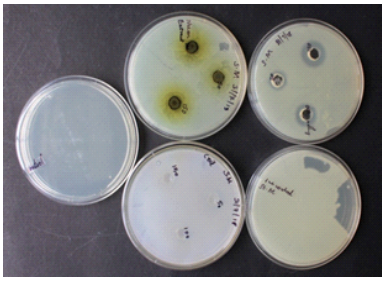


Fig 2: Zone of inhibition of *Streptococcus mutans* against neem extract, citronella oil and sodium hypochlorite.

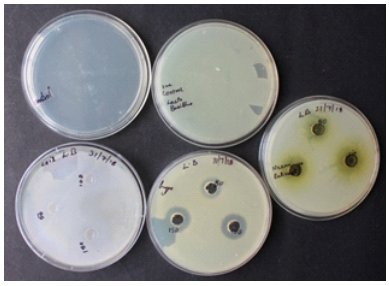


Fig 3: Zone of inhibition of *Lactobacillus acidophilus* against neem extract, citronella oil and sodium hypochlorite.

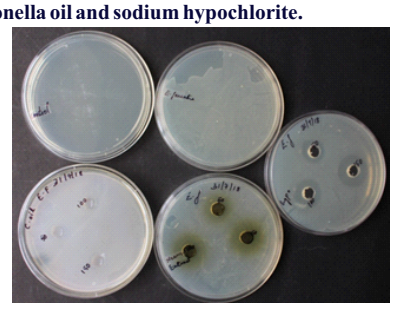


Fig 4:Zone of inhibition of *Enterococcus faecalis* against neem extract, citronella oil and sodium hypochlorite.

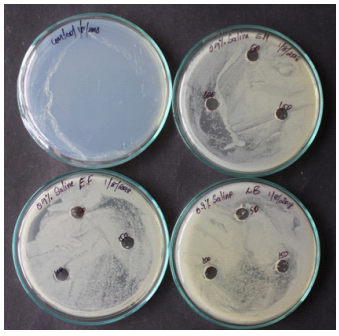


Fig 5: Average of zone of inhibition in Saline

Table 1: zone of inhibition of *Enterococcus faecalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* against neem extract

Sl. no	Antibacterial agent-Neem extract	Zone of Inhibition(cm)					
		Enterococcus faecalis		*Lactobacillus acidophilus*		*Streptococcus mutans*	
		1	2	1	2	1	2
1.	50µl	1.7	1.6	1.8	1.8	1	0.2
2.	100µl	2.1	1.9	1.9	1.9	0	0.9
3.	150µl	2.2	1.7	2.1	2.2	1.9	0.3

Table 2: Average zone of inhibition of *Enterococcus faecalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* against neem extract

Sl. no	Antibacterial agent-Neem extract	Avg Zone of Inhibition(cm)		
		Enterococcus faecalis	*Lactobacillus acidophilus*	*Streptococcus mutans*
4.	50µl	1.65	1.8	0.6
5.	100µl	2	1.9	0.45
6.	150µl	1.95	1.5	1.1

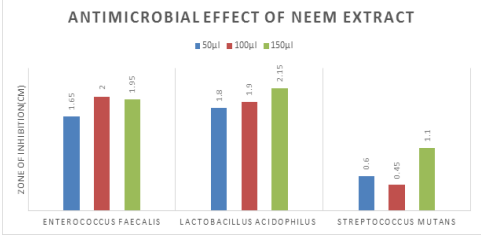


Fig 13: Average zone of inhibition in neem extract

Inference:

1. Neem extract showed good antimicrobial activity against all the organisms used in the analysis. Furthermore, 100µl of neem extract exhibited consistent antimicrobial effect. Hence it is recommended to use herbal neem extract in dental procedures.

