ISSN- O: 2458 - 868X, ISSN –P: 2458 - 8687

International Journal of Medical Science and Innovative Research (IJMSIR)

IJMSIR : A Medical Publication Hub

Available Online at: www.ijmsir.com

Volume – 2, Issue – 4, July - August - 2017, Page No. : 121 - 133

Evaluation of Antimicrobial Efficacy of Herbal Alternatives Against Enterococcus Faecalis as Root Canal Irrigant: An In-Vitro Study

[1]Dr. Vivek Rai, MDS, Department of Conservative Dentistry and Endodontics

[2]Dr. Abhinay Agarwal, Reader, Department of Conservative Dentistry and Endodontics, Teerthanker Mahaveer Dental College and Research Center, Moradabad U.P., India.

[3]Dr. Sumit Sabharwal, Senior Lecturer, Department of Conservative Dentistry and Endodontics, Seema Dental College and Hospital, Rishikesh.

[4]Dr. Tazeen Rahman, BDS, General Clinician.

[5]Dr. Anshdeep Singh, Reader, Department of Conservative Dentistry and Endodontics, Seema Dental College and Hospital, Rishikesh.

[6]Dr. Pulkit Gupta, Senior Lecturer, Department of Conservative Dentistry and Endodontics, Seema Dental College and Hospital, Rishikesh.

Correspondence Author: Dr. Abhinay Agarwal, Reader, Department of Conservative Dentistry and Endodontics, Teerthanker Mahaveer Dental College and Research Center, Moradabad U.P., India.

Conflicts of Interest: None to Declare

Introduction

Complete elimination of microorganism from the root canal system is the most important objective of the root canal treatment. For many years, intracanal irrigants have been used as an adjunct to enhance the antimicrobial effect of cleaning and shaping of the root canal **(1).** Irrigants can augment mechanical debridement by flushing out debris, dissolving tissue, and disinfecting the root canal system. Thus, irrigation becomes one of the most important determinant in the healing of the periapical tissues **(2).** Substantial number of bacteria have been identified in the infected root canal but *Enterococcus faecalis* which is a gram positive facultative anaerobic bacteria, often survives in the root canal as a single microorganism without the support of the other bacteria and multiply causing infection that stimulates local bone resorption. *Enterococcus faecalis* is responsible for the failure of root canal treatment and is resistant to calcium hydroxide due to its proton pump. They also have the capability to invade the dentinal tubules and adhere to the collagen in the presence of the human serum **(3).** A constant increase in the antibiotic resistant strains and tissue toxicity of the conventional irrigants has therefore, prompted researchers to look for herbal alternatives **(4),** due to their antimicrobial activity, biocompatibility, anti-inflammatory and antioxidant properties **(5).**

Azadirachta indica (Neem) is a commonly seen medicinal tree in India, which is considered holy. It is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity **(6,7)** Several pharmacological activities and medicinal applications of various parts of neem are well known **(5,7).** Interest on this substance is based on its properties like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, analgesic and immuno-stimulant activity **(6,7).** Furthermore, it also has an anti-adherence activity by altering bacterial adhesion and ability of organism to

colonize. It has been shown that neem is highly effective in the treatment of periodontal disease. Its biocompatibility to human periodontal ligament fibroblasts is an important factor favoring its clinical application **(6).**

Turmeric [*Curcuma longa*] is extensively used as a spice, food preservative and coloring material in India, China and South East Asia. It has been used in traditional medicine for the treatment of numerous diseases. A recent report suggested that curcumin exhibits phototoxic effect against gram positive and gram negative bacteria **(6,8).** Curcumin [diferuloylmethane], the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities **(5,8)** The aim of the present study was to give a comparative evaluation of the antimicrobial efficacy of Neem, Turmeric, combination of neem and turmeric, in their various concentrations at varying time intervals against *E. faecalis* when compared with 2% chlorohexidine.

Methodology

The in-vitro study was carried out in the Department of Conservative Dentistry & Endodontics, Saraswati Dental College, Lucknow, India in collaboration with the Post Graduate Department of Microbiology, King George's Medical University, Lucknow, Uttar Pradesh.

