



ANTIMICROBIAL EFFICACY OF HERBAL AGENTS AGAINST E. FAECALIS IN COMPARISON TO SODIUM HYPOCHLORITE

Dentistry

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ABSTRACT

Aim: To evaluate antimicrobial efficacy of herbal agents against E faecalis to sodium hypochlorite **Objectives:** To evaluate the antimicrobial efficacy of NaOCL and different irrigating herbal agents (Neem, Green tea, Sunthi, Tulsi, Aleo vera, Turmeric, Miswak, Triphala, Propolis) against E. faecalis **Material And Methods:** CAZ (1:1:1), CAZ (1:2:1), CAZ (2:1:2), CAZ (1:1:2), NAOCL, NEEM, TRIPHALA, SUNTHI, GREEN TEA, MISWAK, ALEO VERA, TURMERIC **Result :** CAZ (1:1:1) > CAZ (1:2:1) > CAZ (2:1:2) > CAZ (1:1:2) > NAOCL > NEEM > TRIPHALA > SUNTHI > GREEN TEA > MISWAK > ALEO VERA > TURMERIC.

KEYWORDS

Cissampelos pareira, Acorus calamus, Zanthoxylum alatum (CAZ), NAOCL, NEEM, TRIPHALA, SUNTHI, GREEN TEA, MISWAK, ALEO VERA, TURMERIC, antimicrobial.

INTRODUCTION

Herbal medicines are the upcoming modalities in dental procedure. In modern days herbal medicaments when used in the form toothpastes and mouthwashes help to reduce the accumulation of plaque reducing the microflora and overall improves the immune response of oral health.

Herbal substitutes are readily and affordably accessible. It has a long storage life and is not toxic.

Herbs that is Cissampelos pareira, Acorus calamus, Zanthoxylum alatum, Azadirachta indica A. Juss (neem), Camellia sinensis (green tea), Triphala, Zingiber officinalis Rose (sunthi) had been tested as irrigating solution. They have exhibited antimicrobial property.

The study was in vitro experimental study. An approval was obtained from Institutional Ethical Committee DMIMS.

Aim

To evaluate antimicrobial efficacy of herbal agents against E faecalis to sodium hypochlorite

Objectives

1) To evaluate the antimicrobial efficacy of NaOCL and different irrigating herbal agents (Neem, Green tea, Sunthi, Tulsi, Aleo vera, Turmeric, Miswak, Triphala, Propolis) against E. faecalis.

II] Materials used for Preparation of herbal irrigants:

- 1) Cissampelos pareira, Acorus calamus, Zanthoxylum alatum (1:1:1)
- 2) Cissampelos pareira, Acorus calamus, Zanthoxylum alatum (1:2:1)
- 3) Cissampelos pareira, Acorus calamus, Zanthoxylum alatum (2:1:2)
- 4) Cissampelos pareira, Acorus calamus, Zanthoxylum alatum (1:1:2)
- 5) NAOCL
- 6) NEEM
- 7) TRIPHALA
- 8) SUNTHI
- 9) GREEN TEA
- 10) MISWAK
- 11) ALEO VERA
- 12) TURMERIC
- 13) TULSI
- 14) PROPOLIS

Properties of New herbal solution

Sr. No.	Herb Name	Scientific Name	Composition	Advantages
1.	Patha	Cissampelos pareira Family : Menispermaceae	isoquinoline are major constituents of C. pareira. Flavanoid, bisbenzylisoquinoline, benzylisoquinoline, tropoloisoquinoline, aporphine, azafluoranthene	The herb is as a medicament for cardiovascular diseases, dysentery and sores, rheumatism, ulcers and fevers, ulcer, wound, rheumatism, fever, asthma, cholera, diarrhoea, inflammation, snakebite, malaria, rabies, and also recommended for blood purification. C. pareira is also used to kill bacteria. The methanol root extract of Cissampelos pareira had a broad spectrum inhibitory activity against both bacteria that is gram positive and gram negative including K. Pneumonia, S. aureus, and E. coli.
2.	Acorus calamus	Acorus calamus (healing plant) Family : Araceae	Flavanoid, A- and B-asarones, which are thought to be responsible for their antimicrobial activities. Methyl eugenol ether	Shows antibacterial, antifungal, antiyeast, insecticides and herbicides. It had no antibacterial activity except E.coli.
3.	Timur or Tejohawa	Zanthoxylum alatum Family: Rutaceae	The chief content of oil is beta-linalool, alpha-limonene, diepoxide, alpha-pinene, D-limonene spilanthol.	Small twigs of the plant are chewed and used as tooth stick for cleaning teeth. It is rated quite high for this purpose as it create a characteristic tingling sensation in the mouth and also activated the salivary glands. Also used in cough, asthma, difficult breathing,

