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Comparative Evaluation of the Antimicrobial Effectiveness of Natural Extracts on Enterococcus Faecalis: An In-Vitro Study

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ABSTRACT:

Background: Enterococcus faecalis (E faecalis) is a major cause of persistent periradicular lesions in the endodontically treated teeth. In recent literature, natural extracts have received considerable attention as potential antimicrobial agents.

Objective: The aim of the current study is to compare the antimicrobial effectiveness of natural extracts namely Propolis, Essential oil of Artemisia pallens, and Black seed oil with that of 2% Chlorhexidine gluconate (CHX Gluconate) against E faecalis.

Materials & Methods: This comparative study was conducted on 40 samples (n=10 for each Group I: Propolis, Group II: Essential oil of Artemisia pallens, Group III: Black seed oil, and Group IV: 2% Chlorhexidine Gluconate). The methods briefly involved inoculation of lawn cultures of E faecalis (ATCC-29212) into Mueller Hinton agar plates. The test irrigants were added to the wells made on the agar media, and an Agar Diffusion Test (ADT) was performed for all samples. The comparisons of the bacterial zones of inhibition (ZOI) were done between groups. ANOVA with a post hoc Tukey's HSD test was used for statistical analysis of obtained data.

Results: The ZOI scores of Group I (Propolis), Group II (Essential oil of Artemisia pallens), Group III (Black seed oil) and Group IV – (CHX Gluconate) were 22.5, 32.60, 34.70 and 24.55 respectively.

Conclusion: The Black seed oil demonstrated the maximum antimicrobial activity against Enterococcus faecalis and could be used as an alternative to conventional root canal irrigants.

Keywords: Black Seed Oil, Essential Oil, Natural Extracts, Propolis, Root Canal Irrigants.

1. Introduction

A successful endodontic therapy involves removal of residual pulp tissue, dentinal debris and complete eradication of microflora from the root canal system. Enterococcus faecalis (E

Faecalis), a gram-positive, facultative anaerobe is reported to survive in root canals even after root canal treatment, leading to persistent periradicular infection (PPI) [1].
There are numerous irrigants employed to disinfect the root canal systems. The most commonly used is Sodium hypochlorite, which has excellent antimicrobial and tissue dissolving properties [2]. Chlorhexidine gluconate (CHX Gluconate) is another widely used irrigant, with a known abroad-spectrum antimicrobial agent known for its substantivity and relatively low toxicity [3]. However, the unpleasant taste, tissue toxicity, instrument corrosion, and tooth discoloration keep CHX Gluconate away for its routine use [4].
Natural extracts, such as Propolis has recently been conferred as a potential alternative to conventional irrigants [5]. It is a natural wax-like resinous substance extracted by honey bees from trees (poplar, conifer, willow, beech, horse-chestnut and clusia flowers, which is used in dentistry [6]. Its antimicrobial activity is attributed to its flavonoid material and used as a root canal irrigant [5,7]. *Artemisia pallens* (Davana), an aromatic herbaceous plant has been first used in Indian folk medicine to treat diabetic mellitus. *Artemisia species* are well documented for their medicinal and antioxidant properties [8]. The essential oil of *Artemisia pallens* possesses antispasmodic, antibacterial, antifungal, and stimulant properties [9]. *Nigella sativa*, also known as black seed, ("miracle herb of the century") owing to healing, antidepressant and many other medicinal properties. Several showed that Thymoquinone, a key ingredient of black seed, have antimicrobial, anti-inflammatory, and analgesic properties[10].
We recognized the non-entity in the literature, on studies assessing the efficacy of such natural extracts as a root canal irrigant. Thus, this current study was undertaken to compare the antimicrobial effectiveness of natural extracts (Propolis, Essential oil of *Artemisia pallens*, Black seed oil) with 2% Chlorhexidine gluconate against *E faecalis.*

2. Materials and Methods

This in-vitro study was conducted after obtaining the ethical clearance from Institutional ethical committee. The comparative testing was performed between 4 groups, namely- Group I: Propolis, Group II: Essential oil of *Artemisia pallens*, Group III: Black seed oil, and Group IV: 2% Chlorhexidine gluconate which acted as controls. A sample of n=10 was taken conveniently for each group, making the final tested sample of 40. The materials obtained for the invitro study were Strains of *Enterococcus faecalis* [ATCC 29212] (Goleccha Diagnostics, Chennai, India), Propolis (Hi-Tech Natural Products Ltd, New Delhi, India), Greenish Baraka Black seed oil (Greenish Trades Pvt Ltd, Chennai, India), 2% Chlorhexidine gluconate (Azure Laboratories, Kochi, India). Mueller–Hinton agar plates (HiMedia, Mumbai, India).