Preparation and Collection of Plant Extracts

Fresh Rhizomes of *Circuma longa* grown organically without the use of pesticides and fresh Neem Leaves were procured from the lush gardens of Saraswati Dental College and Hospital, Lucknow.The Extraction procedure was carried out at Biotech Park in Biotechnology City, Lucknow.

Fresh Leaves of Neem (*Azardica indica*) (130 g) were dried in a tray drier (55 g) and then ground in a grinder and underwent soxlet extraction followed by filtration of the crude extract. solvent recovery (aqueous) was done in rotavapour at 60^{o}c in vacuum **(9)** Thus the pure extract (10 g) was in the ratio herb: extract = 5:1.Similarly Fresh Rhizomes of Turmeric (*Curcuma longa*) (280g) were Dried in a Trey Drier (50g) and Ground in a grinder and underwent soxlet extraction followed by filtration of the crude extract. Solvent recovery (aqueous) was done in rotavapour at 60^{o}c in vacuum **(9)** Thus the pure extract (12 g) was in the ratio herb:extract = 5:1.The prepared Neem extract was considered 100%. 1 g of the extract was measured on the electronic weighing machine, which was then brought to workable consistency by adding 1 ml of distilled water. Hence the dilution percentage stood by at 50% This was further diluted to 25% by adding the Nutrient Broth containing the microbial inocula **(10-12,5,4)** The prepared Turmeric extract was considered 100%. 1 g of the extract was measured on the electronic weighing machine, which was then brought to workable consistency by adding 1 ml of Dimethyl Sulfoxide (DMSO), because the active component of *Circuma longa* is soluble in organic solvents only **(13-16,5,17,4)** Hence the dilution percentage stood by at 50%. This was further diluted to 25% by adding the Nutrient Broth containing the microbial inocula. Preparation of Neem + Turmeric Extract was prepared by adding equal parts of 25% of Neem and Turmeric extracts in their soluble forms. Brain Heart Infusion Broth, Blood agar, Macconkey agar media plates were prepared **(18-20)** and stored in a refrigerator till further use.

Strain of *Enterococcus faecalis* (ATCC29212) was obtained in single use disposable vials from American Type Culture Collection (Manassas,VA) by Hi media Laboratory (Mumbai, India). It was subcultured in BHI broth and incubated at 37^{o}C for 24 hours in aerobic conditions. The broth was visualized for the presence of

turbidity indicating the bacterium growth, which was then maintained in the Department of Microbiology, KGMU till further use.

For morphologically similar colonies, BHI broth containing *E. faecalis* was touched using a sterile wire loop and the growth was transferred to Blood agar plate by streak method and incubated at 37^{o}C in an incubator for about 24 hours in aerobic conditions. This stock culture plate was stored in the refrigerator for further culture preparations and stored in a refrigerator till further use. **(18-20)**

The density of the selected test organism *i.e. Enterococcus faecalis* (S/C 519114) (Fig.5) was to adjusted equal to that of 0.5 McFarland standards (1.5×10^{8} CFU) by adding them to Nutrient Broth (Brain Heart Infusion) for *Enterococcus faecalis* (S/C 519114).A 24 hour old culture was used for the preparation of bacterial suspension, 0.5 McFarland standards (1.5×10^{8} CFU) were used as a reference to adjust the turbidity of the microbial suspension **(18-20).**

The microbial analysis was done by using:-

A) The Broth micro-dilution test for MIC values and then visualization of turbidity in the inoculated tubes to check for bacterial growth **(18-20).**

B) The Broth macro-dilution (Tube dilution) susceptibility test for MBC values and then sub-culturing on an Macconkey Agar medium **(18-20).**

A) Determination of Minimum Inhibitory Concentration (MIC) –

This was done using the Broth micro-dilution MIC testing **(18-20)** and stored in a refrigerator till further use.

