Comparitive Evaluation of Antimicrobial Efficacy of Ocimum Sanctum, Tridax Procumbens and Mango Kernel Extracts With Sodium Hypochlorite and Chlorhexidine as Rootcanal Irrigants - An Invitro Study

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Abstract

Enterococcus faecalis and Candida albicans have a major role in the etiology of retreatment cases. It is frequently found in high percentage of root canal failures and can be seen as single organism or as a major component of the mixed flora.Various investigations have demonstrated that thorough and complete debridement of rootcanal system is impossible with mechanical driven or hand instrumentation to eliminate the microorganisms and their byproducts. Hence, endodontic preparation should be supported by irrigants for enhanced disinfection. The use of conventional irrigants especially sodium hypochlorite (NaOCl) is highly efficient in eliminating E.faecalis biofilm. But its major disadvantage is its tissue toxicity and in addition it doesnot remove all of the smear layer, for which reason plant products that are consumed orally for varied medicinal purpose are assayed for their antibiotic properties.

The present study resulted in a conclusion that combination of 2% chlorhexidine and mango kernel shows superior antimicrobial efficacy to that of 5.25% NaOCl,& 2 % Chlorhexidine against strains of E.faecalis & C.albicans and also shows that the new bio irrigant has good shelf life and rapid onset of action.

Keywords : *Bio irrigants, Mango kernel, Sodium hypochlorite, Chlorhexidine, Agar study*

Introduction

E.faecalis have a major role in the etiology of persistent periradicular lesions after root canal treatment. It is frequently found in high percentage of root canal failures & can be seen as single organism or as a major component of the mixed flora.

Various investigations have demonstrated that thorough and complete debridement of rootcanal system is impossible with mechanical driven or hand instrumentation to eliminate the micro organisms and their byproducts. Hence, endodontic preparation should be supported by irrigants for enhanced disinfection. The use of conventional irrigants especially sodium hypochlorite (NaOCl) and Chlorhexidine is highly efficient in eliminating Enterococcus faecalis biofilm. But its major disadvantage is its tissue toxicity and discoloration respectively, for which reason plant products that are consumed orally for varied medicinal purpose are assayed for their antibiotic properties.

Ocimum sanctum is traditionally used as a medicinal plant in day to day practice in Indian homes for various ailments. Tulsi leaves contains eugenol , a phenolic compound which may be attributed to antimicrobial,antidiabetic & anticancer properties. Magnifera indica called mango in English. Magniferin , a major C-glucosylxanthone found to occur in M.indica stem,bark,leaves,heartwood, roots & fruits. The antibacterial activity of mango kernel may be attributed to tannins present in them.Tridax procumbens commonly known ad coatbuttons or tridax daisy belongs to daisy family. A new flavonoid procumbenetin isolated from aerial parts of Tridax procumbens has been characterized as 3,6,dimethoxy 5,7,2,3,4 pentahydroxy flavone 7-0-ß-D glucopyranoside. It is known for several potential therapeutic activities like antiviral,antioxidant,antibiotic

efficacies, wound healing activity & anti inflammatory activity.

The present qualitative study therefore aimed to assess and compare the antibiotic efficacy of Magnifera indica KERNEL(MANGO KERNEL), Ocimum sanctum (TULSI) LEAVES , Tridax procumbens(TRIDAX DAISY) leaves with that of sodium hypochlorite and chlorhexidine individually and in various combinations against Enterococcus faecalis and Candida albicans

Objectives

- To assess the antibacterial efficacy of extracts of Ocimum sanctum, Tridax procumbens and Magnifera indica kernel against Enterococcus faecalis and Candida albicans
- To compare the antibacterial efficacy of combination of plant extract mixture and 5% NaOCl, chlorhexidine solution against Enterococcus faecalis and Candida albicans
- To check the shelf life of irrigants

MATERIALS REQUIRED

- LEAF EXTRACTS OF Ocimum scantum (50µg/Ml)
- LEAF EXTRACTS OF Tridax procumbens (50µg/Ml)
- KERNEL EXTRACTS OF Magnifera indica (50µg/Ml)
- 5.25 % SODIUM HYPOCHLORITE(GDP CHLORAXID)
- 2% CHLORHEXIDINE (RC CHLOR)
- Enterococcus faecalis (ATCC STRAIN 29212)
- Candida albicans (ATCC STRAIN 10231)
- DIMETHYL SULFOXIDE
- STERILE PAPER DISKS
- 90 % ETHANOL
- VERNIER CALLIPERS

