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Comparative Evaluation of the Antimicrobial Activity of *Piper Betel Leaf*, *Phyllanthus Emblica*, *Lawsonia Inermis* Extracts as Irrigating Solutions : An *In-Vitro* Study

Dr. Deepak P Bhayya[1]
[1]Professor and Head,
Department of Paediatric and Preventive Dentistry, Hitkarini Dental College and Hospital,
Jabalpur, India

Dr. More Shubham Bapurao[2]
[2]Post Graduate Student,
Department of Paediatric and Preventive Dentistry, Hitkarini Dental College and Hospital, Jabalpur, India

Dr. Prabhat Kumar [3]
[3]Reader,
Department of Paediatric and Preventive Dentistry,
Hitkarini Dental College and Hospital,
Jabalpur, India

Dr. Saurabh Tiwari[4]
[4] Senior Lecturer,
Department of Paediatric and Preventive Dentistry,
Hitkarini Dental College and Hospital, Jabalpur,
Jabalpur, India

Dr. Anushka Waghmare[5]
[5]Post Graduate Student,
Department of Paediatric and Preventive Dentistry,
Hitkarini Dental College and Hospital,
Jabalpur, India

Dr. Aditi Mor[6]
[6] Post Graduate Student,
Department of Paediatric and Preventive Dentistry,
Hitkarini Dental College and Hospital,
Jabalpur, India

Dr. Anushika Singh[7]
[7]Post Graduate Student,
Department of Paediatric and Preventive Dentistry,
Hitkarini Dental College and Hospital,
Jabalpur, India

Abstract:- Introduction: The *Enterococcus faecalis* and *Candida albicans* are resistant species in infected root canals causing treatment failures. Sodium hypochlorite is an irrigant with many disadvantages. Thus, a harmless irrigant can always be a choice. Objectives: The aim of the study is to evaluate and compare the antimicrobial activity of *Piper betel* leaf, *Phyllanthus emblica, Lawsonia inermis* as an irrigating solution. Materials & Method: The extract of *Piper betel* leaf, *Phyllanthus emblica, Lawsonia inermis* were prepared and zones of inhibition were measured against *E. faecalis* and *C. albicans* and were compared with sodium hypochlorite and normal saline (Control group).Results: The results revealed that *Phyllanthus emblica* showed the highest antibacterial and 0.5% show anti fungal activity and highest zone of inhibition against *E. Faecalis* and *C. Albicans* among all the groups. Conclusion: *Piper betel, Phyllanthus emblica, Lawsonia inermis* shows effective antimicrobial and antifungal activity against *E. Faecalis* and *C. Albicans*.

Keywords:- *Microorganism, Irrigant, Zone of Inhibition.*

I. INTRODUCTION

Bacteria are the principal causative factor in the development of pulp and periapical diseases (Kakehashi *et al.* 1965, Moller *et al*. 1981, Sundqvist 1992).[1,2,3] They have identified a substantial number of bacterial species as inhabitants of the oral cavity (Bik *et al*. 2010)[4]. *Enterococcus faecalis* and *Candida albicans* is the dominant species associated with secondary infections and appears more resistant to endodontic treatment (Rôças *et al*. 2004).[5] The primary aim of root canal treatment is therefore to eliminate bacteria from the infected root canal system and to prevent re-infection (Chugal *et al*. 2001).[6] Thorough cleaning removes infected soft and hard tissues, provides sufficient access for irrigants and creates for delivery of medicaments and the subsequent canal filling (Haapasalo *et al*. 2005).[7] A broad antimicrobial spectrum against anaerobic and facultative micro-organisms, biofilms and the ability to remove the smear layer during instrumentation or dissolve it once it has formed are amongst the key requirements of endodontic irrigants (Zehnder 2006).[8] They should be nontoxic and

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noncaustic to periodontal tissues (Zehnder 2006).[8] At present, sodium hypochlorite (NaOCl) appears to be the most suitable because it fulfills most of the requirements for an ideal endodontic irrigant (Zehnder 2006).[8] Its excellent antimicrobial activity makes it the irrigating solution of choice to treat teeth with pulp necrosis (Ayhan *et al*. 1999)[9]. But it has several undesirable characteristics such as tissue toxicity, allergic potential and disagreeable smell and taste (Caliskan *et al*. 1994).[10] To overcome problems associated with currently used irrigants, use of natural plant extracts as endodontic irrigants might interest professionals as part of a growing trend to seek natural remedies in dental treatment.[11]