				and rheumatism
4.	Sarjik shar	Impure Carbonate of Soda (Kshara)	Salt composed of sodium and bicarbonate ions.	Reduces cariogenicity of these bacteria. Sodium bicarbonate also has bactericidal properties against bacterial cariogenic pathogens. Bicarbonates used in chewing gums as well as it enhances salivary flow to flush out food debris.
5.	Yavak shar	Impure Carbonate of potash (Kshara)	Salt composed of potassium and carbonate.	It is an inorganic compound. Salts of tartar or pearl ash are more refined forms of potash, which is principally potassium carbonate. It has antibacterial activity of oils, free fatty acids, potassium salt and fatty acid methyl esters.
6.	Madhu	Honey	High concentration of sugar, Flavonoids and Aromatic acids, High Hydrogen peroxide, sugar.	- Natural honey is safe for the tissue. It is sterile in nature. Also, it has antibacterial, antiseptic and hygroscopic properties. - It provide relief from pain and soothing effect on skin when it applied topically. There is an active enzyme in honey that remains active in the absence of saliva but is inactivated by salivary enzymes, which support its effect on root canal dentine.

Preparation of Materials

- Drugs were procured from Mahatma Gandhi Ayurvedic College and Hospital and Research Institute, Salod, Wardha (MGACHRC). All the drugs were authenticated from Department of Rasashastra and Bhaishajya Kalpana from Mahatma Gandhi Ayurvedic College, Hospital and Research Centre, Salod (Hirapur), Wardha.
- Approved with a licence number NG/AYU/002/14 by Dattatrya Ayurveda Rasashala.

Culturing Method Test microorganism

E-faecalis, the microbial strains that are used for the study. These strains were acquired from Microbiology Department (JNMC Wardha, Maharashtra).

Agar Medium

Media which were used are Brain heart infusion broth, Nutrient Broth, Mullerhinton, blood agar and Sabouraud's dextrose agar. For E-faecalis Blood agar was used as enriched media and Brain heart infusion broth for cultivation.

Agar Diffusion Assay

To determine antimicrobial activity of herbal irrigatin Agar well diffusion method was used. In this method Colonies of 'E faecalis' was inoculated in nutrient broth overnight. All testing materials were placed in Petri dishes each with fast filter paper dip in it section. Freshly prepared herbal irrigants was placed in petri dishes than fast filter paper were kept on muller Hilton agar plate and incubation done than zone of inhibition was calculated (in specific culture media) for 24 hours.

Statistical Analysis

The data and the presence of F-values and P-values, it looks like an ANOVA (Analysis of Variance) test was applied to the first group (CAZ (1:1:1)).

ANOVA is used to determine if there are any statistically significant differences between the means of three or more independent groups. Here's why ANOVA might be used in this context:

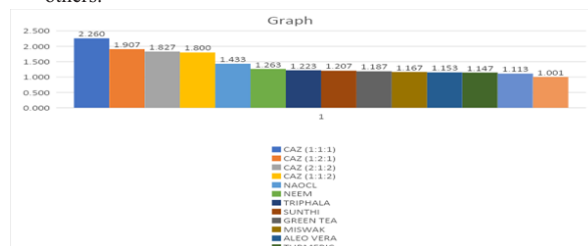
- Multiple Groups:** You have multiple groups (different treatments or conditions) and you want to compare their means.
- Variance Comparison:** ANOVA compares the variance between groups to the variance within groups to determine if the observed differences in means are statistically significant.

Why ANOVA Was Applied:

- Comparing Means Across Groups:** You likely want to assess if there are significant differences between the means of different groups or treatments.
- Statistical Significance:** ANOVA helps determine if the differences observed in the sample means are due to the treatment effect or just random chance.

Interpreting the Results:

- F-value:** Represents the ratio of variance between groups to the variance within groups. A higher F-value indicates a greater likelihood that the group means are different.
- P-value:** Indicates the probability of observing the data (or something more extreme) if the null hypothesis (that all group means are equal) is true. A P-value less than 0.05 typically suggests that at least one group mean is significantly different from the others.