Table 3: Zone of inhibition of *Enterococcus faecalis, Lactobacillus acidophilus* and *Streptococcus mutans* against lemongrass oil

Sl. no	Antibacterial agent-Lemongrass oil	Zone of Inhibition(cm)					
		Enterococcus faecalis		*Lactobacillus acidophilus*		*Streptococcus mutans*	
		1	2	1	2	1	2
7.	50µl	0	0.3	3	0.2	0	0
8.	100µl	0	0.4	3	0.2	0	0.2
9.	150µl	0	0.5	3.3	0.3	0	0.2

Table 4: Average zone of inhibition of *Enterococcus faecalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* against lemongrass oil

Sl. no	Antibacterial agent-Lemongrass oil	Avg Zone of Inhibition(cm)		
		Enterococcus faecalis	*Lactobacillus acidophilus*	*Streptococcus mutans*
10.	50µl	0.15	1.6	0
11.	100µl	0.2	1.6	0.1
12.	150µl	0.25	1.8	0.1

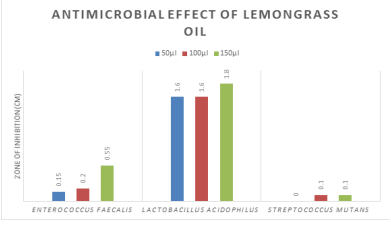


Fig14: Average zone of inhibition in lemongrass oil

Inference:

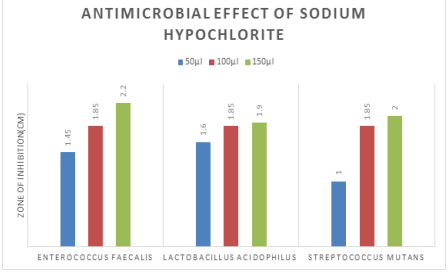
The above analysis proves lemon grass oil is not as effective as neem extract. It showed antimicrobial effect only to *E. faecalis*. While *L. acidophilus* and *S. mutans* exhibited resistance to lemon grass oil.

Table 5: Zone of inhibition of *Enterococcus faecalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* against Sodium Hypochlorite

Sl. no	Antibacterial agent- Sodium Hypochlorite	Zone of Inhibition(cm)					
		Enterococcus faecalis		*Lactobacillus acidophilus*		*Streptococcus mutans*	
		1	2	1	2	1	2
13.	50µl	1.5	1.4	1.5	1.7	1.4	1.6
14.	100µl	1.7	2.0	1.9	1.8	1.8	1.6
15.	150µl	2.2	2.2	1.9	1.9	2.1	1.9

Table 6: Average zone of inhibition of Enterococcus faecalis, Lactobacillus acidophilus and Streptococcus mutans against Sodium hypochlorite

Sl.no	Antibacterial agent- Sodium hypochlorite	Avg Zone of Inhibition(cm)		
		Enterococcus faecalis	*Lactobacillus acidophilus*	*Streptococcus mutans*
16.	50µl	1.45	1.6	1
17.	100µl	1.85	1.85	1.85
18.	150µl	2	1.9	2



Inference:

Sodium hypochlorite showed good zone of inhibition against all selected microorganisms 0.9% saline

Table 7: Zone of inhibition of *Enterococcus faecalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* against Physiological saline

Sl. no	Antibacterial agent-Physiological Saline(0.9%)	Zone of Inhibition(cm)					
		Enterococcus faecalis		*Lactobacillus acidophilus*		*Streptococcus mutans*	
		1	2	1	2	1	2
19.	50µl	0	0	0	0	0	0
20.	100µl	0	0	0	0	0	0
21.	150µl	0	0	0	0	0	0

Table 8:Average Zone of inhibition of *Enterococcus faecalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* against Physiological saline

Sl. no	Antibacterial agent-physiological saline(0.9%)	Avg Zone of Inhibition(cm)		
		Enterococcus faecalis	*Lactobacillus acidophilus*	*Streptococcus mutans*
22.	50µl	0	0	0
23.	100µl	0	0	0
24.	150µl	0	0	0

Inference:

Physiological saline(0.9%) used in the study didn't show any antimicrobial property against the bacteria *Enterococcus faecalis* *Lactobacillus acidophilus* and *Streptococcus mutans*