The alcoholic extract of Turmeric and the aqueous extract of Neem and Neem + Turmeric were serially diluted to four different concentrations of 25%, 12.5%, 6.25% and 3.125% to find out their antibacterial activity against *Enterococcus faecalis* (S/C 519114) by using the Broth dilution method, where the test tubes containing the extracts in different dilutions were then inoculated with 0.1ml suspension of the test organism.2% Chlorhexidine Digluconate was taken as the positive control group, which was introduced into the inoculated test tubes instead of the plant extracts. Nutrient broth was used as the Negative Control group. After 24 hours of incubation at 37^{o}C, the test tubes were observed for turbidity. The least concentration where no turbidity is observed, was determined and noted as the Minimum Inhibitory Concentration value **(18-20).**

B) Determination of Minimum Bactericidal Concentration (MBC) –

This was done using the Broth macro-dilution (Tube dilution) susceptibility test **(18-20)** and then by sub-culturing on Macconkey Agar medium **(19)** and stored in a refrigerator till further use. In this technique, the content of the test tubes containing the extract in their serial dilution of 25%, 12.5%, 6.25% and 3.125% respectively were inoculated with 0.1ml suspension of the test organism for periods of 15 mins, 30 mins, 45 mins and 1 hour respectively and then the contents of the tubes were streaked using a sterile wire loop on the Macconkey Agar plates and incubated at 37^{o}C for 24 hours and then checked for the growth of the bacterial colony forming units **(20)** 0.2% Chlorhexidine Digluconate was taken as the positive control group and was introduced into the inoculated test tubes instead of the plant extracts for time intervals of 15 mins, 30 mins, 45 mins and 1 hour respectively. The lowest concentration for each of the extracts and the positive control group, which showed no bacterial growth was recorded and noted as the Minimum Bactericidal Concentration **(18,19)**

Results

Tables 1, 2, 3 and 4 represent the Minimum Bacterial Concentration in Neem extract, Turmeric extract, combination of Neem and turmeric extract and Chlorhexidine Group respectively.

Table 1: Minimum Bacterial Concentration in Neem Extract (Group I)

Time Interval	Concentration	Colony Forming Units
15 min	25%	100
30 min	25%	200
45 min	25%	200
1 hr	25%	100
15 min	12.50%	200
30 min	12.50%	200
45 min	12.50%	200
1 hr	12.50%	180
15 min	6.75%	200
30 min	6.75%	200
45 min	6.75%	200
1 hr	6.75%	200
15 min	3.13%	200
30 min	3.13%	200
45 min	3.13%	250
1 hr	3.13%	200
Number of specimens		16
Minimum		100
Maximum		250
Mean		189.37
Standard deviation		37.50
Median		200
Kolmogorov-Smirnov test		K=0.424; p<0.001

Table 2: Minimum Bacterial Concentration in Turmeric Extract (Group II)

Time Interval	Concentration	Colony Forming Units
15 min	25%	0
30 min	25%	0
45 min	25%	0
1 hr	25%	0
15 min	12.50%	0
30 min	12.50%	0

45 min	12.50%	0
1 hr	12.50%	0
15 min	6.75%	2
30 min	6.75%	50
45 min	6.75%	50
1 hr	6.75%	50
15 min	3.13%	200
30 min	3.13%	50
45 min	3.13%	50
1 hr	3.13%	100
Number of specimens		16
Minimum		0
Maximum		200
Mean		34.50
Standard deviation		53.83
Median		1.00
Kolmogorov-Smirnov test		K=0.290; p=0.001

Table 3: Minimum Bacterial Concentration in Neem+Turmeric Extract (Group III)

Time Interval	Concentration	Colony Forming Units
15 min	25%	150.00
30 min	25%	150.00
45 min	25%	200.00
1 hr	25%	200.00
15 min	12.50%	150.00
30 min	12.50%	200.00
45 min	12.50%	200.00
1 hr	12.50%	180.00
15 min	6.75%	150.00
30 min	6.75%	200.00
45 min	6.75%	150.00
1 hr	6.75%	150.00
15 min	3.13%	150.00
30 min	3.13%	200.00
45 min	3.13%	250.00
1 hr	3.13%	200.00

Number of specimens	16
Minimum	150
Maximum	250
Mean	180
Standard deviation	30.55
Median	190
Kolmogorov-Smirnov test	K=0.274; p=0.002

Table 4: Minimum Bacterial Concentration in 2% Chlorhexidine (Control)

Time Interval	Concentration	Colony Forming Units
15 min	2%	2
30 min	2%	0
45 min	2%	0
1 hr	2%	0
15 min	2%	2
30 min	2%	0
45 min	2%	0
1 hr	2%	0
15 min	2%	0
30 min	2%	0
45 min	2%	0
1 hr	2%	0
15 min	2%	0
30 min	2%	0
45 min	2%	0
1 hr	2%	0
Number of specimens		16
Minimum		0
Maximum		2
Mean		0.25
Standard deviation		0.68
Median		0.00
Kolmogorov-Smirnov test		K=0.518; p<0.001

