Comparison of the Antimicrobial Efficacy of 3 % Sodium Hypochlorite, 2 % Chlorhexidine Gluconate, Neem Leaf Extract, Noni Juice and Guava Leaf Extracts against E Faecalis and C Albicans - In Vitro Study

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Abstract: ***Plants have been utilised for health problems and illness prevention, including epidemics, since the dawn of time. India is noted for its extensive medicinal and herbal plant collection. Phytochemical concentration is high in several of the plants. These phytochemicals can be used to make phytomedicines with positive effects on the human body. Phytomedicines are a godsend in the field of endodontics. Herbal extracts are most commonly employed as endodontic irrigants, although they have the potential to be very popular owing to the adverse effects of synthetic drugs that affect the microbiota. This study focuses on the benefits of using various herbal plant extracts as herbenodontics in root canal irrigation over commercially available most popular irrigants for removing microorganisms.***

Keywords: NaOCl, CHX, E. faecalis, C. albicans

1. Introduction

Endodontic infections have been shown to contain the most well-known microbial species such as *E.faecalis* and *C.albicans*, with research indicating that the retention of both of these microorganisms contributes to root canal treatment failures in the long run. In contrast to initial endodontic infections, which are polymicrobial in origin and dominated by Gram-negative anaerobic rods, secondary infections include just one or a few bacterial species. *Enterococcus faecalis* has been recovered in 23-70 percent of positive cultures and is usually found in monoculture in obturated root canals presenting indications of chronic apical periodontitis.(1) Fungi, on the other hand, make up a minor component of the oral microbiota. Candida species make up the vast majority of the fungal microbiome.(2)*Candida albicans* has been found in the oral cavity in 30-45 percent of healthy persons and 95 percent of patients infected with the human immunodeficiency virus. 2. Both initial infections (5-20%) and chronic infections (25%) of root canal systems show the presence of *Candida albicans*. In addition to mechanical debridement, successful RCT aims for entire removal of sick tissue by disrupting and eliminating all microorganisms, which necessitates frequent, repeated irrigation with intracanal medicaments. Sodium hypochlorite (0.5-5.25 percent), hydrogen peroxide (3 percent) solution, EDTA, chlorhexidine gluconate (0.2-2 percent), and physiologic saline solution are all commonly used in RCT. The most often used medication is sodium hypochlorite, which has an antibacterial action and is an excellent organic tissue solvent without leaving any toxic residues. When used in concentrations ranging from 0.5 to 6%, sodium hypochlorite (NaOCl) is the gold standard in root canal irrigation. The irrigant concentration is still a point of contention: many writers advocate a 5.25 percent concentration of NaOCl (Harrison 1984), while others prefer a lower concentration of 3 percent or even 0.5 percent (Spangberg et al. 1973, Baumgartner and Cuenin 1992).(3) NaOCl has been shown to be efficient against a wide variety of bacteria and to disintegrate both vital and necrotic tissue (Senia et al. 1975).(4) The irrigant is well-known for its antimicrobial properties as well as its ability to dissolve tissue. The effects of NaOCl and its toxicity are dose-dependent.(5) Chlorhexidine gluconate is another often used irrigant that has the substantivity feature. Because of its broad-spectrum antibacterial effect, substantivity, and low toxicity, CHX gluconate has been recommended as a root canal irrigant.(6)Though 2 percent chlorhexidine showed strong antimicrobial activity against *E. faecalis*, its lack of acceptability may be attributed to its failure to dissolve necrotic tissue remains in combination with low antimicrobial action when tested in vivo.(5)Some attempts have been made to investigate the activity of CHX in dissolving organic matter, with the results revealing that both aqueous solution and gel formulations of this chemical are incapable of dissolving pulp tissues. It has a few additional disadvantages, such as a foul taste, is poisonous in nature, causes instrument rust, causes inflammatory and allergic responses, causes tooth discolouration, has a bad taste, causes dry mouth, and is generally inferior than NaOCL in antimicrobial clearance. As a result, research was conducted to find safer and more effective herbal alternative agents. Various natural plant extracts have also been proven to exhibit antibacterial activity, implying that they might be employed as a root canal irrigant. Noni juice, guava, and neem leaf extract have been examined for their antibacterial and antioxidant properties, and it was shown that they have antimicrobial properties against oral infections.

