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Comparative study of anti-microbial efficacy of herbal root canal irrigants *Curcuma longa* (turmeric), *Ocimum sanctum* (basil), *Cocos nucifera* (coconut) against *Enterococcus faecalis* and *Candida albicans*: in vitro study

Garima Sinha[1], Kiran Murthy D[2], Thota Lenin Babu[3], Mrinmoy Chakraborty[4], Vinay Oraon[5], Jyoti Kumar[6]

[1234]Department of Conservative Dentistry and Endodontics, VDCH, Farathiya, Garhwa, Jharkhand
[56]Biotechnology, University Department of Botany, Ranchi University, Ranchi, Jharkhand

Corresponding Author: Garima Sinha, Email: gsinha277@gmail.com

ABSTRACT

The objective is, to compare the antimicrobial efficiency between irrigants of Tulsi (*Ocimum sanctum*) extract, Turmeric (*Curcuma longa*) extract, Coconut water (*Cocos nucifera*), Sodium hypochlorite and Saline against *Enterococcus faecalis* and *C. albicans*. Total number of irrigants sample are five: Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Coconut water (*Cocos nucifera*), Sodium hypochlorite and Saline. The Antibiogram analysis of Tulsi, Turmeric, sodium hypochlorite, saline and Coconut water were performed against two microbial pathogens. A stock culture of ACC 3040, *E. faecalis* strain and MCC 1152, *Candida albicans* were used. Respectively culture was grown overnight at 37℃ in BHI broth (Himedia) and SDA. Microbial growth was checked by changes in turbidity at 24 hours. After that loading of irrigants sample, then plates were incubated in invert position for overnight at 37℃ for bacteria & 26℃ for fungus. After incubating for overnight the plates are observed for the zone of inhibition, which were then measured. The diameter was measuring vertically & horizontally and the average value was calculated. Highest herbal irrigants zone of inhibition against *Candida albicans* was seen for Turmeric (*Curcuma longa*), 20 mm and 12 mm for Tulsi (*Ocimum sanctum*). Maximum zone of inhibition against *Enterococcus faecalis* was seen for Tulsi (*Ocimum sanctum*), 15 mm and 13 mm for Turmeric (*Curcuma longa*). Tested herbal medicine Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Coconut water (*Cocos nucifera*), Sodium hypochlorite and Saline showed inhibitory zones against *E. faecalis* and *C. albicans*. Herbal extract (Tulsi and Turmeric) has shown significantly inhibitory effect against *E. faecalis* and *C. albicans* compared to sodium hypochlorite. Hence, these herbal irrigants can be used alternatively as endodontic irrigants/medicament. Further in vivo modify its content for acceptability by patients.

Keywords: Tulsi extract, Turmeric Extract, Coconut water, Sodium hypochlorite, Saline, *E. faecalis* and *C. albicans*

1. INTRODUCTION

Pulpal disease is prevalent oral diseases of recent times with bacteria or their products entering the pulp being the frequent etiological agents (1). The foci of infection involve of gram positive, gram negative and anaerobic micro-organisms (2). Man does not live by bread alone. In the same way, the pulp system cannot be cleaned and shaped by instruments alone. Along with filing, the second important process while root canal debridement (cleaning & shaping) is irrigation (3). Irrigating solutions are very important during root canal preparation because they support in the cleaning of root canal, lubricating files removing debris, it has anti-microbial effect, provide pulp dissolution without affecting the periapical tissues (4). For the proper cleaning and disinfecting the root canal system, an irrigants should have the ability to disinfect and penetrate dentin and its tubules, provide long-standing antibacterial effect (substantivity), smear layer should be removed, and it should be nonantigenic, nontoxic and noncarcinogenic. Dentin and sealing ability of filling materials should not be hampered and as well as it should be reasonably cost effective, its

application should be convenient and should not cause tooth staining or discoloration. Other desired properties for an ideal irrigants comprise the ability to dissolve pulp tissue and reduce its endotoxins (5). An extensive synthetic antimicrobial agent has been used as an endodontic irrigants (6). The irrigants that are presently used during biomechanical preparation can be divided into antibacterial and decalcifying agents or their combinations.

