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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF VARIOUS HERBAL ROOT CANAL IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS - AN IN VITRO STUDY

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Abstract

Introduction: Antimicrobial resistance has been a global concern. Currently, interest has been focused on exploring antimicrobial properties of plants and herbs. Herbs such as Garlic,Lemon,Guava leaf are used in this study.

Aim: To evaluate the antimicrobial activity of various herbal root canal irrigants against enterococcus faecalis.

Material and methods: Agar diffusion method was used to evaluate the antimicrobial action of different medicines. Sixty samples were segregated into four groups with 15 samples in each: Group I: Garlic extract, Group II: guava leaf extract, Group III: Lemon extract, and Group IV: 5% Sodium hypochlorite. The inhibition zones against E. faecalis were recorded and statistically assessed using one-way analysis of variance (ANOVA) test ($P < 0.05$).

Result: Significant antibacterial effect against E. faecalis was observed with Garlic extract followed by sodium hypochlorite, lemon and Guava leaf extract.

Conclusion: Herbal extracts seemed to be effective against E. faecalis compared to 5% Sodium hypochlorite.

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Introduction:-

Dental caries is the most common infectious disease of childhood and adolescence. World health organization [WHO] in their oral report of 2003 reported dental caries as one of the pandemic diseases affecting all population irrespective of gender, age or socioeconomic status[1]. Even through a lot of preventive measures are available for dental caries, it is still a major threat to population and once it occurs treatment of dental caries is the only option. The treatment option are excavation, restoration or if deep then endodontic treatment. Thus endodontic treatment

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become an inevitable part in the field of dentistry. Role of microorganisms in the initiation and progression of endodontic lesion has been proven long back. So the treatment sequence for the infected pulp of a tooth needs the elimination of infection and protection of tooth from further microbial invasion. Proper instrumentation, irrigation and obturation of root canal are the three main steps which results in the success of a root canal treatment. Out of these three steps, irrigation of root canal is the most important step in eliminating microorganisms from the root canal system. This is because of the fact that mechanical cleaning alone cannot able to eliminate all microorganisms from the root canal system. Moreover, the root canal microbia contains more than 700 different bacterial species; of these commonly isolated species from persistent apical peridontitis is the Enterococcus faecalis. Its long term survival in the root canal system and its ability to adhere to dentin and dentinal tubules and to form communities organized in biofilms contribute persistence after intracanal antimicrobial procedures. Hence an ideal root canal irrigants solution should possess a broad antimicrobial spectrum, high efficacy, ability to digest protein and necrotic tissue, prevent the formation of a smear layer during instrumentation, should possess low surface tension to reach areas inaccessible to the tools [dentinal tubules], offer a long term antibacterial effect, should be non-antigenic, nontoxic and non-cariogenic[2]. In addition to these, it should not possess any adverse effects on dentin or sealing ability of filling materials.

The most commonly used irrigants are sodium hypochlorite and had being this gold standard for antimicrobial effect against E faecalis. Inspite of having many advantages of this materials the most common side effect are tissue toxicity, staining of instruments, irritation to periapical tissue, inability to remove smear layer and has some undesirable smell and taste. This made in search for novel irrigants solutions which is of natural origin. The term "HERBAL" is nowadays become a trade mark in all products. Herbal alternatives have been used in medical and dental science for hundreds of years and have become even more acknowledged today due to their high antibacterial activity, analgesic, biocompatibility, anti-inflammatory, and anti-oxi-dant properties [3] Dentistry is also witnessing these trends in an increasing way due to the beneficial properties, ease of availability and less side effects. Moreover, herbal extracts contains different components such as essential oil, flavonoids, tannins and alcohol through which it attain varying degree of medicinal values.

Guajava, commonly known as guava, belongs to the family Myrtaceae. Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments. Pharmacological investigations indicated that its bark, fruit, and leaves possess antibacterial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, and CNS depressant activities [3]Garlic (Allium sativum L.) belongs to the group of onion and falls within the group of the Liliaceae family. The most frequently used part of the garlic plant for medicinal use are its bulb and cloves. Its odor is pungent and antibacterial activity depends on the sulfur-containing compounds. The major one is allicin which is produced by enzymatic (alliin lyase) hydrolysis of allicin after cutting and crushing of the cloves. Throughout history, many different studies have recognized the use of garlic for prevention and treatment of different diseases. Recent researches have focused on four main areas: Heart disease, cancer, infectious disease, and antioxidant effects. Indeed, garlic is found to have antihypertensive, antioxidant, antiplatelet, antitumor and lipid-lowering actions. This study also points out towards the antimicrobial action of garlic.[4] Herbal alternatives are easy availability, cost-effective, low toxicity, and they have good bactericidal property. Lemon is a medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of Lemon against clinically significant bacterial strains has been reported. Thus herbal extracts of garlic, Lemon, Guava leaves extract being used for the root canal irrigants in past literature and its antimicrobial effect of these irrigants had not been assessed till date. Hence in the light of above knowledge this in-vitro study has been designed with the aim to compare and evaluate the antimicrobial efficacy of different herbal root canal irrigants.