Neem (Azadirachta Indica) is a versatile medicinal plant with antibacterial action throughout a broad spectrum. It is known as the 'sickness reliever' and is considered India's local dispensary.(7) The National Academy of Science in the United States has designated neem as a "tree for tackling world challenges." It's a safe and effective herbal alternative to root canal irrigants like NaOCL. It's biocompatible, doesn't harm tissues, and has antifungal, antiviral, and antibacterial properties.(8)

Guava (Psidium guava) is a popular and nutritious fruit found across India. It's also known as the "poor man's apple" because of its high nutritional content, which is similar to that of an apple.(9) Guava leaf extracts were discovered to exhibit antibacterial activity against oral infections. It removes smear layer effectively when used as a root canal irrigant.(10)

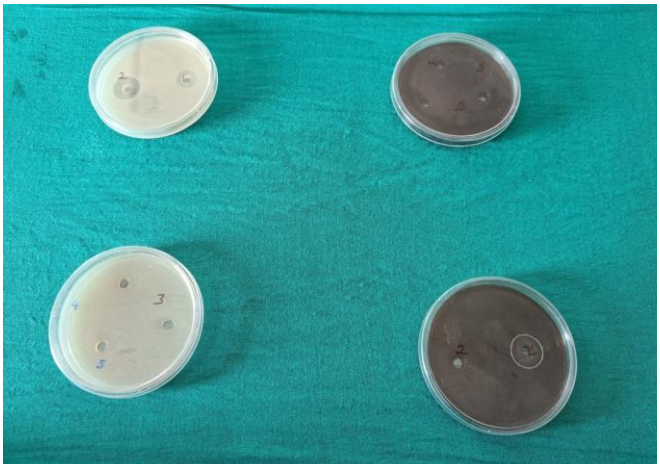
Noni (Morindacitrofolia) in India it is also known as Indian Mulberry or Cheese Fruit. It possesses antibacterial, antifungal, anti-inflammatory, anti-oxidant, and moderate analgesic properties, among others. Noni juice with a concentration of 6% has the potential to be a unique root canal irrigant. It's biocompatible and immune-boosting, and it's unlikely to irritate sensitive tissues.(11)It was one of the first herbal irrigants to be used as a substitute for NaOCl, the gold standard root canal irrigant. When compared to NaOCl, it is about as effective in removing the smear layer. It has been discovered to improve stem cell adhesion and has low toxicity; therefore it may be extensively used in the future over NaOCl in regenerative endodontic operations.

2. Materials and Methods

The in vitro investigation was carried out at the Institute of Dental Sciences, Bareilly, and the Department of Microbiology, Rohilkhand Medical College, Bareilly Uttar Pradesh where neem leaf extract (group I) was prepared. Fresh, mature A. indica leaves were gathered, rinsed with sterile distilled water, and air-dried at room temperature for two weeks. The leaves were then crushed into a fine powder and placed in a muslin fabric bag for extraction using a hot continuous pressure method. The extraction technique was carried out using ethanol (absolute alcohol 99.99 percent v/w). We prepared a 15 percent stock solution by dissolving 150 mg of crude neem extract in 10 ml of dimethyl sulfoxide (DMSO) after obtaining the crude form. Noni juice (group II) was created by diluting 100% juice obtained from fresh noni fruit available in the Bareilly market with 6 percent morindacitrifolia juice.

Production of guava leaves extract (group III) - Guava leaves were taken from the Rohilkhand medical college botanical garden and dried in fresh open air, away from direct sunlight. In a beaker holding 500 mL of sterile distilled water, 50 grammes of powdered leaves were placed. The hot water extract was made by boiling the menstruum in a water bath until it was reduced to around 125ml, or roughly a quarter of its original volume. The resultant liquid was filtered using filter paper after the water content in the extract had completely evaporated.