Preparation of Essential oil of Artemisia pallens*:* The plant was shade dried for 7 days and was ground to powder and soaked in methanol for 48 hours at room temperature with occasional shaking. The extract was filtered, and the residue was soaked with the same volume of the fresh solvent. The procedure was repeated twice to get the maximum extract of constituents. A rotary evaporator at 40^{o} C was used to evaporate the methanol. The preparation was done as per previous standards and opinion of the microbiologists [11]. The crude greenish-color solid mass was removed and essential oil sedimented below was obtained for testing (Figure 1).

E. faecalis culture preparation*:* A pure culture of *Enterococcus faecalis* (ATCC 29212) was grown on Mueller-Hinton (MH) agar plates. E. *faecalis* strain was inoculated into the nutrient broth and was incubated at 37^{o}C for 3 to 4 hours and turbidity adjusted to 0.5

McFarland standards CFU. Under aseptic conditions, standard molten agar was dispensed onto sterile plates and allowed to solidify. A lawn culture of E. *faecalis* was inoculated on the surface of Mueller-Hinton Agar plates with a sterile spreader.
Wells with a diameter of 6 mm were bored in MH Agar plates and 100 µl of the test products were added and incubated at 37ºC for 24 hours. All the plates were examined after incubation for zones of inhibition (ZOI) of bacterial growth, and the diameters of these zones were measured in millimeters (Figure 2).
The obtained results were analyzed and compared with ANOVA and Post hoc Tukey HSD test to find out if there was a significant difference between the mean ZOI of various irrigants used. The $p<0.05$ score was considered statistically significant in all instances.

3. Results

The mean ZOI scores of all tested irrigants are shown in Table 1. The Group III (Black seed oil) showed ZOI of 34.70, followed by Group II (Essential oil of *Artemisia pallens) with a* ZOI of 32.60, Group IV – (CHX Gluconate) with ZOI of 24.55, and finally Group I -Propolis with ZOI of 22.50. Table 2 shows intergroup differences were also statistically significant as per the ANOVA test ($p<0.000$).

Table 1: Descriptive statistics of the study groups

	N	Mean (mm)	SD	SE	95% CI for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Propolis	10	22.50	1.73205	.54772	21.2610	23.7390	19.50	25.00
Essential Oil of AP	10	32.60	1.14988	.36362	31.7774	33.4226	30.50	34.50
Black seed oil	10	34.70	1.25167	.39581	33.8046	35.5954	33.00	37.00
2% CHX Gluconate	10	24.55	1.03950	.32872	23.8064	25.2936	23.00	26.00

SD: Standard Deviation, SE: Standard Error, CI: Confidence Interval

Table 2: One-way Analysis of Variance (ANOVA)

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1068.219	3	356.073	204.362	.000
Within Groups	62.725	36	1.742		
Total	1130.944	39			

The post hoc analysis revealed that the mean ZOI value of group III (Black seed oil) was significantly higher ($p= 0.006$), than that of group II (Essential oil of *Artemisia pallens).* Likewise, the ZOI value of group II was significantly higher ($p=0.000$) than the group IV (2% CHX Gluconate) and group I (Propolis). The post hoc comparison also revealed that the mean ZOI value in the group IV (2% CHX Gluconate) was significantly ($p=0.007$) higher than in the group I (Propolis group). See Table 3.

Table 3: Multiple Comparisons using Post hoc Tukey test

(I) Irrigants	(J) Irrigants	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Propolis	Essential Oil of AP	-10.10000*	.59032	.000	-11.6899	-8.5101

	Black seed oil	-12.20000*	.59032	.000	-13.7899	-10.6101
	2% CHX Gluconate	-2.05000*	.59032	.007	-3.6399	-.4601
Essential Oil of AP	Propolis	10.10000*	.59032	.000	8.5101	11.6899
	Black seed oil	-2.10000*	.59032	.006	-3.6899	-.5101
	2% CHX Gluconate	8.05000*	.59032	.000	6.4601	9.6399
Black Seed Oil	Propolis	12.20000*	.59032	.000	10.6101	13.7899
	Essential Oil of AP	2.10000*	.59032	.006	.5101	3.6899
	2% CHX Gluconate	10.15000*	.59032	.000	8.5601	11.7399
2% CHX Gluconate	Propolis	2.05000*	.59032	.007	.4601	3.6399
	Essential Oil of AP	-8.05000*	.59032	.000	-9.6399	-6.4601
	Black seed oil	-10.15000*	.59032	.000	-11.7399	-8.5601