PLANT EXTRACT PREPARATION(cold extraction)

- Wash Fresh leaves of Ocimum sanctum , Tridax procumbens and mango kernel & dry at room temperature
- Dried leaves and kernel should be pulverized into coarse or medium coarse particles (FIG 1)
- Dried powdered forms(FIG 2) of all plant materials should be suspended in 50% ethanol in sterile conical flasks(FIG 3)
- Suspension of each flask should be placed on magnetic stirrer for 2-4hours
- Supernatants should be filtered through Whatman filterpaper (FIG 4) & dry filtration by evaporating organic solvent through lyophilisation so that influence of extracting agent was eliminated.
- Solutions should be made in 30 % dimethyl sulfoxide(DMSO), DMSO (30%) was used only as a solvent.(FIG 5)
- Pure culture of the test strain E.faecalis & C.albicans should be prepared in blood agar & incubate at 37°c for 24 hours
- Antibiotic activity of extracts against E faecalis & C.albicans should be evaluated using agar disk diffusion method

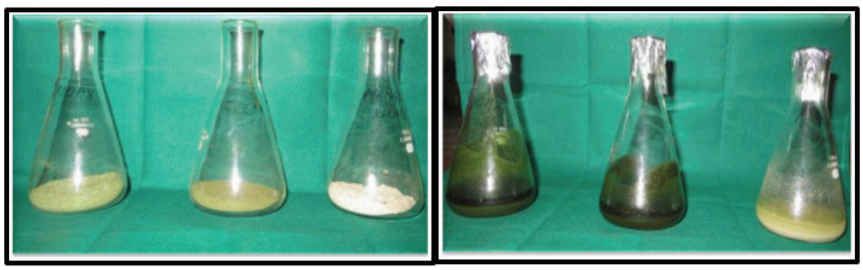


FIG 1

AGAR DIFFUSION TEST

- Freshly prepared inoculum containing bacterial suspension in blood agar were spread over the surface of 20 mL of Muller Hinton agar plate using sterile cotton swabs

- Sterile disks were placed on the plates & labelled

- Disk 1- 50µg/Ml O.sanctum extract

Disk 2 – 50µg/Ml Tridax procumbens extract

Disk 3 – 50µg/Ml mango kernel extracts

Disk 4- combination of extracts ; 1: 1: 1,1:2:1,1:1:2, 2:1:1

Disk 5 – 5 % NaOCl

Disk 6- 2% CHX

Disk 7- 6 month old mango kernel irrigant

Disk 8- mango kernel irrigant and 2% CHX

Disk 9 –DMSO

INOCULATION OF ORGANISMS

Pure culture of the test strain enterococcus faecalis was prepared in sterile brain heart infusion broth and incubated at 37°c for 24 hours.Pure culture of the test strain candida albicans was prepared in potato dextrose agar broth & incubated at 37°c for 24 hours

FIRST PART OF STUDY

- 50mL of working suspension of O.sanctum , Tridax procumbens, mango kernel extracts, various combinations, & same volume of control i.e DMSO as negative control & 5% NaOCl as positive control was delivered into respective disks with a micropipette.

- Plates left for 2-4 hours at room temperature with the lid closed until the extracts diffused in the medium and then incubated at 37°c for 24 hours

- Zones of microbial inhibition around the disks containing the test material should be measured with a pair of vernier callipers & record after incubation

- Shortest distance(mm) from outer margin of well to the initial point of microbial growth should be considered as inhibitory zone

- All assays were performed under aseptic conditions & experiment was repeated three times using three separate culture plates with inocula derived from same initial pure culture