Table 5: Minimum Bacterial Concentration (Overall) in different groups

Group	Number of specimens	Mean	Standard deviation	Minimum CFUs	Maximum CFUs
Group I	16	189.38	37.50	100	250
Group II	16	34.50	53.83	0	200
Group III	16	180.00	30.55	150	250
Control	16	0.25	0.68	0	2
Total	64	101.03	92.29	0	250

Discussion

Enterococcus faecalis has been the focus of increased interest both in medicine and dentistry **(21).** A recognized pathogen in post-treatment endodontic infections, *E. faecalis* is frequently isolated both in mixed flora and in monocultures. *E. faecalis* is probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canal. Eradication of *E. faecalis* from the root canal with chemomechanical preparation and using disinfecting irrigants and antibacterial dressings is difficult **(22).**

In endodontics, *E. faecalis* is rarely present in primary apical periodontitis, but it is the dominant microorganism in root-filled teeth presenting with post-treatment apical periodontitis. It is often isolated from the root canal in pure culture, but it can also be found together with some other bacteria or yeasts Eradication of *E. faecalis* from the root canal remains a challenge, while chlorhexidine and combinations of disinfectants have shown some promise **(22).**

Neem (*Azadirachta indica*) has been found to contain a vast array of biologically active compounds, which are chemically diverse and have got an enormous therapeutic potential **(23-26)**

Although a large number of compounds have been isolated from various parts, a few have been studied. Nimbidin, a major crude bitter principle, extracted from A. *indica* demonstrated several biological activities **(26,11).** From this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated. Nimbidin and sodium nimbidate possess significant dose-dependent anti-inflammatory activity **(25, 26, 11).**

In this study, Neem was included as one of the experimental groups because previous studies **(27,28, 12)**, have proven its anti-adherence activity by altering bacterial adhesion and the ability of organism to decolonize, resulting in Neem having the maximum reduction in adherence of *E. faecalis* to dentin.**(15,29)** The extract of *Azadirachta indica* exhibited pronounced activity against a wide spectrum of bacteria *viz. S. aureus*, *Enterococcus faecalis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis* and even *Streptomycin* resistant strains **(30,10-34)**

The leaves of *Azadirachta indica* possessed good anti bacterial activity, confirming the great potential of bioactive compounds useful for rationalizing the use of this plant extract as a potential endodontic irrigant.**(25,11,30,24,35).**The biocompatibility of Neem extract on human fibroblast cells was evaluated by Sudhakar Benjamin *et al.* and they concluded that the cytotoxic effects of neem extract were significantly less compared to that of conventional endodontic irrigants **(35)**

Turmeric (*Curcuma longa*) is a Rhizome which is used as a spice in Asia. It has been used in Ayurveda and Unani medicines for centuries. They have excellent anti-inflammatory and anti-oxidant activies **(36)** Results from previous studies have hypothesized that turmeric **(6)** has the potential to eliminate the EPS matrix and bacteria**(37).**

Effectiveness of turmeric extract against *E. faecalis* biofilm in root canals were studied and compared to that with conventional irrigants to overcome the disadvantages of conventional irrigants such as unpleasant taste, toxicity, inability to remove smear layer, limited anti bacterial activity and dentrimental effect on dentins structural integrity **(6,38,39,37)**

Future scope and research warrants that Curcumin the chief biological component of turmeric can be used as an irrigant and intra canal medicament **(40,16, 41, 42)**

On the basis of findings from previous studies, the extract of turmeric was hence included in this study as one of the groups because of its anti-inflammatory, anti-oxidant properties, which basically highlight its biocompatibility**(40,38)** and also its anti-bacterial property, especially its efficacy against *Enterococcus faecalis **(17,38,40,39,37,16)***

.