Commercially available 3% sodium hypochlorite (group IV) and 2% chlorhexidine gluconate (group V) were used. Disc Preparation - For each organism, 100 microliters of suspensions of *E. faecalis* and *Candida albicans* were collected from prepared cultures and injected in culture plates with previously laid layers of Mueller Hinton Agar and Blood Agar. These organisms were inoculated over their different medium using a sterile spreader. On the *E. faecalis* and *C.albicans* culture plates, five identical 6mm wells were produced. Only 200l each of the experimental and positive control solutions were added to the wells of each plate. In an incubator, these plates were incubated for 24 hours at 37°C.Plates were tested for zones of bacterial growth inhibition after the incubation time, and the widths of the zones attained by each group against *E.faecalis* and *C.albicans* were measured in millimetres (mm). A calliper was used to measure the antibacterial sensitivity pattern, which was depicted as the zone of inhibition at its maximum diameter, surrounding each well, and the findings were tabulated. To get a statistically significant result, six agar diffusion experiments were performed and inhibition assessed after 24, 48, and 72 hours, respectively.



3. Results

In the preliminary Agar well diffusion study, neem leaf extract had the maximum zone of inhibition and noni juice had minimum zone of inhibition (Figure 1). The diameter of the inhibition zone was in the following order: (5) neem leaf extract > (1) 3% NaOCl > (2) 2% CHX > (3) guava leaf extract >(4) 6% noni juice.

Table 1: Descriptive statistics of zone of inhibition of all the experimental irrigants with mean zone and standard deviation.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
E.faecalis	NaOCl	20	18.0	1.58	0.71	16.04	19.96	16	20
	CHX	20	21.4	1.82	0.81	19.14	23.66	19	24
	NEEM	20	23.8	1.30	0.58	22.18	25.42	22	25
	GUAVA	20	14.6	2.07	0.93	12.03	17.17	12	17
	NONI	20	12.0	1.58	0.71	10.04	13.96	10	14

Table 2: Descriptive statistics of zone of inhibition of all the experimental irrigants with mean zone and standard deviation.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
C.albicans	NaOCl	20	20.0	1.58	0.71	18.04	21.96	18	22
	CHX	20	22.0	1.58	0.71	20.04	23.96	20	24
	NEEM	20	24.4	2.07	0.93	21.83	26.97	22	27
	GUAVA	20	16.0	1.58	0.71	14.04	17.96	14	18
	NONI	20	14.0	1.58	0.71	12.04	15.96	12	16

Table 3: Comparison of groups of experimental irrigants for their zones of inhibition against *Enterococcus Faecalis*

ANOVA						
		Sum of Squares	Df	Mean Square	F-Value	P-Value
E.faecalis	Between Groups	463.760	4	115.940	40.538	<0.001*
	Within Groups	57.200	95	2.860		
	Total	520.960	99			

*statistically significant.

Table 4: Comparison of groups of experimental irrigants for their zones of inhibition against *Candida albicans*

ANOVA						
		Sum of Squares	Df	Mean Square	F-Value	P-Value
C.albicans	Between Groups	363.840	4	90.960	31.804	<0.001*
	Within Groups	57.200	95	2.860		
	Total	421.040	99			

*statistically significant

Table 5: Inter-group comparison of mean difference in the zone of inhibition

Dependent Variable			Mean Difference	Std. Error	P-Value	95% Confidence Interval	
						Lower Bound	Upper Bound
E.faecalis	NaOCl	CHX	3.4	1.0696	0.005*	-5.631	-1.169
	NaOCl	NEEM	5.8	1.0696	0.000*	-8.031	-3.569
	NaOCl	GUAVA	3.4	1.0696	0.005*	1.169	5.631
	NaOCl	NONI	6	1.0696	0.000*	3.769	8.231
	CHX	NEEM	2.4	1.0696	0.036*	-4.631	-.169
	CHX	GUAVA	6.8	1.0696	0.000*	4.569	9.031
	CHX	NONI	9.4	1.0696	0.000*	7.169	11.631
	NEEM	GUAVA	9.2	1.0696	0.000*	6.969	11.431
	NEEM	NONI	11.8	1.0696	0.000*	9.569	14.031
	GUAVA	NONI	2.6	1.0696	0.025*	.369	4.831