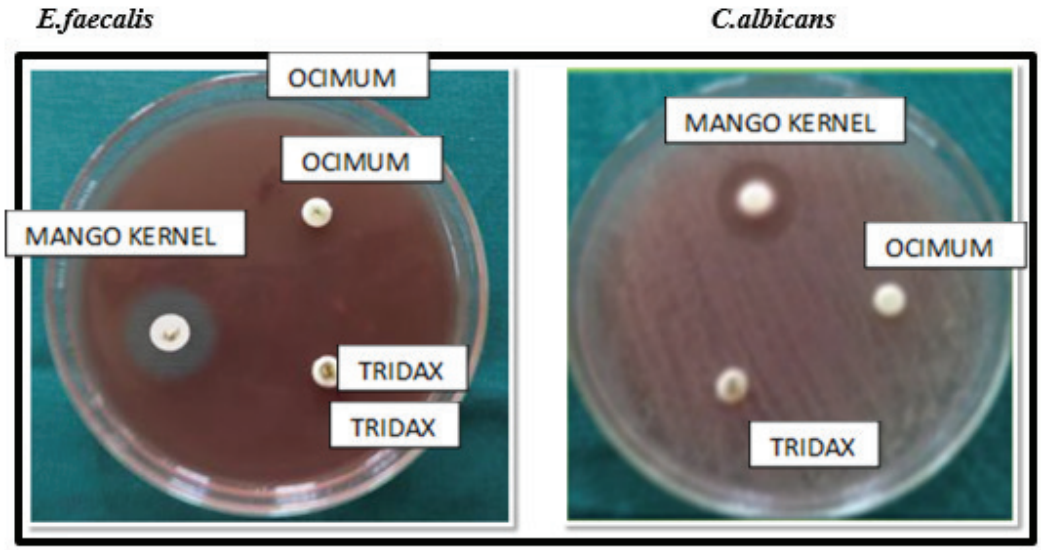
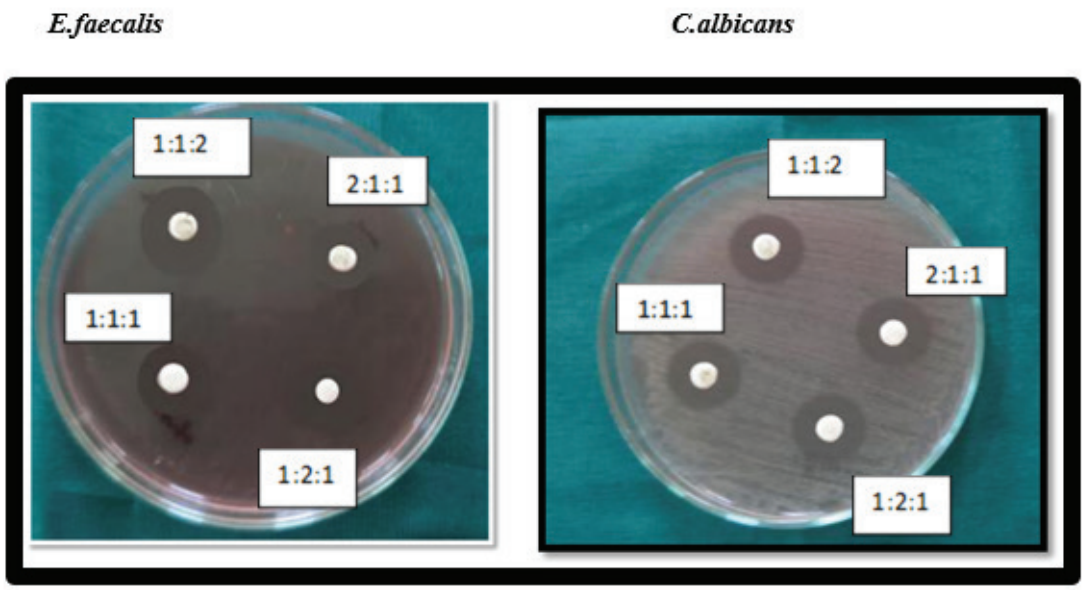


FIG 2



Results of First Part of Study

Table 1

Growth inhibition zones- mean diameters					
	Materials tested	**Mean (mm) (FIG 8) E.faecalis**		**Mean (mm) C.albicans (FIG 8)**	
		1hr	24hr	1hr	24hr
1	OCIMUM SCANTUM	0	0	0	0
2	TRIDAX PROCUMBENS	0	0	0	0
3	MANGO KERNEL	6	12	7	18
4	1:1:1	4	5	5	7
5	1:2:1(O:T:M)	4	5	5	6
6	1:1:2(O:T:M)	5	9	5	10
7	2:1:1(O:T:M)	4	5	5	7
8	DMSO	0	0	0	0
9	SODIUM HYPOCHLORITE	3	13	4	17