These synthetic agents are sodium hypochlorite (NaOCl), chlorhexidine, ethylenediaminetetraacetic acid (EDTA), a mixture of tetracycline, an acid and detergent (MTAD) (7). Because of the increased antibiotic resistance to these antimicrobial agents, toxic and injurious side effects of few common antibacterial agents, there's need of substitute agents that are affordable, non-toxic and effective. It has been known that herbal extracts could be used as efficient endodontic irrigants (8). E. faecalis was selected because it has been shown to be related with failed endodontic cases and has the ability to invade whole length of dentinal tubules to 1100 cm or close to the cementum. It is also significant to authorize the bactericidal action of different disinfection methods against resistant microorganism such as E. faecalis (9). Facultative microorganisms such as *Enterococcus faecalis, Candida albicans,* and *Staphylococcus aureus* have been considered as the greatest resistant species in the oral cavity and may be a probable cause of failure of root canal treatment. Persistent periarticular lesions after root canal treatment, *E. faecalis* is associated with it.

C. albicans and *S. aureus* may be associated with failed endodontic therapy and can be considered as dentinophilic microorganism. *C. albicans* is found in 18% of the retreatment infections, whereas *S. aureus* is found in 0.7%-15% of the cases (10). Individuals are becoming aware of side effects of synthetic drugs and resistant pathogens, there is an urgent need to develop new antimicrobial agents. For the control of numerous human pathogens, on toxic and eco-friendly herbal products are more in demand. Traditional treatments include medicinal plants for numerous human diseases for thousands of years. Medicine made from these plant products have advantage of being simple, effective and exhibit broad spectrum activity (11). As there are approximately 500000 plant species occurring worldwide, in which only 1% has been phytochemically examined, having a great potential for ascertaining new bioactive compounds from these sources.

Therefore, the purpose of this article is to introduce some examples of traditional medicinal plant extracts or phytochemicals having ability to hinder the growth of oral pathogens, and lessen the signs of oral diseases (12). The bacterium shows certain issues such as lytic enzymes, cytolysin, pheromones, and lipoteichoic acid (13, 14) which reduce the action of lymphocytes, potentially causative to endodontic failure (15). The source of modern medicine is the traditional medicine (16). Medicines in that are in kind of traditional medicine are turmeric and triphala (17). The numerous advantages of using natural irrigants, desired to take the present research for the evaluation of the antibacterial properties of herbal extracts, that are Turmeric, Tulsi, Coconut water, sodium hypochlorite and saline, as irrigants during root canal treatment, compared with conventional irrigating solutions.

2. MATERIALS AND METHODS

Sample Collection: Total number of samples are five: Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Coconut water (*Cocos nucifera*), Sodium hypochlorite and Saline. The Tulsi sample was collected from the Vananchal Dental college & Hospital, Garhwa, where the plant of Tulsi, was grown. From there the healthy plants of Tulsi, leaves were collected. And Turmeric and coconut water was bought from the market of Garhwa.Sodium hypochlorite and saline were bought from Ranchi, Jharkhand. Solvents Used. For the preparation of plant extracts, organic solvents were used.

During extraction solvents diffuse in to the solid plant material and solubilize compound with similar polarity. The following five solvents were used for herbal extraction out of which first three are organic solvents-Methanol, Ethyl Acetate, Chloroform, Ethanol and Hot Water (18). Preparation of Plant Extract Samples (Tulsi, Turmeric, coconut water) were collected. The leaves of plants were separated and washed properly. The rhizomes of turmeric were washed properly. The samples were placed in room temperature. After complete dry the sample was grinded in mortar-pestle to get their powder form. The sample's powder was separately mixed with ethanol solvent in 1:10 ratio. After mixing, the solution was place in incubator shaker for.

After completion of 72 hours the sample was filtered by the help of filter paper in a washed and air-dried beaker. Note- The weight of blank beaker was taken in order to calculate the difference after collecting the filtrate.

The obtained filtrate was then placed in to room temperature and incubated for complete dry. The weight of beaker having the solid filtrate was then measured in order to calculate the difference. Then final weight of the plant extract was calculated. Pathogens used the Antibiogram study of Tulsi, Turmeric, sodium hypochlorite, saline and Coconut water were performed against two microbial pathogens which are listed below- In order to become a controlled and standard suspension of the organism the following procedure was accepted. A stock culture of ACC 3040,*E. faecalis* strain (National Centre for Microbial Resource, NCMR, National Centre for Cell Science, Pune, India) and MCC 1152, *Candida albicans* strain (National Centre for Microbial Resource, NCMR, National Centre for Cell Science, Pune, India) were used. Separately culture was grown overnight at 37℃ in BHI broth and 26℃ for fungus(Himedia). Microbial growth was checked by changes in turbidity at 24 hours.