The CAZ (1:1:1) group, the P-value is less than 0.01, indicating that the differences between this group and others are statistically significant.

Graph 1 - Antimicrobial efficacy of different herbal root canal irrigant against E faecalis.

Group	N	Minimum	Maximum	Mean	Std. Deviation	F-value	P-value
CAZ (1:1:1)	25	1.90	2.50	2.2040	.18592	167.3	<0.0
CAZ (1:2:1)	25	1.50	2.70	1.9240	.38974	3	1
CAZ (2:1:2)	25	1.80	2.80	1.8720	.20108		
CAZ (1:1:2)	25	1.40	2.80	1.8480	.25351		
NAOCL	25	1.20	2.70	1.5920	.31480		
NEEM	25	.75	2.45	1.3140	.34747		
TRIPHALA	25	1.05	2.45	1.3060	.24678		
SUNTHI	25	.90	2.40	1.2892	.28314		
GREEN TEA	25	.70	1.90	1.2600	.27386		
MISWAK	25	1.00	1.40	1.1600	.14142		
ALEO VERA	25	.80	1.80	1.1560	.20224		
TURMERIC	25	.80	1.40	1.1520	.14468		
TULSI	25	.90	1.40	1.1200	.16583		
PROPOLIS	25	.70	1.40	1.0040	.14283		

Table of statistical data related to different groups or treatments, possibly from an experiment. The columns include the group name, number of samples (N), minimum and maximum values, mean, standard deviation, F-value, and P-value.

Here's a brief overview of what each term indicates:

- Group:** The treatment or condition being tested.
- N:** Number of samples in each group.
- Minimum:** The lowest value recorded in that group.
- Maximum:** The highest value recorded in that group.
- Mean:** The average value of the measurements in that group.
- Std. Deviation:** A measure of the variation or dispersion of the data.
- F-value:** A ratio used in ANOVA to determine if there are significant differences between group means.
- P-value:** Indicates the probability that the observed differences are due to chance; a value less than 0.05 typically indicates statistical significance.

The F-value and P-value are only provided for the first group (CAZ (1:1:1)), suggesting that statistical significance has been tested for this group, but not for the others. If you need to perform ANOVA or another statistical test for the remaining groups, you would calculate the F-value and P-value based on the variance between and within groups

Antimicrobial efficacy of different herbal root canal irrigant against *E. faecalis* The "CAZ (1:1:1)" group had a minimum value of 1.90, a maximum of 2.50, a mean of 2.2600, and a standard deviation of 0.15946. In the "CAZ (1:2:1)" group, the minimum was 1.50, the maximum was 2.60, the mean was 1.9067, and the standard deviation was 0.33693. For the "CAZ (2:1:2)" group, the minimum value was 1.80, the maximum was 1.91, the mean was 1.8273, and the standard deviation was 0.04698.

The "CAZ (1:1:2)" group showed a minimum value of 1.50, a maximum of 2.10, a mean of 1.8000, and a standard deviation of 0.13628. In the "NAOCL" group, the minimum was 1.20, the maximum was 1.60, with a mean of 1.4333 and a standard deviation of 0.13973.

For the "NEEM" group, the minimum value was 1.15, the maximum was 1.35, the mean was 1.2633, and the standard deviation was 0.05164. The "TRIPHALA" group had a minimum of 1.10, a maximum of 1.35, a mean of 1.2233, and a standard deviation of 0.09232. The "SUNTHI" group showed a minimum of 1.00, a maximum of 1.30, a mean of 1.2067, and a standard deviation of 0.07988.

In the "GREEN TEA" group, the minimum was 1.00, the maximum was 1.70, with a mean of 1.1867 and a standard deviation of 0.20307. The "MISWAK" group had a minimum of 1.00, a maximum of 1.40, a mean of 1.1667, and a standard deviation of 0.14475.

The "ALEO VERA" group showed a minimum value of 1.00, a maximum of 1.60, a mean of 1.1533, and a standard deviation of 0.16417. For the "TURMERIC" group, the minimum value was 1.00, the maximum was 1.40, with a mean of 1.1467 and a standard deviation of 0.11872. The "TULSI" group had a minimum of 0.80, a maximum of 1.30, a mean of 1.1133, and a standard deviation of 0.17265.