Second Part of Study

Freshly prepared 50 ml mango kernel extract and 50 ml 6 month old mango kernel extracts were pipetted out to disks and compared for both enterococcus faecalis and candida albicans. combination of chlorhexidine and mango kernel extract in 1:1 ratio was pipetted out to the disk and compared with sodium hypochlorite, chlorhexidine as positive control & dmso as negative control

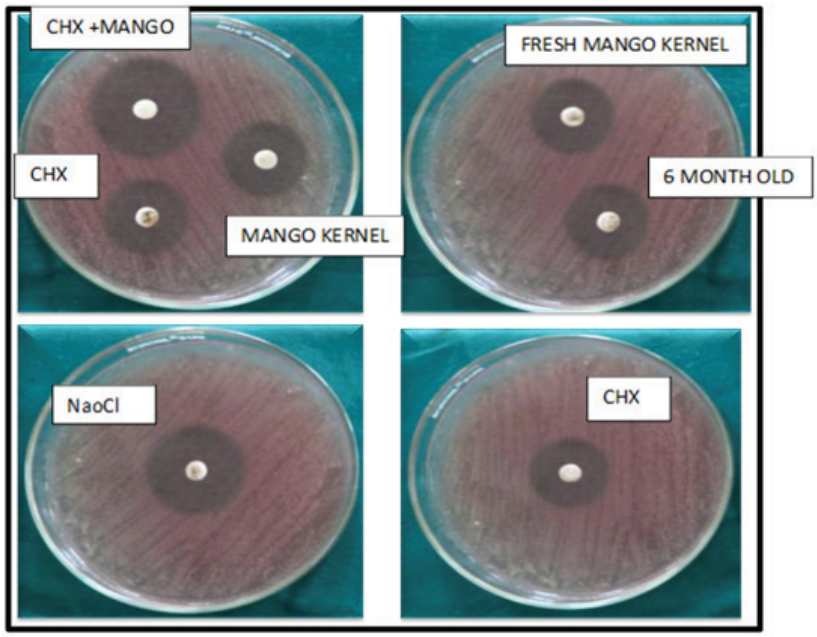


FIG 3

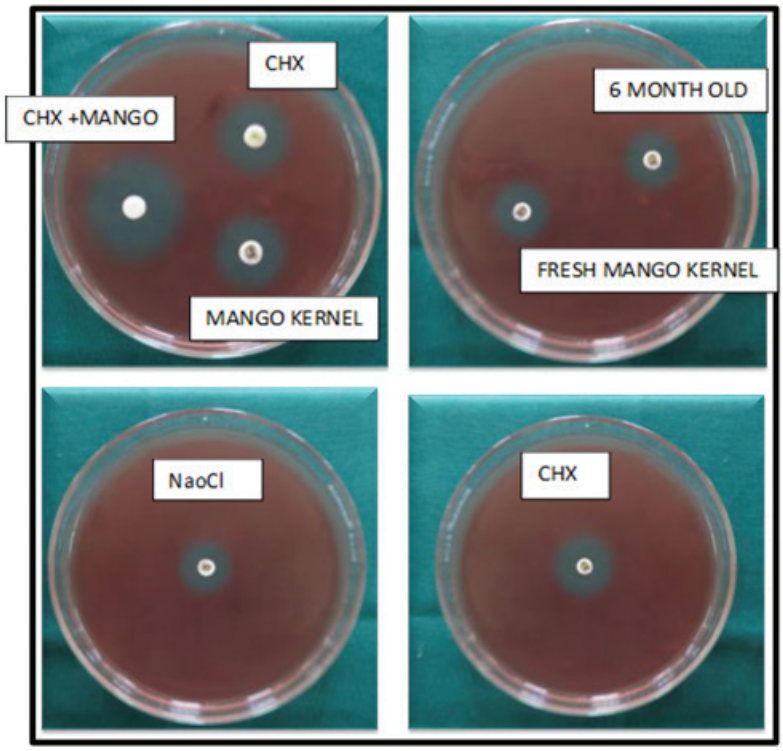


FIG 4

Results of Second Part

Table 2

	Mean (mm) E faecalis (FIG 10)		Mean (mm) C.albicans (FIG 9)	
	1 HOUR	**24 HOUR**	**1 HOUR**	**24 HOUR**
FRESH MANGO KERNEL	6	16	8	18
6 MONTH OLD M.KERNEL	7	16	8	18
CHLORHEXIDINE	7	16	8	18
SODIUM HYPOCHLORITE	5	15	4	16
DMSO	6	16	5	17
CHLORHEXIDINE + M KERNEL	0	0	0	0
DMSO	8	19	8	20

Discussion

Nature has bestowed a very rich botanical wealth,and a large number of diverse types of plants grow in different parts of world. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment for infectious diseases, and simultaneously they also mitigate many of the side effects that are often associated with synthetic antimicrobials [1].Hence, the purpose of the present study was to evaluate the role of antimicrobial agents of plant origin in elimination of root canal infections.