Chlorhexidine is a synthetic cationic bis-guanide that consists of two symmetric 4-cholorophenyl rings and two biguanide groups, connected by a central hexamethylene chain **(43)** Its antibacterial efficacy, especially against enterococcus faecalis is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls, thereby altering the cells' osmotic equilibrium. This increases the permeability of the bacterial cell wall, which allows the CHX molecule to penetrate into the bacteria **(43,44).** In the present study, Chlorhexidine was taken as a positive control group because of its proven efficacy against the test organism of choice (*Enterococcus faecalis*) **(45,46,47,48).**

Very few studies have been conducted, wherein the antibacterial efficacies of Neem, Turmeric and their combination have been tested against *Enterococcus faecalis*, as endodontic irrigants. In the present study, the 100% extract of Neem, which was viscous in nature, was diluted with distilled water, in order to bring it to a workable consistency, in order to fulfill the prerequisites of the Broth Dilution Method to be used **(17)** The 100% extract of Turmeric required an organic solvent such as dimethyl sulfoxide(DMSO) for its dilution **(17)**. DMSO has no antibacterial properties by itself and did not affect the properties of the extract of turmeric in any manner **(17)**

The anti-microbial efficacy of the extracts was tested using the Broth Dilution Method, which is the standard technique for checking the minimum bactericidal concencentration (MBC) **(18-20)**

The turbidity of the tested solutions was determined to check the minimum inhibitory concentration (MIC), as this is the standard procedure followed **(18-20)** Even though a vast number of studies have been conducted on extracts of Neem and Turmeric using the Serial Dilution Method to check their anti-microbial efficacies against *Enterococcus faecalis*,**(15,29,17,49)** a very few studies have taken into account, certain important parameters such as their MBC at different time intervals of 15 minutes, 30 minutes, 45 minutes and 1 hour. Hence, the present study focuses on this important aspect in order to simulate the clinical procedures.

A combination of Neem and Turmeric has also been used as one of the experimental groups. This facet has not been explored till date in the context of newer herbal endodontic irrigants.

The results of the present study suggested that, at a concentration of 12.5 and 25%, Turmeric was most

effective significantly at all time intervals of 15 mins, 30 mins, 45 mins and 1 hour where no colonization was seen ($p<0.001$) followed by 2%Chlorhexidine, where few colony forming units were evident at the 15 minute time interval. Chlorhexidine was equally effective as Turmeric for the rest of the time intervals tested ($p<0.001$). The anti-microbial efficacy of Neem extract was relatively less than that of Turmeric and Chlorhexidine, where colonization was observed at all the time intervals ($p<0.001$) .The anti-microbial efficacy of the combination of extracts of Neem and Turmeric was observed to be the least among all the groups in all the tested time intervals ($p<0.002$).

At a concentration value of 12.5% of the tested extracts, the same trend was observed, with Turmeric and 2% CHX at all the time intervals ($p<0.001$). As with the previous concentration, here also, the Neem extract ($p<0.001$) as well as the combination of extracts of neem and turmeric ($p<0.002$) were found to be relatively less effective (Fig.15-30).

At a concentration of 6.25% of the tested extracts, 2% CHX was observed to be most effective among the tested solutions, as the extract of Turmeric had shown evidence of colonization at all the tested time intervals. The extract of neem and the combination of the extracts of neem and turmeric were relatively as ineffective as in the previously tested concentrations at all time intervals.

At a concentration of 3.125% of the tested extracts, 2% CHX was again observed to be most effective among the tested solutions, ($p<0.001$) as the extract of Turmeric had shown multiple variance in levels of colonization, but still showed better anti-microbial efficacy compared to the extract of neem and the combination of extracts of neem and turmeric which as seen previously, were relatively the least effective in their respective groups at all the tested time intervals ($p<0.159$).

As established in the above study, Turmeric attained its Minimum Bactericidal Concentration at 12.5% and was slightly more effective than 2% CHX at the 15 minute time interval and equally effective at the 30 minute time interval.

The Minimum Bactericidal Concentration of Neem was observed to be at 25% at time intervals of 15 minutes and also at 1 hour, which was still relatively less effective when compared to turmeric and 2% CHX.

However, the MBC for the combination of extracts of Neem and Turmeric was observed to be at a concentration of 25% at a time period of 30 minutes, and hence was relatively the least effective preparation among all the groups.

This also suggested that, when turmeric was used in combination with neem, its anti-bacterial efficacy was significantly reduced and that turmeric as a separate entity is a better option.