*statistically significant

Table 6: Inter-group comparison of mean difference in the zone of inhibition

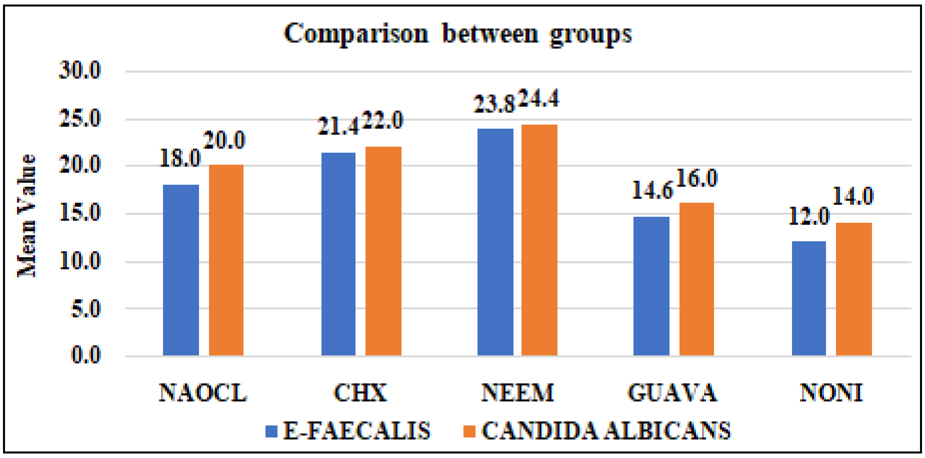
Dependent Variable			Mean Difference	Std. Error	P-Value	95% Confidence Interval	
						Lower Bound	Upper Bound
C.albicans	NaOCl	CHX	2.0	1.0696	0.076	-4.231	.231
	NaOCl	NEEM	4.4	1.0696	0.001*	-6.631	-2.169
	NaOCl	GUAVA	4.0	1.0696	0.001*	1.769	6.231
	NaOCl	NONI	6.0	1.0696	0.000*	3.769	8.231
	CHX	NEEM	2.4	1.0696	0.036*	-4.631	-.169
	CHX	GUAVA	6.0	1.0696	0.000*	3.769	8.231
	CHX	NONI	8.0	1.0696	0.000*	5.769	10.231
	NEEM	GUAVA	8.4	1.0696	0.000*	6.169	10.631
	NEEM	NONI	10.4	1.0696	0.000*	8.169	12.631
	GUAVA	NONI	2.0	1.0696	0.076*	-.231	4.231

*statistically significant

Table 7: Group comparison of mean in the zone of inhibition

	E-FAECALIS	*CANDIDA ALBICANE*	
	Mean ± SD	Mean ± SD	P-value
NaOCl	18.0 ± 1.58	20.0 ± 1.58	0.081#
CHX	21.4 ± 1.82	22.0 ± 1.58	0.593#
NEEM	23.8 ± 1.3	24.4 ± 2.07	0.599#
GUAVA	14.6 ± 2.07	16.0 ± 1.58	0.264#
NONI	12.0 ± 1.58	14.0 ± 1.58	0.081#

#statistically significant



4. Discussion

The primary goal of root canal therapy is to remove germs from the root canal and avoid recontamination during the recovery phase. After mechanical debridement, the biofilm in anatomically problematic places such as fins, lateral or furcal canals, apical deltas, webs, and isthmus may remain undisturbed. As a result, in order to achieve comprehensive disinfection of the canal system, irrigant solutions must be used in addition to the mechanical equipment.

By virtue of genetic polymorphism and dentin binding capabilities, *E. faecalis*, a gram-positive bacteria, although if present in minute quantities, plays a major role in producing periradicular infections and root canal failures. According to studies, E. *faecalis* is found in 4 to 40% of root canal infections, with a ninefold increase in treatment failures.(12)*C.albicans*is a dentinophilic yeast that thrives in infected canals and peri-radicular tissues.(13) Because of the following reasons, *E. faecalis* was chosen as one of the test microorganisms in this experiment: (I) It is known as the associated pathogen in endodontically treated teeth with chronic apical periodontitis (ii) it is resistant to sodium hypochlorite (NaOCl) (iii) it may colonise the whole width of dentinal tubules (iv) and it grows fast.(14)

Persistent bacteria such as *E. faecalis* and *Candida albicans* in root canals even after therapy cause treatment failure. As a result, appropriate treatment techniques must incorporate both traditional intracanal irrigating medications such as sodium hypochlorite and chlorhexidine as well as newer herbal items such as azadirecta indica, guava leaf extract, and Morindacitrifolia fruit juice.(15)