Lastly, in the "PROPOLIS" group, the minimum value was 0.70, the maximum was 1.12, with a mean of 1.0013 and a standard deviation of 0.12082. Results shows highly significant value with F-value 167.4 & Pvalue, 0.01.

Pair comparison	Post -hoc test HSD test
CAZ (1:1:1) vs CAZ (1:2:1)	<0.01
CAZ (1:1:1) vs CAZ (2:1:2)	<0.01
CAZ (1:1:1) vs CAZ (1:1:2)	<0.01
CAZ (1:1:1) vs NAOCL	<0.01
CAZ (1:1:1) vs NEEM	<0.01
CAZ (1:1:1) vs TRIPHALA	<0.01
CAZ (1:1:1) vs SUNTHI	<0.01
CAZ (1:1:1) vs GREEN TEA	<0.01
CAZ (1:1:1) vs MISWAK	<0.01
CAZ (1:1:1) vs ALEO VERA	<0.01
CAZ (1:1:1) vs TURMERIC	<0.01
CAZ (1:1:1) vs TULSI	<0.01
CAZ (1:1:1) vs PROPOLIS	<0.01

Descriptive statistics for Antimicrobial efficacy of different herbal root canal irrigant against *E. Faecalis*.

Antimicrobial efficacy of different herbal root canal irrigant with sodium hypochlorite against *E. faecalis* were observed. For all the herbal medicaments were selected. They are Acorus calamus, Zanthoxylum alatum, Cissampelos pareira, Neem, Triphala, Sunthi, Green Tea, Miswak, Aloe vera, Turmeric, Tulsi and Propolis. These medicaments were tested against *E. Faecalis*.

DISCUSSION

Acorus calamus, Zanthoxylum alatum, Cissampelos pareira, Neem, Triphala, Sunthi, Green Tea, Miswak, Aloe vera, Turmeric, Tulsi and Propolis were selected for the study. These were tested in pure form for evaluating the efficacy against *E. faecalis*.

The significant results were found to be with Acorus calamus,

Zanthoxylum alatum, Cissampelos pareira, Neem, Triphala, Sunthi and Green tea and Non-significant results were found with Miswak, Aloe Vera, Turmeric, Tulsi and Propolis. Studies done on Aloe Vera by Sahebi S et al concluded that, the efficacy of aloe vera is same as that of normal saline with effective against *E. faecalis* biofilm. The aloe vera latex contains anthraquinone which is associated cytotoxicity, genotoxicity and carcinogenicity in humans. According to research on turmeric conducted by Prabhakar J et al, the antimicrobial activity of turmeric gradually declines over the course of 3 - 7 days. This result is a result of the dentin's capacity to buffer the curcumin's activity. Studies done on Miswak by Gazi M et al stated that Miswak decreases phosphate and Ph, saturation of the saliva with calcium; promotes demineralization of tooth enamel. Studies done on Tulsi by Chaurasia A et al concluded that Tulsi in its composition contains eugenol and essential oils. Our study already contains Eugenol so Tulsi was excluded from our study. Studies done on Propolis by Ahangari Z et al concluded that the use of propolis in root canal can change the colour of the clinical tooth crown. Resin is one of the active ingredients of miswak that will result in monoblock formation which is difficult for re-treatment.

Inference

Acorus calamus, Zanthoxylum alatum, Cissampelos pareira, Neem, Triphala, Sunthi, Green Tea, Miswak, Aloe vera, Turmeric, Tulsi and Propolis were evaluated for antimicrobial efficacy. They were evaluated through antimicrobial testing. These herbal medicaments were selected based on there antimicrobial properties. When evaluated Acorus calamus, Zanthoxylum alatum, Cissampelos pareira, Neem, Triphala, Sunthi, Green Tea showed statistically significant results with *E. Faecalis*. Whereas, Miswak, Aloe vera, Turmeric, Tulsi and Propolis showed

Statistically non-significant results. Medicaments which showed statistically non-significant results were excluded from the study. All drugs had comparatively better antimicrobial property. Therefore, these drugs were selected. The antimicrobial efficacy of different herbal root canal irrigant agints *E. faecalis* rank as, the superior most is in combination of CAZ (1:1:1) > CAZ (1:2:1) > CAZ (2:1:2) > CAZ (1:1:2) > NAOCL > NEEM > TRIPHALA > SUNTHI > GREEN TEA > MISWAK > ALEO VERA > TURMERIC.

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