DISCUSSION

A successful endodontic treatment depends upon complete removal of microbiota from the root canal system Chemical intracanal irrigators and medicaments can debride infected tissues and eradicate microorganisms from the root canal system. (14)

Low oxygen tension, less nutrient availability, and enormous bacterial interactions lead to predominant colonization of facultative anaerobic species prevailing in the root canals(15)

In persistent periradicular infections, Enterococcus faecalis had been isolated in about 24%–77% cases that perpetually resulted in failure of root canal therapy . This is due to their ability to survive at high alkaline environment and deeper tubular invasion. It grows through adhering on biofilm and colonizes on to the surface.(16)

The most common gram positive organisms in endodontic infection include E faecalis, streptococcus (*Streptococcus mitis, Streptococcus gordonii, Streptococcus anginosus, Streptococcus oralis*) and lactobacillus(*Lactobacillus paracasei* and *Lactobacillus acidophilus*)(17)

A broad antimicrobial spectrum against anaerobic and facultative microorganisms, biofi lms and ability to remove smear layer during instrumentation or dissolve it once it has formed are among the main requirements of endodontic irrigants. They should be non-toxic and non-caustic to tissue(18)

The most effective and commercially used is sodium hypochlorite. However, it has several undesirable characteristics such as tissue toxicity, risk of emphysema, allergic potential, disagreeable smell and taste.To overcome the problems associated with currently used irrigants, the use of natural plant extracts as endodontic irrigants might be of interest to professionals as part of a growing trend to seek natural remedies in dental treatment.[19,20,21]

The selection of the tested plants was based on their antimicrobial efficacy. The tested plant extracts fractions were concentrated to dryness under reduced pressure using rotary device to give the dry fractions of each solvent. They were dissolved in DMSO to facilitate testing and usage as an endodontic irrigant, since DMSO doesn't possess any antimicrobial effect.

The antimicrobial potential in this study was tested using the agar well-diff usion method .The well-diffusion method has been used in this study due to the larger amount of tested material which can be placed in the wells, as reported by Essawi and Srour (22)

Sodium hypochlorite (5.25% NaOCl) was used as a positive control because it is a well-known antimicrobial endodontic irrigant,while saline was used as a negative control. The agar well-diff usion method was used to screen the antimicrobial action of the plant extracts and the most potent plant extracts were additionally tested by MIC test using agar disc diffusion method. MIC determination is the most commonly employed procedure to evaluate the physiological effects of an anti-microbial agent on microorganisms, and correlation of product concentration and effect The minimal/minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the visible growth of bacteria. [23]

In this study the zones of inhibition of bacterial growth attained by NaOCl were greater than that obtained for other extracts for the tested organisms.This indicates that it has the highest efficacy against the tested organisms than other herbal agents.

However,the zones of inhibition of bacterial growth obtained by neem extract against E. faecalis, Smutans and lactobacillus are significantly greater than lemon grass extract and almost same as sodium hypochlorite in this study.Thus neem leaf extract is as effective as sodium hypochlorite as root canal irrigant against selected all organisms. 100µl of neem extract exhibited consistent antimicrobial effect. Hence it is recommended to use herbal neem extract in dental procedures lemon grass extract is effective only against E faecalis not against Smutans and lactobacillus

Antimicrobial activity of neem may be attributed to the presence of triterpinoids nimbidin, nimbolide, and nimbin reported to be present in Azadirachta indica.[24,25] These compounds have been reported to have antimicrobial activity.(26)

Agar diffusion method to study antimicrobial efficacy of neem implies that it has significant antimicrobial activity against endodontic pathogens.[28,29,30] Tyagi *et al*.[31] have found neem to have a lower antimicrobial efficacy than 5% NaOCl .Although we found neem to have significant antimicrobial activity against E faecalis Smutans and L acidophlus, our findings cannot be directly compared with those of Ghonmode *et al*.(27) and Hegde *et al*.(29) as their study was carried out on *Enterococcus faecalis* and *C. Albicans*, which are commonly found in endodontic reinfection cases. Our results are in agreement with Dutta *et al*.[032] who found no difference in antimicrobial efficacy of neem and 2.5% NaOCl against anaerobic bacteria. Our findings also clinically validate those of Mistry *et al*.[33] who found neem to be very effective against *Streptococcus mutans* and *Staphylococcus aureus* in an agar diffusion model.