Agar Disk Diffusion & Antimicrobial Optimization Test Agar disk diffusion is one of the methods to study the antimicrobial or antibiotic activity against various microorganisms. The activity is determined by measuring the diameter of zone of inhibition in mm (19). Incubation & determination of Zone of Inhibition (ZOI) After loading of sample, the plates were incubated in straight position for overnight at 37℃ for bacteria & 26℃ for fungus. After incubating for overnight the plates are observed for measuring the zone of inhibition. The diameter was measuring vertically & horizontally and the average value was calculated.

3. RESULTS AND DISCUSSIONS

IRRIGANTS: Total number of irrigants arefive,Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Coconut water (*Cocos nucifera*), Sodium hypochlorite and Saline.

Figure 1: Irrigants- Sodium hypochlorite, Turmeric, Saline, Tulsi and Coconut water

The 100%, 90%, 80%, 70%, 60%, 50%, 40%,30%, 20% and 10% concentrations of the herbal extract showed inhibitory activity against *Enterococcus faecalis* and *C. albicans*; however, 60% to 100% concentration of Turmeric and Tulsi showed best activity with a zone of inhibition of 9mm, 10 and 8 mm, 8 mm respectively. The positive control i.e., Sodium hypochlorite and saline showed the mild zones of inhibition (graph 1 and 2).

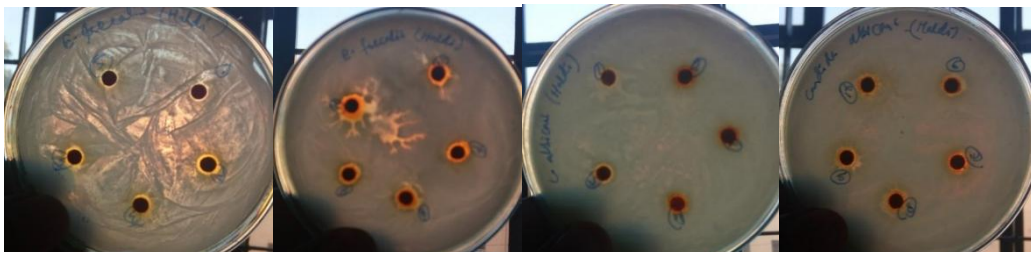
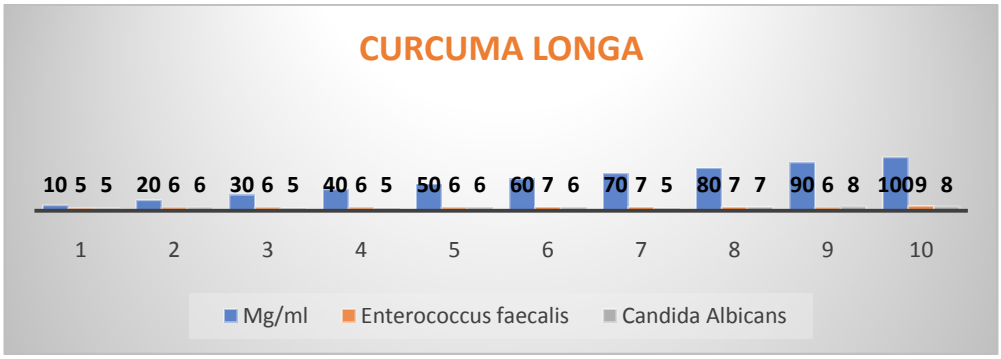
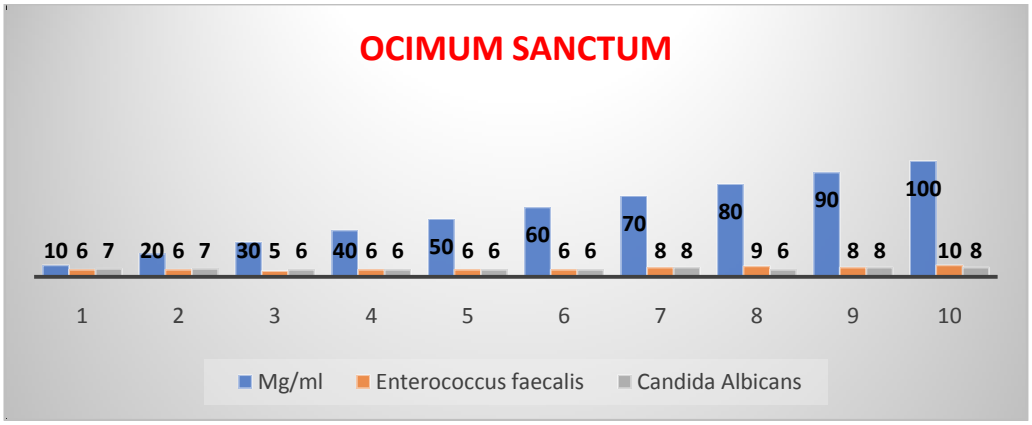