The Minimum Inhibitory Concentration was calculated by checking for turbidity of the solutions at a time period of 24 hours, which suggested that 25% extracts of neem, turmeric and 2% CHX were equally effective, as no turbidity was observed.

However, the combination of extracts of neem and turmeric showed turbidity, which was evidence of bacterial colonization after 24 hours, rendering it comparatively less effective.

Conclusions

Within the limitation of this study, it can be concluded that the use of turmeric as an endodontic irrigant can be promising. Use of neem extract in elimination of *E. feacalis* is still debatable as complete elimination did not take place. Combination of neem+turmeric extract was also relatively less effective in removing *E. feacalis*, thus suggesting that they might have no synergistic action.

Furthermore, studies on animal model and *in-vivo* are required to access the efficacy of herbal irrigants.

References

[1]. Emad A. Shalaby, N. F. Nasr and Samia M. El Sherief. An *in vitro* study of the antimicrobial and antioxidant efficacy of some nature essential oils. Journal of Medicinal Plants Research Vol. 5(6), pp. 922-931.

[2]. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. IEJ 2010; 43(5): 419-23.

[3]. Madhu Pujar, Saleem Makandar Herbal Usage In Endodontics- A Review IJCD. January 2011(2).

[4]. J. Prabhakar, M. Senthilkumar, M.S. Priya, K. Mahalakshmi, VG Senthilkumar. 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: JOE — Volume 36, Number 1, January 2010.

[5]. Enzo A. Palombo. TraditionalMedicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases Evidence-Based Complementary and Alternative Medicine ,Volume 2011

[6]. Prasanna Neelakanthan, Nithya Jagannathan, Nabeel Nazar. Ethnopharmacological approach in Endodontic treatment: A focused review. Int J Drug Dev & Res, Oct-Dec 2011,3(4): 68-77.

[7]. Kausik Biswas, Ishita Chattopadhyay, Ranajit K. Banerjee and Uday Bandyopadhyay. Biological activities and medicinal properties of neem (*Azadirachta indica*). Current science, vol. 82, no. 11, 10 june 2002.

[8]. Ishita Chattopadhyay, Kaushik Biswas, Uday Bandyopadhyay and Ranajit K. Banerjee. Turmeric and curcumin: Biological actions and medicinal applications. Current science, VOL. 87, NO. 1, 10 JULY 2004.

[9]. Sukhdev Swami Handa, Suman Preet Singh Khanuja, *Extraction Technologies for Medicinal and Aromatic Plants* United Nations Industrial Development Organization and the International Centre for Science and High Technology, 2008.

[10]. Maragathavalli S, Brindha S, Kaviyarasi N.S, B.Annadurai,B.& Gangwar S.K. Antimicrobial activity in leaf extract of neem (azadirachta indica linn.) I.J.S.N., VOL.3(1)2012:110-113.

[11]. Ms. P. Chaturvedi, Dr. A. Bag, Dr. V. Rawat, Dr. N. S. Jyala. Antibacterial Effects of Azadirachta indica Leaf and Bark Extracts in Clinical Isolates of Diabetic Patients. NJIRM. Jan-March 2011; Vol. 2(1):5-9.

[12]. Nayak Aarati, Nayak Ranganath, G Soumya B, Bhat Kishore, Kudal Kishore. Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extract of neem (Azadirachta indica) – An *in vitro* study. IJRAP 2011(1) 230-235.

[13]. Mehvish Saleem, Betty Daniel, Murli K. Antimicrobial activity of three different rhizomes of *Curcuma longa* &*Curcuma aromatica* on uropathogens of diabetic patients. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 3, Issue 4, 2011.

[14]. Saranya .R , Bikash Jyoti Borthakur , Ganesan .S , Swathika .B Antimicrobial efficacy of Curcuma longa (Turmeric) extract e-Journal of Dentistry Jan - Mar 2014 Vol 4 Issue 1.

[15]. Thilla S Vinothkumar, Mohamed I Rubin, Lakshmi Balaji, Deivanayagam Kandaswamy. *In vitro* evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. Journal of Conservative Dentistry | Mar-Apr 2013 | Vol 16 | Issue 2.

[16]. Reshma Suvarna, Sham S Bhat and K. Sundeep Hegde. Antibacterial Activity of Turmeric against *Enterococcus faecalis* -An *In vitro* Study. *Int.J.Curr.Microbiol.App.Sci* (2014) 3(2): 498-504.