Red betel leaf (*Piper crocatum*) is one of the natural materials that might be used as an ingredient of root canal irrigation.[12,13] Red betel leaf contains saponin compounds characterized as "surfactants" which can lower the surface tension so that it can dissolve organic materials, inorganic materials, and microorganisms in the root canal.[14,15] As root canal irrigation material, red betel leaf infusion must have a certain concentration, but until now there has been no research on it.[16]

Phyllanthus emblica (syn. *Emblica officinalis*) is commonly known as amla or Indian gooseberry. In Ayurveda, *P. emblica* has been extensively used, both as edible (tonic) plants and for its therapeutic potentials. It is highly nutritious and is reported as an important dietary source of vitamin C, minerals and amino acids.[17] The extract of *Phyllanthus emblica* has shown to have chief activity against all grampositive, gram-negative and resistant- bacteria and also antifungal, and antiviral activities.[18]

The henna plant, *Lawsonia inermis Linn*, is a plant known for healing attributes and is now the subject of intense scientific study. Though they used it for various purposes, the antifungal property was not yet investigated. It belongs to the family of *Lythraceae*. In Sudan, it is traditionally used to develop a red or black coloring to hands, feet, and hair on some occasions such as weddings and religious festivals. The phytochemical analysis of the plant leaves revealed anthroquinones as major constituents which are commonly known to possess antimicrobial activity.[19]

All the new materials intended for human usage should go through a series of in-*vitro* and in-*vivo* tests to understand its effectiveness and biocompatibility. Thus, taking the properties of these herbal extracts, this study has been undertaken with the aim to evaluate and compare the antimicrobial activity of three different plant extracts namely *Piper betel* leaf, *Phyllanthus Emblica*, *Lawsonia inermis* as an irrigating solution.

II. MATERIALS AND METHOD

The microorganisms used in this study were *Enterococcus faecalis* and *Candida albicans*. The study was conducted in a microbiology laboratory (Excellent Bio Research Solutions Pvt. Ltd. Jabalpur, India) to evaluate and compare the antimicrobial activity of *Piper betel* leaf, *Phyllanthus emblica*, *Lawsonia inermis* plant extracts as an irrigating solution on *Enterococcus faecalis* and *Candida albicans* which is responsible for root canal failure. The plant material was collected and authentified by the lab and washed thoroughly with water and dried under shade. Dried pieces were powdered in a grinder and the residue obtained after the aqueous extraction was air dried, and powder was extracted with ethanol by soxhelation process. The extract evaporated to dryness at a controlled temperature (45°C), to gain an effective concentration of 2ml.

The substances used in the study were divided into following groups:

- Group 1: *Phyllanthus emblica* Extract (n=10)
- Group 2: *Lawsonia inermis* Extract (n=10)
- Group 3: *Piper Betel* Extract (n=10)
- Group 4: 3% Sodium hypochlorite (n=10)
- Group 5: 0.9% NaCl (n=10)

Minimum inhibitory concentration of *Piper betel* leaf, *Phyllanthus emblica*, *Lawsonia inermis* extract was evaluated by the agar well diffusion method. The lowest dilution inhibiting growth was taken as the minimum inhibitory concentration (MIC). The growth of bacteria was checked through the antimicrobial activity testing using the cultures of *E. faecalis* (ATCC 29212) and *C. albicans* which were maintained on Muller-Hinton agar media at 37°C for 24-48 hours. The lowest dilution at which bacterial and fungal growth was halted was measured in millimeter using zone measurement scale (HiMedia).

III. RESULTS

The results obtained were statistically analyzed using one-way ANOVA followed by Tukey honest significant difference (HSD) test.

Normal Saline did not show any inhibitory activity and thus was not included in the analysis of the data.

The mean zone of inhibition for the Amla for *E. Faecalis* was 21.12 ± 1.45 mm Sodium Hypochlorite (18.43 ± 1.63mm), Henna (12.49 ±0.96 mm) and *Piper Betel* Leaf (11.65 ± 1.63mm). The test result shows there was significant difference between the groups with F value 49.01 and p value 0.01.(Table 1)

Table 1: Comparison of Antibacterial activity against *E. faecalis* by different plant extract and Sodium Hypocholorite

Group	mm	SD	F value	P value
Amla	21.12	1.45	49.11	**0.01* (HS)**
Heena	12.50	1.18		
***Piper betel* Leaf**	11.65	0.96		
Sodium Hypochlorite	18.50	1.63		