Figure 2: Turmeric (mg/ml)- Show Zone of Inhibition of *E. faecalis* and *C. albicans*



Graph 1: Show Zone of Inhibition results of *Curcuma longa*

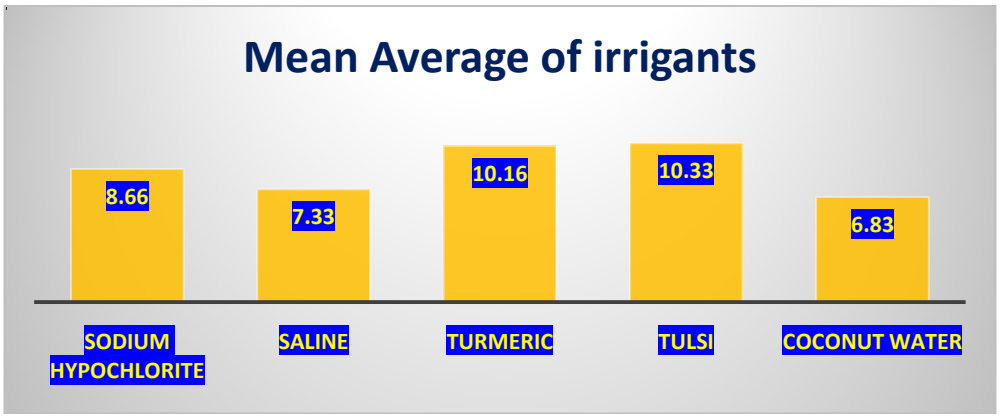
Figure 3: Tulsi (mg/ml)- Show Zone of Inhibition of *E. faecalis* and *C. albicans*



Graph 2: Show Zone of Inhibition results of *Ocimum sanctum*

Table 1: Inhibitory zone against *Enterococcus faecalis* by various root canal irrigants

Irrigants	Plate1	Plate 2	Plate3	Plate4	Plate 5	Plate6	Mean Average
Sodium Hypochlorite	10mm	10mm	8mm	6mm	10mm	8mm	52/6= 8.66
Saline	6mm	10mm	7mm	5mm	8mm	8mm	44/6 =7.33
Turmeric	10mm	11mm	13mm	6mm	11mm	10mm	61/6=10.16
Tulsi	15mm	12mm	13mm	6mm	8mm	8mm	62/6=10.33
Coconut water	6mm	6mm	8mm	5mm	8mm	8mm	41/6=6.83



Graph 3: Average Zone of Inhibition results of *E. faecalis* by various root canal irrigants

The results presented in Table 1 reveal that concentrated Tulsi showed the greatest zone of inhibition of 15, 13,12 mm against *Enterococcus faecalis*and the 50% concentration showed the best zone of inhibition of Turmeric and Tulsi same as 12mm against *Enterococcus faecalis.*

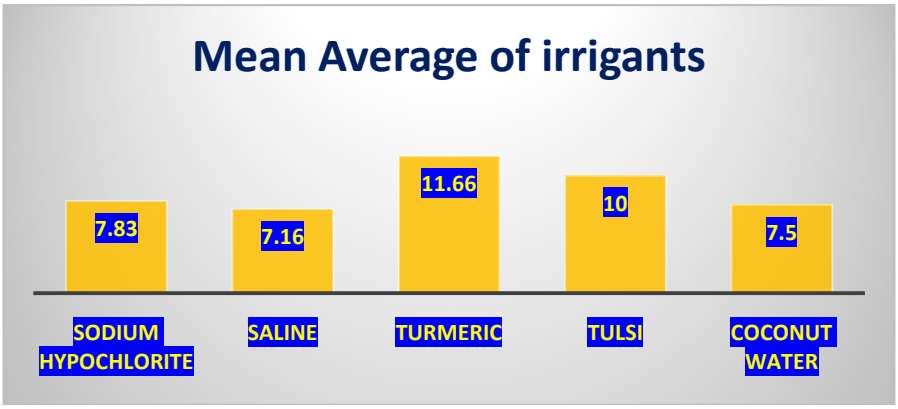
Table 2: Anova test of *E. faecalis*

Anova: Single Factorcalculation of *Enterococcus faecalis*				
SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Sodium Hypochlorite	6	52	8.666667	2.666666667
Saline	6	44	7.333333	3.066666667
Turmeric	6	61	10.16667	5.366666667
Tulsi	6	62	10.33333	12.26666667
Coconut water	6	42	7	1.2