[17]. Mithra N Hegde, Shishir Shetty, Mahalaxmi Yelapure, Amit Patil. Evaluation of Antimicrobial Activity of Aqueous and Hydro-Alcoholic Curcuma Longa Extracts against Endodontic Pathogens. IOSR Journal of Pharmacy Mar.-Apr. 2012, Vol. 2(2) pp: 192-198.

[18]. Connie.R.Mahon,George Manuselis. Textbook of Diagnostic Microbiology (Second edition) Mc Hills Company, 2000.

[19]. Steve. K.Alexander, Dennis Streete, Mary Jane Niles. Laboratory exercises in organismal molecular microbiology. Mc Hills Company.

[20]. Prescott, Harley,klein. Microbiology, 5th edition. 2000.

[21]. Imam Hashmat, Hussain Azad and Ajij Ahmed. Neem (Azadirachta indica A. Juss) A Nature's Drugstore: An overviewInternational Research Journal of Biological SciencesVol. 1(6), 76-79, October (2012).

[22]. Isabelle Portenier, Tuomos M.T. Waltimo & Markus Haapasalo. Enterococcus faecalis – the root canal survivor and 'star' in post treatment disease. Endodontic Topics 2003, 6, 135–159.

[23]. Amal Kumar Ghimeray, Cheng-Wu Jin, Bimal Kumar Ghimire and Dong Ha Cho. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta Indica* A. Juss grown in foothills of Nepal. African Journal of Biotechnology Vol. 8 (13), pp. 3084-3091.

[24]. Raja Ratna Reddy Y, Krishna Kumari C, Lokanatha O, Mamatha S, Damodar Reddy C Antimicrobial activity of Azadirachta Indica (neem) leaf, bark and seed extracts (2013) Int. J. Res. Phytochem. Pharmacol., 3(1), 1-4

[25].Saradhajyothi Koona, Subbarao Budida. Antibacterial Potential of the Extracts of the Leaves of *Azadirachta indica* Linn. Not Sci Biol, 2011, 3(1)65-69.

[26]. Tomar Lokeshwar,Sharma pankaj, Bachwari mukesh, Bansal Vishnu. Review on neem: thousand problems one solution IRJP 2011, 2(12)97-102.

[27]. Lilian Eiko Maekawa, Roberta Lamping, Sidnei Marcacci Marcos Yasunori Maekawa, Maria Renata Giazzi, Cristiane Yumi Kogaito. Antimicrobial activity of chlorophyll-based solution on Candida albicans and Enterococcus faecalis. RSBO v. 4, n. 2, 2007 – 37.

[28]. Venkateswara Rao Chava, SM Manjunath, AV Rajanikanth, N Sridevi. The Efficacy of Neem Extract on Four Microorganisms Responsible for causing Dental Caries viz Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis and Streptococcus sanguis: An *in vitro* Study. The Journal of Contemporary Dental Practice. November-December 2012; 13(6):769-772.

[29]. Dhanya Kumar N.M. Preena Sidhu The antimicrobial activity of azardirachta indica, glycyrrhiza glabra, cinnamum zeylanicum, syzygium aromaticum, accacia nilotica on streptococcus mutans and enterococcus faecalis. Endodontology.

[30]. Gajendra singh P. Rathod, Bhavika M. Kotecha, Rohit Sharma, Hetal Amin, P.K. Prajapati. *In vitro* Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (Azadirachta indica L.) and Tulsi (Ocimum sanctum L.). International Journal of Pharmaceutical & Biological Archives .2012; 3(3):582-586.

[31]. Venkateswara Rao Chava, SM Manjunath, AV Rajanikanth, N Sridevi. The Efficacy of Neem Extract on Four Microorganisms Responsible for causing Dental Caries viz Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis and Streptococcus sanguis: An *in*

vitro Study. The Journal of Contemporary Dental Practice. November-December 2012; 13(6):769-772.

[32]. Srinivas Kumar Chandrabhatla, Vabbalareddy Rajasekhar, Sai Gautam Nalam, Jayalakshmi Pandrank. Natural Medicaments in Endodontics.Journal of Oral Research & Review. August 2012; Volume 4(2):25-32.