Sundaram *et al*.[34] and Prasad *et al*.[35] in 2016 also tested the herbal irrigants including neem for their antibacterial efficacy and had suggested its potential role in the future of endodontics.

In this study lemon grass extract is effective only against E faecalis which is in agreement with the findings of Abuzied and Eissa (36)

Inference: Commercial antimicrobial agent, Sodium hypochlorite was taken as a positive control, for comparing the efficacy of the herbal extracts. Neem extract manifested antimicrobial efficacy as good as Sodium hypochlorite. Sodium hypochlorite showed good zone of inhibition against all selected microorganisms.

CONCLUSION:

In the current study comparative analysis of the antibacterial efficacy of lemongrass oil and neem extract was made with Sodium hypochlorite and physiological saline as controls against Enterococcus faecalis, Lactobacillus acidophilus and Streptococcus mutans. Neem extract showed better results compared to the lemongrass oil. Hence neem extract can be used as dental root canal irrigant.

REFERENCES

1. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am. 2010;54:291–312. [PubMed]
2. Mohammadi Z, Abbott PV. Antimicrobial substantivity of root canal irrigants and medicaments: A review. Aust Endod J. 2009;35:131–9.[PubMed]
3. Siqueira JF, Jr, Rocas IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod.2000;26:331–4. [PubMed]
4. Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.
5. Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis - the root canal survivor and 'star' in post treatment disease. Endod Top.2003;6:135–59.
6. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32:93–8. [PubMed]
7. C H Stuart ,S.A.Schwartz ,TJ Beeson ,CB Owatz ,Enterococcus faecalis :its role in root canal treatment failure and current concept in retreatment ,J.Endod 32(2006)93-98
8. P Garg ,SP Tyagi, DJ Sinha ,U P Singh ,V Malik ,E R Maccune ,Comparison of antimicrobial efficacy of propolis ,Morinda citrifolia ,Azadirachta indica ,triphala green tea polyphenols and 5.25% sodium hypochlorite against Enterococcus faecalis biofilm ,Saudi Endod .J4(2014)122-127
9. Madhu Pujar, ChetanPatil, AjayKadam. Comparison ofantimicrobial efficacy of Triphala, Green tea polyphenols and3% Sodiumhypochlorite on Enterococcus faecalis biofilims formed on tooth substrate – In vitro. JIOH 2011; Vol 3 (2) :23-29
10. Kamat S, Rajeev K, Saraf P. Role of herbs in endodontics. An update. Endodontology 2011;23:98-102. .
11 Jain A, Basal E. Inhibition of Propionibacterium acnes-induced mediators of inflammation by Indian herbs. Phytomedicine 2003;10:34-8.
12. Okpanyi SN, Ezeukwu GC. Anti-inflammatory and antipyretic activities of Azadirachta indica. Planta Med 1981;41:34-9.
13 P Satthanakul , S Taweechaisupapong, J Paphangkorakit , M Pesee , P Timabut W, Khunkitti Antimicrobial effect of lemongrass oil against oral malodour micro-organisms and the pilot study of safety and efficacy of lemongrass mouthrinse on oral malodour.J Appl Microbiol 2015;118:11-7
14. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigation solutions. J Endod. 1975;4:127–35.[PubMed]
15. Murray PE, Farber RM, Namerow KN, Kuttler S, Garcia-Godoy F. Evaluation of Morinda citrifolia as an endodontic irrigant. J Endod 2008;34:66-70
16 Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old Enterococcus faecalis biofilms in dentin canals. J Endod 2012;38:1376-9.
17 L Lakshmi Narayanan and C Vaishnavi Endodontic microbiology J Conserv Dent 2010;13:233-239