Because of its antibacterial activity and tissue dissolving potential, NaOCl has been regarded the irrigant of choice for root canal irrigation since its inception. NaOCl's high pH interferes with cytoplasmic membrane integrity and causes biosynthetic changes in cellular metabolism, which contributes to its antibacterial character. NaOCl's tissue dissolving activity and dissolve rate are related to its concentration [16]Not only does NaOCl's antibacterial activity, tissue dissolving capacity, and smear layer removal ability rise with concentration, but so does its caustic potential and toxicity.(17)

NaOCl has a number of drawbacks, including toxic effects on important tissues, which can lead to hemolysis, skin ulceration, and necrosis. When NaOCl comes into contact with the eyes of the patient or the operator, it causes instant discomfort, excessive watering, extreme burning, and erythema. It also produces periapical swelling and edoema of the tissues when extruded through the apical foramina. [2]

CHX has been proposed as an endodontic irrigant because of its antibacterial properties, lower cytotoxicity than NaOCl, substantivity, and effective clinical performance.(18) However, it has drawbacks such as tissue irritation and the generation of reactive oxygen species.(19)

When tested against *Candida albicans*, chlorhexidine at varied doses exhibited wider zones of inhibition at higher concentrations (2 percent), comparable to the findings of Estrella et al.(20)

Because of its broad-spectrum antibacterial effect, substantivity, and low toxicity, CHX gluconate has been recommended as a root canal irrigant. However, the inability of CHX to dissolve tissue has been identified as a significant drawback. Some attempts have been made to investigate the activity of CHX in dissolving organic matter, with results revealing that both aqueous solution and gel formulations of this chemical are incapable of dissolving pulp tissues.(2)

Tanins found in Morindacitrifolia contribute to its antibacterial effect due to their toxicity, which causes bacteria and fungus to be destroyed; another component is its high pH. (3.5).(21) The leaf extracts of *A.indica* displayed substantial antibacterial activity against all of the test microorganisms, according to Rajshekharan et al. The inhibitory actions of the leaf extracts, on the other hand, were organism and solvent dependent. Both Gram-positive and Gram-negative bacteria were inhibited by the leaf extracts.(22)

Furthermore, Botelho et al. (2002) and Behl et al. (2008) concluded from their experiments and trials that A. indica is highly effective in the treatment of periodontal disease, demonstrating its biocompatibility with human periodontal ligament (PDL) fibroblast; it has anti-adherence activity by altering bacterial adhesion and has been beneficial in the prevention of microorganism colonisation inside the root canals.The use of neem as an endodontic irrigant may be beneficial since it is unlikely to cause significant harm to patients, like NaOCl accidents can. When compared to 3 percent NaOCl and 2 percent CHX, neem extract at a concentration of 0.94 percent against *E. faecalis* and 1.88 percent against *Candida albicans* demonstrated considerable antibacterial activity.(2)

Guava leaf extracts may have antibacterial properties due to flavonoids such as mosin glycosides, quercetin, and quercetin glycosides. The polygalacturonase inhibitory proteins in the guava plant cell walls are thought to be responsible for the resistance to bacterial infections. The aqueous extracts of guava leaf can significantly reduce the adherence of the plaque biofilm's early organisms.(5)

Tanins found in Morindacitrifolia contribute to its antibacterial effect due to their toxicity, which causes bacteria and fungus to be destroyed; another component is its high pH. (3.5).

When tested against *Candida albicans*, Morindacitrifolia juice at various quantities revealed bigger zones of inhibition at higher concentrations (100 percent). There was no restraint (0.75 percent, 1.5 percent). Some antibacterial activity was seen at a concentration of 3%. Wang et al., findings are comparable to ours.(23)

5. Conclusion

This was a preliminary study of the antimicrobial efficacy of an indigenously herbal irrigant against *E. faecalis*. & *Candida albicans*. The results indicate that, within the limitations of this study, Neem leaf extreact has the most potential to be used as an herbal alternative in root canal irrigation. However additional research and clinical trails are needed to prove feasibility of the result.

References

[1] Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ free and conventional laboratory rats. *J South Calif Dent Assoc.* 1966; 34:449-51.

[2] Prasad SD, Goda PC, Reddy KS, Kumar CS, Hemadri M, 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. Journal of Dr. NTR University of Health Sciences. 2016 Apr 1;5(2):104.

[3] Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. International endodontic journal. 2009 Apr;42(4):288-302.

[4] Hülsmann M, Hahn W. Complications during root canal irrigation–literature review and case reports. International endodontic journal. 2000 May;33(3):186-93.