At present, sodium hypochlorite (naocl) appears to be the most suitable gold standard irrigant because it fulfils most of the requirements for an ideal endodontic irrigant [2].but it has several undesirable characteristics such as tissue toxicity, allergic potential and disagreeable smell and taste [3]. Regarding the organic tissue dissolution and bacterial inhibition, a 5.25% naocl solution is more effective in a shorter time within 2 minutes[4]. Chlorhexidine digluconate (CHX) is widely used in disinfection in dentistry because of its good antimicrobial activity. CHX does not possess some of the undesired characteristics of sodium hypochlorite .however, CHX has no tissue-dissolving capability and therefore it cannot replace sodium hypochlorite. Several studies have compared the antibacterial effect of NaOCl and 2% CHX against intracanal infection and have shown little or no difference between their antimicrobial effectiveness. No adverse effects have been published regarding CHX use as irrigant or intracanal medicament[5]

To overcome problems associated with currently used irrigants, 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

Tridax procumbens Linn. (Family-Asteraceae; common name-Dhaman grass) (T. procumbens) is common herb found in India. It is denoted by different names; in English as Mexican Daisy, in ayurvedic as Jayanti, in siddha/tamil as Vettukkaaya-thalai and in folk as Akala kohadi. It was reported to treat various aliments, such as bronchial catarrh, dysentery, diarrhea, preventing hair loss, and to check hemorrhage from cuts . Pharmacological studies have shown that T. procumbens possess properties like-anti inflammatory, hepatoprotective, wound healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effects. Earlier workers have reported that the presence

of dexamethasone, luteoline glucotureolin,-sitosterol, flavone, glycoside and quercetin in this plant. This study aims to investigate the phytochemical and antibacterial effect of T. procumbens[6]

The active constituents of O. sanctum are tannins (4.6%) and essential oil (up to 2%). The essential oil consists principally of eugenol (up to 62%) and methyleugenol (up to 86%)[7] .Studies have demonstrated that eugenol is effective against both gram-negative and gram-positive bacteria by causing protein leakage [8]. The antimicrobial mechanism of tannins is due to their ability to form complexes with enzymes or substrates required by microorganisms for their functioning or may be related to its action on the cell membrane of the microorganisms [9]. The reduced efficacy of O. sanctum as compared to other groups in this study may be attributed to the lower concentration of active ingredients.

The mango seed kernels constituted about 18% of the total fruit and had 5% protein, 6–7% crude fat, 0.19–0.44% tannins, iodine value of 34–44 and saponification number . Oleic acid (42%) and stearic acid (39%) were the principal fatty acids in the oil.. The essential amino acid index and protein quality index were high, thus indicating the good quality of the protein in mango seed kernel[10].

MECHANISM OF ANTIMICROBIAL ACTION OF TANNINS[11]

- INHIBITION OF EXTRACELLULAR MICROBIAL ENZYMES
- DEPRIVATION OF THE SUBSTRATES REQUIRED FOR MICROBIAL GROWTH
- DIRECT ACTION ON MICROBIAL METABOLISM THROUGH INHIBITION OF OXIDATIVE PHOSPHORYLATION

The present study demonstrates the antimicrobial efficacy of mango kernel equivalent TO 5.25% NaoCl against strains of Enterococcus faecalis and Candida albicans, which may well be replaced by this potential herbal extract as endodontic irrigant to overcome the deleterious effects of the conventional irrigants (NAOCL AND CHLORHEXIDINE) on dentine. Mango kernel irrigant shows rapid onset of action within 1 hour compared to other groups which highlights strong antimicrobial efficacy.this reveals, it requires less contact time compared to naocl for its antimicrobial action. This study highlighted the shelf life of new bio irrigant.