[33]. Hannah Rosaline, Kandaswamy D, Gogulnath D, Rubin MI. Influence of various herbal irrigants as a final rinse on the adherence of Enterococcus faecalis by fluorescence confocal laser scanning microscope. Journal of Conservative Dentistry Aug 2013; Vol 16; (4):352-355.

[34]. Wasudeo Namdeo Ghonmode, Omkar D Balsaraf, Varsha H Tambe, K P Saujanya, Ashish kumar K Patil, Deepak D Kakde. Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against E. feacalis . Journal of International Oral Health. Nov-Dec 2013; 5(6):61-6

[35]. Vabbalareddy Rajasekhar, Sai Gautam Nalam ,Jayalakshmi Pandrank.Natural Medicaments in Endodontics.Journal of Oral Research & Review.August 2012;Volume: 4(2):25-32.

[36]. Anamika Bagchi. Extraction of Curcumin. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) Volume 1, Issue 3 (Sep-Oct. 2012), PP 01-16.

[37]. Chamele and Chetan Bhat . Efficacy of turmeric extract as an intracanal medicament in deciduous teeth against Enterococcus faecalis. Int.J.Curr.Microbiol.App.Sci (2014) 3(9) 17-25.

[38]. Prasanna neelakantan, Chandana subbarao, Subash sharma, Chandragiri venkata subbarao, Franklin G Arcia-godoy & James l gutmann. Effectiveness of curcumin against Enterococcus faecalis biofilm. Acta Odontologica Scandinavica, 2013; Nov; 71(6):1453-7.

[39]. Vibha Hegde Dhaval P. Kesaria Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E. Faecalis and C. Albicans . Endodontlogy June 2013 25(2):38-45.

[40]. Sharmila Devi Devaraj, Prasanna Neelakantan Curcumin- pharmacological actions and its role in dentistry . Asian J Pharmaceut Res Health Care Volume 6 Issue 1 19-22.

[41]. Chhibber S, Sharma N Medicinal and Therapeutical Potential of Neem (Azadirachta indica): A Review International Journal of Scientific and Research Publications, Volume 4, Issue 5, May 2014.

[42]. Chaturvedi T P. Uses of turmeric in dentistry: An update. *Indian J Dent Res* 2009; *20*: 107 9.

[43]. Arumugam Velmurugan, Manavalan Madhana Madhubala1, Sreekrishnapillai Bhavani, Kasinathan Subbaian Satheesh Kumar, Srinivasan Sai Sathyanarayana, Natanasikamani Gurucharan.An in-vivo comparative evaluation of two herbal extracts Emblica officinalis and Terminalia Chebula with chlorhexidine as an anticaries agent:A preliminary study. Journal of Conservative Dentistry. Dec 2013; Vol 16(6):546-549.

[44]. Matthias Zehnder. Root Canal Irrigants. JOE — Volume 32, Number 5, May 2006. Markus Haapasalo, Ya Shen, Wei Qian, Yuan Gao. Irrigation in Endodontics Dent Clin N Am 54 (2010) 291–312.

[45]. Jamile B. Taheri1, Somayyeh Azimi, Nasrin Rafieian and Hosein Akhavan Zanjani. Herbs in dentistry.International Dental Journal 2011; 61: 287–296

[46]. Amir Moeintaghavi, Hamidreza Arab, Mehrangiz Khajekaramodini. *In vitro* Antimicrobial Comparison of Chlorhexidine,Persica Mouthwash and Miswak Extract. The Journal of Contemporary Dental Practice. 2011(10):10024-1111.

[47]. Uday Kumar, Hina Seth,Sai Ramesh, Keshav Singla. Comparison of the Antibacterial Efficacy of Tea Tree Oil with 3%Sodium Hypochlorite and 2%Chlorhexidine

against E.faecalis: An *in vitro* study. Joural of Contemporary Dentistry. December 2013;3(3):117-120.

[48]. Matthias Zehnder. Root Canal Irrigants. JOE — Volume 32, Number 5, May 2006.

[49]. Ojezele Matthew Obaineh, Agunbiade Shadrach Oludare, Adeosun Abiola Muhammad. Screenning of extracts of *Hibiscus sabdariffa* and *Azardirachta indica* for bioactive compounds. International Journal of Traditional and Herbal Medicine. Vol., 1 (5), 153-158, 2013