Materials and Methods:-

15 Agar plate with 4 samples in each agar plate are used in this study.

Preparation of guava leaves extract:-

Leaves of guava were obtained and dried in fresh open air protecting from direct exposure to sunlight. A 50 g of powdered leaves were taken into a beaker containing 500 ml of sterile distilled water. Hot water extract was prepared by heating this in a water bath till menstruum reduced to about 125 ml which is about one-fourth of the original volume. After the complete evaporation of the water content from extract, the resulting liquid was filtered using filter paper.[5]

Garlic extract :-
In order to prepare fresh garlic (Allium sativum) extract, fresh garlic was purchased from the local market, deskinned and was washed 5-6 times in sterile distilled water. It was then blended in a sterilized mortar and pressed with gauze. This extract was centrifuged at 12,000 rpm for 10 min and then filtered with a 0.45 mm filter to obtain raw garlic extract.[4]

Lemon extract:-
Fresh lemon fruits were obtained from the local market. The fruits were washed in running tap water, rinsed with sterile distilled water, and cut open with a sterile knife and the extract obtained by squeezing was filtered (using Whatman filter paper No. 1) to remove the seeds and pulp. The fresh extract was diluted by mixing 5 ml of fresh lemon extract with 5 ml of sterile distilled water to obtain 1:1 diluted lemon extract.[6]

Sodium hypochlorite:-
The chemical was obtained commercially 5% Sodium hypochloriteand used as per established protocol.

Agar-diffusion test:
The bacterial stock culture Enterococcus faecalis was obtained and culture was grown overnight in brain heart infusion (BHI) broth and inoculated in Mueller- Hinton agar plates. Inoculation was performed by using sterile swab brushed across the media. One round well, 4mm of depth X 6mm of diameter was punched in each agar plate and the prepared irrigants were added to the wells. Agar plates were incubated at 37^0 C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm. The results were tabulated and statistically analyzed using analysis of variance (ANOVA) [7,8]

Results:-

On statistical analysis group2 Garlic irrigant showed the best result with the mean value 24.466 followed by Group 4 sodium hypochlorite with mean value 15.466 followed by lemon extract with mean value 13.400 and group 1 guava leaf extract with mean value 6.400 **(Table 1)**

Graph -1 shows the mean distribution of study samples with the standard deviation. Out of total samples of 60, Group 1 guava (n=15) shows a mean of 6.40, Group 2 garlic (n=15) showed a mean of 24.466, Group 3 lemon (n=15) had a mean of 13.400 and Group 4 sodium hypochlorite (n=15) 15.466.

On applying one way ANOVA, we found that inhibition zone in all the groups was statistically significant (p=0.001) **(Table 2)**

When intercomparison of various groups were done using Tukey's test (Post Hoc tests) Groups i's Guvava versus Garlic, Guava versus Lemon, Guava versus Sodium hypochlorite, Garlic versus Lemon, Garlic versus Sodium hypochlorite, Lemon versus Sodium hypochlorite and was statistically significant (p=0.001) **(Table 3, Graph 2).**

Groups	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Guava Extract (GROUP-1)	6.400	1.352	0.349	5.651	7.148	5.00	9.00
Garlic Extract (GROUP – 2)	24.466	1.552	0.400	23.607	25.326	22.00	28.00
Lemon Extract (GROUP-3)	13.400	1.121	0.289	12.779	14.020	11.00	15.00
Sodium Hypo (GROUP-4)	15.466	4.517	1.166	12.964	17.968	11.00	27.00

Table -1:- Mean values of inhibition zone in different groups.

ANOVA Table

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	2495.067	3	831.689	128.423	0.001 (Sig)
Within Groups	362.667	56	6.476		
Total	2857.733	59			

Table 2:- Comparison of Means of inhibition zone in all groups.

Graph 1:- Bar diagram showing mean antimicrobial efficacy of all the groups.

Post Hoc Analysis

Intergroup Comparison	Mean Diff	Std Error	P value	Significance
Guava vs Garlic (GROUP-1 vs 2)	18.066	0.349	0.001	Significant
Guava vs Lemon (GROUP-1 vs 3)	7.000	0.349	0.001	Significant
Guava vs Sodium Hypo (GROUP-1 vs 4)	9.066	0.349	0.001	Significant
Garlic vs Lemon (GROUP-2 vs 3)	11.066	0.289	0.001	Significant
Garlic vs Sodium Hypo (GROUP-2 vs 4)	9.000	1.166	0.001	Significant
Lemon vs Sodium Hypo (GROUP-3 vs 4)	2.066	0.289	0.030	Significant

Table 3:- Intergroup Comparison of means of inhibition zone by Post-hoc Test.

Graph 2:- Bar diagram showing Intergroup Comparison of means of inhibition zon

Discussion:-

Herbal dentistry has gained its footage through various dental products like herbal mouthwashes, dentifrices, irrigating solutions, oral rinses and topical anaesthetics etc. In recent endodontics because of the limitations of most of the commercial intracanal medicaments used such as cytotoxicity, unpleasant taste, odor and their inability to eliminate bacteria from dentinal tubules, trend of recent medicine to use biologic medication extracted from natural plants is drawing a lot of attention. The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity, and lack of microbial resistance reported. Literature has shown that herbs can have a promising role as root canal irrigants. However, there is a need for evidence based research for use of herbal products in endodontics, particularly when it is used in children.