The post Hoc Tuckey test shows Amla had the maximum antibacterial activity against *E.faecalis* which shows statistically significant difference with all the other groups. Sodium hypochlorite was the gold standard shows more antimicrobial activity then Henna and Piper leaf extract. The henna and *piper betel* leaf also shows antibacterial activity against *E. Feacalis* .(Table 2)

Table 2: Post Hoc test for antibacterial activity against *E. faecalis*

Comparison Group	Mean difference	P value
Amla vs heena	2.62	0.00(HS)
Amla vs piper Betel	8.62	0.00(HS)
Amla vs Sodium Hypochlorite	9.47	0.00(HS)
Heena vs Piper	0.85	0.15 (NS)
Heena vs Sodium Hypochlorite	6.00	0.00(HS)
Piper Betel vs Sodium Hypochlorite	6.85	0.00(HS)

The mean zone of inhibition for the Amla for *C. albicans* was 24.19 ± 1.62 mm, Sodium Hypochlorite (17.85± 1.14 mm) , Piper leaf (16.28 ±1.14 mm) and Henna (15.15 ± 1.06 mm).The test result shows there was significant difference between the groups with F value 17.65 and p value 0.01. (Table 3)

Table 3: : Comparison of Antifungal activity against *C. Albicans* by different plant extract and Sodium Hypocholorite

Group	mm	SD	F value	P value
Amla	24.19	1.62	17.65	**0.01* (HS)**
Heena	15.15	1.06		
***Piper betel* Leaf**	16.28	1.14		
Sodium Hypochlorite	17.85	1.14		

In the post Hoc Tuckey test all the groups shows statistically significant difference. Amla had the maximum antifungal activity against *C. albicans.* Sodium hypochlorite was the gold standard shows more antimicrobial activity then Henna and Piper leaf extract. The henna and piper leaf also shows antifungal activity against *E. feacalis* (Table 4).

Table 4: Post Hoc test for antifungal activity against *C. albicans*

Comparison Group	Mean difference	P value
Amla vs Heena	9.04	0.00(HS)
Amla vs piper betel	7.91	0.00(HS)
Amla vs Sodium Hypochlorite	6.34	0.00(HS)
Heena vs Piper betel	1.13	0.03 (S)
Heena vs Sodium Hypochlorite	2.70	0.00(HS)
Piper vs Sodium Hypochlorite	1.57	0.00(HS)

IV. DISCUSSION

Root canal irrigation plays an important role in the removal of the smear layer and kills pathogenic bacteria. We use irritants for various purposes as tissue solvent, disinfection, removal of debris and lubrication. *E. faecalis* and *C. albicans* are gram-positive bacteria responsible for root canal infection.

In present study 1mg/mL *Phyllanthus emblica* show highest antibacterial and 0.5% show antifungal activity and highest zone of inhibition against *E. faecalis* and *C. albicans* than other two plant extract and Sodium Hypochlorite. They observed similar results in agreement with the fact of strong antibacterial and antifungal efficacy observed by Hossain M. *et al.* (2012).[18] This result could be attributed to the properties of *Phyllanthus emblica* such as Vitamin C (Ascorbic acid), Tannins, Alkaloids, Flavanoid, Gallic acid, Methyl gallate and Ellagic acid-4-Orhamnoside.

In present study 0.5mg/mL *Lawsonia inermis* show antibacterial and show anti fungal activity and less zone of inhibition against *E. faecalis* and *C. albicans* than *Phyllanthus emblica* and Sodium hypochlorite. This result could be possibly because of the chemical Lawsone which is antibacterial in effect, betulinic acid, hennadiol, tannins and phenolic compounds. In a similar study, Henna showed effective antibacterial activity found by Kannahi M. *et al.*(2013).[20] The observation agreed with the fact of strong antifungal efficacy of *Lawsonia inermis* (10mg/mL) as observed Suleiman A. *et al.* (2014).[19]

In present study 1mg/mL *Piper betel* extract as an irrigant solution showed antibacterial and antifungal activity and minimal zone of inhibition against *E. faecalis* and *C. albicans* observed when compared with other two plant extracts and Sodium Hypochlorite. Similarly, effective concentration of the red betel leaf infusion for cleaning smear layer observed by Pangabdian F. *et al.* (2012).[15]

V. CONCLUSION

Within the limitations of this study, it appears that *Piper betel*, *Phyllanthus emblica*, *Lawsonia inermis* shows effective antimicrobial and antifungal activity against *E. faecalis* and *C. albicans.* Further in-*vivo* studies should be conducted to validate the data of the present study.

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