18. M. Zehnder, "Root canal irrigants," Journal of Endodontics, vol. 32, no. 5, pp. 389–398, 2006.
19 Murray PE, Farber RM, Namerow KN, Kuttler S, Garcia-Godoy F. Evaluation of Morinda citrifolia as an endodontic irrigant. J Endod.2008;34:66–70. [PubMed]
20. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, ukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate: An in vitrostudy. J Endod. 2010;36:83–6. [PubMed]
21. Mohammadi Z. Sodium hypochlorite in endodontics: An update review.Int Dent J. 2008;58:329–41. [PubMed]
22. Love RM. Enterococcus faecalis – A mechanism for its role in endodontic failure. Int Endod J 2001;34:399-405
23. WR Roberts, M Addy.Comparison of the in vivo and in vitro antibacterial properties of antiseptic mouthrinses containing chlorhexidine, alexidine, cetyl pyridinium chloride and hexetidine. Relevance to mode of action.. JClinPeriodontol. 1981;8:295–310. [PubMed]
24. Mohammad MS, Forough M. Investigation of compounds from (Azadirachta indica) neem. Asian J Plant Sci 2007;6:444-5.
25. Girish K, Shankara S. Neem a green treasure. Electron J Biol 2008;4:102-11.
26. Saxena, D., Saha, S. G., Saha, M. K., Dubey, S., & Khatri, M. (2015). An in vitro evaluation of antimicrobial activity of fi ve herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against Enterococcus faecalis. Indian Journal of Dental Research, 26(5), 524.ISO 690.
27 Ghonmode WN, Balsaraf OD, Tambe VH, Sanjanya KP, Ashishkumar KP, Kakde DD. Comparison of the antibacterial efficiency of neem leaf extract, grape seed extract and 3% Sodium hypochlorite against E.faecalis- an invitro study. J Int Oral Health. 2013;5(6):61–66. [PubMed]
28 Ghonmode WN, Balsaraf OD, Tambe VH, Saujanya KP, Patil AK, Kakde DD. Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against E. feacalis – An in vitro study. J Int Oral Health. 2013;5:61–6. [PMC free article] [PubMed]
29 Hegde V, Kesaria DP. Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E. fecalis and C. albicans – An in vitro study. Endodontology.2013;25:38–45.
30 Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity ofAzadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctumand 2% chlorhexidine gluconate on common endodontic pathogens: An in vitrostudy. Eur J Dent. 2014;8:172–7. [PubMed]
31 Tyagi SP, Sinha DJ, Garg P, Singh UP, Mishra CC, Nagpal R. Comparison of antimicrobial efficacy of propolis, Morinda citrifolia, Azadirachta indica (Neem) and 5% sodium hypochlorite on Candida albicans biofilm forme
32 Dutta A, Kundabala M. Comparative anti-microbial efficacy of Azadirachta indica irrigant with standard endodontic irrigants: A preliminary study. J Conserv Dent. 2014;17:133–7. [PubMed]
33 Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of Azadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. Eur J Dent 2014;8:172-7
34 Sundaram D, Narayanan RK, Vadakkepurayil KA. Comparative evaluation on antimicrobial effect of honey, neem leaf extract and sodium hypochlorite as intracanal irrigant: an ex-vivo study. J Clin Diagn Res 2016;10:ZC88-91.
35 Prasad SD, Goda PC, Reddy KS, Kumar CS, Hemadri M, Ranga Reddy DS. Evaluation of antimicrobial efficacy of neem and aloe vera leaf extracts in comparison with 3% sodium hypochlorite and 2% chlorhexidine against E. faecalis and C. albicans. J NTR Univ Health Sci 2016;5:104-10.
36 Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D. In vitro evaluation of fi ve diff erent herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent 2013;16:167-70.