*Mean Difference is significant at 0.05 level

4. Discussion

The failure of endodontic therapy due to poor mechanical instrumentation is considerably reducing owing to advances in surgical instruments and enhanced visual tools. However, instruments alone do not eliminate the microbes from the root canal system [12]. *E faecalis* is a bacterium that has shown resistance to a broad range of antimicrobial agents and is well known for its persistence and recolonization within the root canals which frequently results in endodontic failure [13]. To improve the success of root canal treatment, complete removal of infected tissue and elimination of total microbes is essential, along with those having resistance. This necessitates the usage of updated and effective antimicrobial irrigants for clinical practice. Sodium hypochlorite and 2% Chlorhexidine gluconate have long been utilized due to their antimicrobial and organic tissue dissolving properties. However, they have a few potential adverse effects limit their use currently, leading us to relentless search for safer, or natural irrigants. A meta-analysis of randomized controlled trials comparing the antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection revealed that both irrigant demonstrated comparable antimicrobial efficacy [14]. Hence, 2% Chlorhexidine gluconate was used as a control in the present study.

The results obtained from the present study revealed that the antimicrobial effectiveness of black seed oil against E. *faecalis* was found to be the highest among the tested irrigants. These findings are in line with previous research, which report that black seed oil was 2.5% more effective than sodium hypochlorite in terms of antimicrobial activity against E. *faecalis*. The reason for this could be attributed to the thymoquinone, the bioactive component of black seed oil (*Nigella sativa*) which prevents bacterial biofilm formation by reducing the metabolic oxidative activity of E. *faecalis* [15]. Previous research found that thymoquinone exhibits resistance modifying activity by inhibiting the pump efflux of multi-drug resistant bacteria, resulting in antibiotic aggregation in the bacterial cell [16].

In the current study, the essential oil of *Artemisia pallens* was shown to have significant antimicrobial activity against E. *faecalis*, but it was less effective than black seed oil. However, it had superior antimicrobial activity than the 2 % Chlorhexidine gluconate. In agreement with our findings, the previous study reported that the essential oil of *Artemisia pallens* exhibit medium to high efficacy against the E. *faecalis* [17]. In the present study, propolis showed a decent antimicrobial effect against E. *faecalis*, but its efficacy was not greater than 2% Chlorhexidine gluconate. This is similar with the findings of an earlier study, which reported that while propolis exhibited remarkable antibacterial activity, but it was not more effective than 2% Chlorhexidine gluconate [5].

There above findings are novel reporting on the antimicrobial efficacy of natural formulations such as black seed oil and essential oil of *Artemisia pallens*. Also, the comparisons of these ingredients with 2 % Chlorhexidine gluconate were not done routinely and thus, the results cannot be substantiated or paralleled with those of any other studies. This novelty adds up to the strength of the study. The study's limitation is that it was carried out in-vitro with relatively small sample size of culture. Also, the study settings, could not replicate the biofilm protection provided by E. *faecalis*. More preclinical and clinical studies are required to assess their biocompatibility, safety and to modify their content for patient acceptability.

5. Conclusion

Within the limitations of this study, it can be concluded that the natural extracts such as black seed oil and essential oil of Artemisia pallens have higher antimicrobial efficacy against E. faecalis than 2% Chlorhexidine gluconate, and might be used as an alternative to commonly used sodium hypochlorite and chlorhexidine gluconate.

6. References

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Figure Legends

Figure 1: Extract of Essential oil of Artemisia pallens

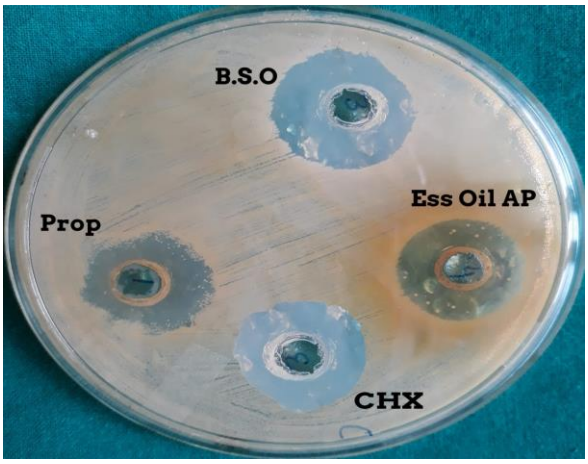

Figure 2: Agar plate- well diffusion test against E. faecalis