[5] Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, Mariodan JP. Comparative evaluation of antimicrobial activity of QMiX, 2.5% Sodium Hypochlorite, 2% Chlorhexidine, Guava Leaf extract and Aloevera extract against Enterococcus faecalis and Candida albicans–An in-vitro Study. Journal of clinical and diagnostic research: JCDR. 2016 May;10(5):ZC20.

[6] Oliveira DP, Barbizam JV, Trope M, Teixeira FB. In vitro antibacterial efficacy of endodontic irrigants against Enterococcus faecalis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2007 May 1;103(5):702-6.

[7] Rajasekaran C. Investigations on antibacterial activity of leaf extracts of Azadirachta indica A. Juss (Meliaceae): a traditional medicinal plant of India. Ethnobotanical leaflets. 2008;2008(1):161.

[8] Babaji P, Jagtap K, Lau H, Bansal N, Thajuraj S, Sondhi P. Comparative evaluation of antimicrobial effect of herbal root canal irrigants (Morindacitrifolia, Azadirachta indica, Aloe vera) with sodium hypochlorite: An in vitro study. Journal of International Society of Preventive & Community Dentistry. 2016 May;6(3):196.

[9] Joseph B, Priya M. Review on nutritional, medicinal and pharmacological properties of guava (Psidium guajava Linn.). International Journal of pharma and bio sciences. 2011 Jan;2(1):53-69.

[10] Ramesh S. Antibacterial Efficacy of Psidium Guajava Leaf Extract on E. feacalis-In Vitro Study. Annals of Medical and Health Sciences Research. 2021 Jun 10.

[11] Pujar M, Makandar SD. Herbal usage in endodontics-A review. International journal of contemporary dentistry. 2011;2(1).

[12] B, Vacheva R. In vitro study of the effectiveness of intracanal irrigants on candida albicans. J of IMAB 2007; 13:3-7.

[13] Ashraf H, Samiee M, Eslami G, Ghodse Hosseini MR. Presence of Candida Albicans in Root Canal System of Teeth Requiring Endodontic Retreatment with and without Periapical Lesions. Iranian Endodontic Journal. 2007;2(1): 24-28.

[14] Mathew J, Pathrose S, Kottoor J, Karaththodiyil R, Alani M, Mathew J. Evaluation of an indigenously prepared herbal extract (EndoPam) as an antimicrobial endodontic irrigant: An ex vivo study. Journal of international oral health: JIOH. 2015 Jun;7(6):88.

[15] Singh M, Singh S, Salgar AR, Prathibha N, Chandrahari N, Swapna LA. An in vitro comparative evaluation of antimicrobial efficacy of propolis, Morindacitrifolia Juice, sodium hypochlorite and chlorhexidine on Enterococcus faecalis and Candida albicans. J. Contemp. Dent. Pract. 2019 Jan 1;20(1):40-5.

[16] Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *J Endod*. 1992;18(12):605-12.

[17] K P Shetty, S V Satish, K Kilaru, K C Ponangi, V R Venumuddala, P. Ratnakar. Comparative evaluation of the cytotoxicity of 5.25% sodium hypochlorite, 2% chlorhexidine and mixture of a tetracycline isomer, an acid and a detergent on human red blood corpuscles: an in-vitro study. *Saudi Endod J.* 2014;4(1):1-6

[18] Leonardo MR, Tanomaru Filho M, Silva LA, Nelson Filho P, Bonifácio KC, Ito IY. *In vivo* antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod 1999;25(3):167-71.

[19] Chapple IL. Reactive oxygen species and antioxidants in inflammatory diseases. J Clin Periodontol 1997;24(5):287-96.
[20] Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Brazilian dental journal. 2002;13(2):113-117.
[21] Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G. Morindacitrifolia (Noni): a literature review and recent advances in Noni research. Acta PharmacologicaSinica. 2002 Dec 1;23(12):1127-1141.
[22] Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, *et al*. Investigations on antibacterial activity of leaf extracts of Azadirachta indica A.Juss (Meliaceae): A traditional medicinal plant of India. Ethnobot Leaflets 2008;12:1213-7.
[23] Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G. Morindacitrifolia (Noni): a literature review and recent advances in Noni research. Acta PharmacologicaSinica. 2002 Dec 1;23(12):1127-1141.