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	57.46667	4	14.36667	2.924016282	0.041124	2.75871
Within Groups	122.8333	25	4.913333			
Total	180.3	29				

Table 3: Inhibitory zone against *Candida albicans* by various root canal irrigants

Irrigants	Plate1	Plate2	Plate3	Plate4	Plate5	Plate6	Mean Average
Sodium Hypochlorite	10mm	6mm	8mm	6mm	8mm	9mm	47/6=7.83
Saline	7mm	6mm	8mm	5mm	8mm	9mm	43/6=7.16
Turmeric	20mm	12mm	12mm	7mm	9mm	10mm	70/6=11.66
Tulsi	12mm	10mm	15mm	7mm	8mm	8mm	60/6=10
Coconut water	8mm	7mm	7mm	5mm	10mm	8mm	45/6=7.5



Graph 4: Average Zone of Inhibition results of *C. albicans* by various root canal irrigants

Table 4: Anova test of *C. albicans*

Anova: Single Factor calculation of *Candida albicans*				
SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Sodium Hypochlorite	6	47	7.833333	2.566667
Saline	6	43	7.166667	2.166667
Turmeric	6	70	11.66667	20.26667
Tulsi	6	60	10	9.2
Coconut water	6	45	7.5	2.7

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	89.66667	4	22.41667	3.037489	0.036002	2.75871
Within Groups	184.5	25	7.38			
Total	274.1667	29				

The results presented in Table 3 reveal that concentrated Turmeric showed the greatest zone of inhibition of 20mm against *Candida albicans* the in 50% concentration Tulsi showed the best zone of inhibition of 12mm against *candida albicans.*

Highest herbal irrigants zone of inhibition against *Candida albicans* was seen for Turmeric (*Curcuma longa*), 20 mm and 12 mm forTulsi (*Ocimum sanctum*). Maximum zone of inhibition against *Enterococcus faecalis* was seen forTulsi (*Ocimum sanctum*), 15 mm and 13 mm forTurmeric (*Curcuma longa*).

Anova reports:
P value = 0.000 (calculated) is less than .05 (confidence interval).As we have strong evidence to support that we can reject null hypothesis.There is a significant difference between the five groups.So, it can be concluded that at least one of the means is different in the given five group. P value of *Enterococcus faecalis*and *Candida albicans* in the given five group of irrigants is0.041124 and 0.036002 respectively (Table 2 & 4).

The current research related the antibacterial properties of herbal with conventional endodontic irrigants. The agar disc diffusion test used in this study is valuable for evaluating and comparing the *in-vitro* antimicrobial actions of irrigants before performing more advanced tests; many studies have used this technique for evaluation of antibacterial effects of many endodontic irrigants (20).

Other issues that may limit the dynamics and variability of agar diffusion test includes control and standardization of the inoculum density, moments at which the results are read, choice of agar, incubation temperature of the plates, and reading of inhibition zone (21,22,23).Two among the three tested organisms i.e.,*E.faecalis*and *C. albicans* could be completely killed by the extract of *Curcuma longa* within 1 hour, the time usually spent for chemo mechanical preparation of the root canals, Morgana Eli Vianna,2004, (24). The chemical composition of Tulsi is highly complex, containing many nutrients and other biologically active compounds (25). Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in Ocimum sanctum, perhaps is largely responsible for the therapeutic potential of Tulsi(26) Coconut water's unique nutritional profile makes an excellent oral rehydration, enhances immune function, possesses anti-aging properties, decreased swelling, relieve spasm, root canal irrigants (antiviral, antifungal and antimicrobial properties) and storage media for avulsed tooth(27).Sodium hypochlorite (household bleach) is the most commonly used root canal irrigants. It is an antiseptic and inexpensive lubricant that has been used in dilutions ranging from 0.5% to 5.25%.Normal saline is an isotonic solution to the body fluids and is being universally used as an irrigating material in all the surgical procedures very effect Endodontic treatment is also a type of surgical procedure, so normal saline is routinely used here.

CONCLUSION

Tested herbal drug Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Coconut water (*Cocos nucifera*), Sodium hypochlorite and Saline showed inhibitory zones against *E. faecalis* and *C. albicans*. Herbal extract (Tulsi and Turmeric) has shown significantly inhibitory effect against *E. faecalis* and *C. albicans* compared to sodium hypochlorite. Hence, these can be used alternatively as endodontic irrigants/medicament. Further *in vivo* modify its content for acceptability by patients.

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