Among the many herbs the most extensively researched herbs are Garlic extract, Guava leaf extract, lemon extract.

E. faecalis was taken in the present study because it has been identified as the most common species in root canal diseases. The present study has shown maximum inhibitory effect against *E. faecalis* by Garlic extract followed by Sodium hypochlorite, Lemon extract and then Guava extract.

Our result is validated by other studies which showed similar antimicrobial property of garlic extract comparable to 2% sodium hypochlorite against *E.faecalis* microorganism **Z Ambareen , S Konde, Sl Raj , Kumar N C (2015)**[9]and **Seema Yadav, Niyati A. Trivedi, Jagat D. Bhatt (2015)**[10] Moreover Fresh garlic juce has definite antimicrobial activity against Escherichia coli and Staphylococcus aureus.

The antimicrobial efficacy of Group 3 Lemon herbal irrigant with mean value 13.400 found to be statistically lower than that of Garlic herbal irrigant with mean value 24.466 and sodium hypochlorite with mean value 15.466 but was statistically better than group 1 guava herbal irrigant with mean value 6.400 in reduction of *E.faecalis*. While contradictory results have been discussed in the study done by **Ramamurthi Arularasi Aberna, G. Mohan, S. Saranya (2022)**[6] stated that undiluted lemon extract demonstrated better anti-enterococcal activity than conventional irrigant sodium hypochlorite. It is because they had used 2% sodium hypochlorite and in our study we had taken 5% sodium hypochlorite. Another study done by **Maruti J Dhanavade, Chidamber B Jalkute, Jai S Ghosh and Kailash D. Sonawane (2011)**[11] stated that lemon peel extract with ethanol shows higher antimicrobial

activity against *Pseudomonas aeruginosa* , *Salmonella typhimurium* and *Micrococcus aureus* in comparison with the extracts of lemon peel in other solvents like methanol and acetone.

The antimicrobial efficacy of Group 4 Sodium hypochlorite irrigant found to be statistically lower than that of Garlic herbal irrigant but was statistically better than group 1 guava extract and group 3 lemon extract.

The antimicrobial efficacy of Group 1 guava irrigant found to be statistically lower than group 3 Lemon extract but both where statistically significant.

In the present study, garlic extract effectively killed E. faecalis. Garlic is one of the greatest health tonics and has proven medicinal properties. It contains a substance called allicin which is equivalent to that of penicillin (1mg of allicin is equated to that of 15 IU of penicillin) **Suresh Pandey et al (2018)**[12] stated that Allicin can destroy cell wall and cell membrane of root canal bacteria. Garlic inhibit the growth of oral pathogens such as E.faecalis and also against Streptococcus mutans which is the causative organism of dental caries.[12]

In the present study, NaOCl effectively killed E. faecalis. **Soujanya Goud (2018)**[13] NaOCl exerts its antibacterial effect by inducing the irreversible oxidation of the sulfhydryl groups of essential bacterial enzymes, resulting in disulfide linkages, with the consequent disruption of the metabolic functions of bacterial cells. It also has deleterious effects on bacterial DNA, which involve the formation of chlorinated derivatives of nucleotide bases.[9]

The antibacterial activity of lemon juice is due to the acidic pH that could affect the charges of the amino acids that constitute the peptidoglycan and active site of enzymes of bacteria. **Somnath De et al (2017)**[14] had evaluated Antimicrobial activity of guava extract is attributed to the presence of flavonoids such as mosin glycosides, quercetin and quercetin glycosides in Guava leaf **Gaurav Jain (2020)**[5]

Therefore, in the present study it can be concluded that Garlic extract can be used as alternative to 5% sodium hypochlorite and lemon extract and guava also showing good result so it can be also considered as endodontic irrigants in primary dentition. We recommend further studies to authenticate these results. Clinical trials should be made so as to check other properties in the intraoral environment.

Conclusion:-

Within the limitations of this in vitro study it is found that All the endodontic irrigants viz guava extract, garlic extract, Lemon extract and 5% sodium hypochlorite used in this study showed antibacterial efficacy against E.faecalis. Garlic herbal endodontic irrigant showed maximum antibacterial efficacy against E. faecalis than 5% sodium hypochlorite, lemon and guava herbal irrigant. 5% Sodium hypochlorite irrigant showed better antibacterial efficacy against E. faecalis than Lemon and Guava herbal irrigant. Guava herbal irrigant showed least antibacterial efficacy against E. faecalis than Garlic, Lemon and 5% Sodium hypochlorite irrigant. Hence, it is concluded that freshly prepared garlic extract proved be the best antibacterial efficacy against E. faecalis when compared with 5% Sodium hypochlorite irrigant and Lemon and Guava herbal irrigant. So Garlic can be used as a better alternative to other endodontic irrigants used in this study.

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