Six month old mango kernel irrigant shows equal efficacy to that of freshly prepared irrigant. We also compared the combination of 2% chlorhexidine with mango kernel irrigant and it shows maximum antimicrobial efficacy.so by using this combination we can try to avoid complications with maximum antimicrobial actions. The present study is only an invitro study, which is a stepping stone in the evolution of the new bio irrigant.further ex vivo, invivo studies are required to launch the new bio irrigant to our clinics

Conclusion

WITHIN THE LIMITATION OF THE STUDY, IT WAS CONCLUDED THAT,

- COMBINATION OF 2% CHLORHEXIDINE AND MANGO KERNEL SHOWS SUPERIOR ANTIMICROBIAL EFFICACY TO THAT OF 5.25% NaOCl,& 2 % CHLORHEXIDINE AGAINST STRAINS OF E.FAECALIS & C.ALBICANS
- MANGO KERNEL IRRIGANT IS HAVING GOOD SHELF LIFE
- MANGO KERNEL IRRIGANT IS HAVING RAPID ONSET OF ACTION

DATA AVAILIBILITY STATEMENT : Previously reported research data were used to support this study and are available at google scholar. These prior studies (and datasets) are cited at relevant places within the text as references 6-10

CONFLICTS OF INTEREST : The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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References

[1] Rios, J.L. et al.Medicinal Plants and Antimicrobial Activity.. Journal of Ethnopharmacology.2005:80-84.

[2] Zehnder, Matthias, Root Canal Irrigants. Journal of Endodontics .2006;32(5): 389 – 398

[3] Caliskan MK et al .Allergy to sodium hypochlorite during root canal therapy: a case report.International Endodontic Journal .1994;27:163

[4] Marion, Jefferson & Manhães et al . Efficiency of different concentrations of sodium hypochlorite during endodontic treatment. Literature review. Dental Press Endodontics.2012; 2: 32-37.

[5] Gomes BP. Chlorhexidine in endodontics, Braz Dent J. 2013;24(2):89-102

[6] Ali, Mohammed et al. A new flavonoid from the aerial parts of Tridax procumbens.2001; 72 (3): 313–5.

[7] Maheshwari et al.Essential oil of sacred basil (Ocimum sanctum). Indian Perfumer .1987;31:137-145.

[8] Oyedemi SO et al. The proposed mechanism of bactericidal action of eugenol, α-trepinol and γ-terpinene against Listeria monocytogenes, Streptococcus pyogenes, Proteus vulgaris and Escherichia coli. Afr J Biotechnol 2009; 8: 1280-6

[9] Akiyama H et al . Antibacterial action of several tannins against Staphylococcus aureus. J. Antimicrobial Chemotherapy, 2001;48 (48):487-491

[10] Arunajatesan et al . Antibacterial efficacy of Mangifera indica L. kernel and Ocimum sanctum L. leaves against Enterococcus faecalis dentinal biofilm, , J Conserv Dent. 2013; 16(5): 454–457

[11] Richard W. Hemingway et al .Plant Polyphenols: Synthesis, Properties, Significance. - Science .2012 : 1053

[12] A Geethashri et al. Comparative evaluation of biofilm suppression by plant extracts on oral pathogenic bacteria. Journal of Applied Pharmaceutical Science.2014 ;4(3):20-23 ·

[13] M.A. AUGUSTIN and E.T. LING.Composition of Mango Seed Kernel, Pertanika 1987;10(1): 53-59

[14] Kittiphoom, S. and Sutasinee, S.Mango seed kernel oil and its physicochemical properties,IFRJ .20(3):1145-1149

[15] Muhammad Nadeem & Muhammad et al. Promising features of mango (Mangifera indica L.) kernel oil: a review, J Food Sci Technol .2016; 53(5):2185–2195

[16] Zahed Mohammadi et al.Antimicrobial efficacy of chlorhexidine as a root canal irrigant: a literature review, Journal of Oral Science, 2014;56(2):99-103

[17] Jefferson J. C. Marion.Efficiency of different concentrations of sodium hypochlorite during endodontic treatment.Literature review, Endod. 2012;2(4):32-7

[18] Grossman's Endondontic Practice,12th edition

[19] Ingles Endodontics 6th Edition

[20] Cohen's Pathways of the Pulp -10th edition