RESEARCH ARTICLE

Comparative Evaluation of Antimicrobial Efficacy of Zinc Oxide Eugenol with Zinc Oxide Mixed with Three Herbal Products to be Used as Root Canal Filling Material: An *In Vitro* Study

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Abstract

Introduction: Primary teeth with pulpal involvement and those having periapical issues should be retained until their normal exfoliation because their premature loss may lead to adverse aberrations in the future dentition. Root canals harbor different types of microorganisms and root canal infections generally are polymicrobial in nature. One of the most common and preferred root canal filling material which is commonly used for primary teeth is zinc oxide eugenol (ZOE) cement.

Aims and objectives: To evaluate and compare the antimicrobial efficacy of ZOE with zinc oxide powder mixed with *Morinda citrifolia* extract, *Aloe vera* extract, and neem extract against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans.*

Materials and methods: The material used in the study were zinc oxide powder, eugenol liquid, *M. citrifolia* extract, *A. vera* extract, neem extract, petroleum jelly (Vaseline). The zinc oxide powder was mixed with minimum inhibitory concentration (MIC) percentage value of herbal extract.

Result: Zinc oxide eugenol showed strong inhibitory effect against *S. aureus* and *C. albicans.* For *P. aeruginosa,* zinc oxide+*M. citrifolia* showed strong inhibitory. Petroleum jelly (Vaseline) was used as control agent which showed no inhibitory effect.

Conclusion: The test root canal filling materials, i.e., ZOE, zinc oxide powder mixed with *M. citrifolia* extract, *A. vera* extract, and neem extract , respectively showed varied antimicrobial activity against the microorganisms tested, i.e., *S. aureus*, *P. aeruginosa,* and *C. albicans.*

Keywords: *Aloe vera*, Antimicrobial efficacy, *Morinda citrifolia*, Neem, Primary teeth, Zinc oxide eugenol.

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Introduction

Primary teeth with pulpal involvement and those having periapical issues should be retained until their normal exfoliation because their premature loss may lead to adverse aberrations in the future dentition.[1] When the pulp becomes irreversibly infected or necrotic root canal treatment is indicated for children. For proper success of the endodontic treatment, various materials with good antimicrobial properties are used as root canal filling materials in primary teeth.[2]

Root canals harbor different types of microorganisms and their microflora has been a topic of discussion and such root canal infections generally are polymicrobial in nature[1] therefore, it is important to identify which microorganisms are present. Very few studies have documented regarding the bacterial species that are present in primary teeth with pulp necrosis as well as periapical infections; more so because many of these are anaerobic microorganisms which are difficult to culture.[3] One of the most common fungus encountered during root canal treatment is *C. albicans*, their incidence account for 18% in cases of retreatments and 21% in primary infections.[4]

One of the most common and preferred root canal filling material which is mostly used for primary teeth is ZOE cement;[5] however, ZOE cannot be considered the ideal material for root canal filling due to its restricted antimicrobial action[6] and also it tends to resorb at a very slower rate than the roots of the primary teeth.[7] Such shortcomings have led in quest of newer alternative materials which can be used for root canal filling materials in primary teeth.[7]

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Scientists and medical professionals have realized the importance of traditional, herbal/natural products, and their use in the contemporary world;[8] amongst them are "*phytochemicals*" which are exclusively isolated from plants (considered as prospective alternatives).[9]

M. citrifolia belongs to family Rubiaceae. The synonyms used are Indian Mulberry, Ba Ji Tian, Nono or Nonu, Cheese Fruit, and Nhau. It has various medical uses such as antibacterial, antiviral, analgesic, anti-tumor, antihelminthes, anti-inflammatory, hypertensive, and immune-enhancing effects. Fruit juice is used in, diabetes, arthritis,

muscle pain, cardiovascular disease, menstrual disorder, and gastrointestinal disturbances.[8,10,12]

A. vera belongs to the family Alliaceae. The synonyms used is *Aloe barbadensis* Miller. It has anti-inflammatory and anticancer activity.[13-17]

Neem belongs to the family Meliaceae. The synonyms used is *Azadirachta indica.* It has various medical uses such as immunomodulatory, antifungal, antihyperglycemic, antiulcer, antimalarial, anti-inflammatory, antibacterial, antiviral, and antioxidant.[18–21]

Therefore, the present study was undertaken in order to evaluate and compare the antimicrobial efficacy of ZOE with zinc oxide mixed with *M. citrifolia* extract, *A. vera* extract, and neem extract against *S. aureus, P. aeruginosa,* and *C. albicans* microorganisms, which are mostly isolated from infected root canals of primary teeth.

Aims and Objectives

- To evaluate the antimicrobial efficacy of ZOE against *S. aureus, P. aeruginosa*, and *C. albicans*
- To evaluate the antimicrobial efficacy of zinc oxide powder mixed with M. citrifolia extract, A. vera, extract and neem extract against *S. aureus, P. aeruginosa, and C. albicans*
- To compare the antimicrobial efficacy of ZOE vs zinc oxide powder mixed with *M. citrifolia* extract, *A. vera* extract, and neem extract against *S. aureus, P. aeruginosa, and C. albicans*

Materials

The material used in the study were zinc oxide powder, eugenol liquid, *M. citrifolia* extract (Konark Herbals and Health Care, Daman), *A. vera* extract (Konark Herbals and Health Care, Daman), neem extract (Konark Herbals and Health Care, Daman), and petroleum jelly (Vaseline). The microbial strains were *S. aureus* (ATCC 25923), *P. aeruginosa*. (ATCC 27853), and *C. albicans* (ATCC 10231).

Methods

Determination of Minimum Inhibitory Concentration[22-24]

Minimum inhibitory concentration of *M. citrifolia* extract, *A. vera* extract and neem extract were calculated against *S. aureus*, *P. aeruginosa,* and *C. albicans*. A standard inoculum of single colony of *S. aureus, P. aeruginosa,* and *C. albicans* microorganism was passed in nutrient broth and was incubated at 37°C for 24 hours. The turbidity was adjusted by comparing with Mc Farland 0.5 standard. The zinc oxide powder and liquid herbal extracts as per the MIC obtained of all root canal filling materials used in the study which were standardized according to the formulae given by Tchaou et al.[6] and Reddy et al.[1] The control material used was petroleum jelly (vaseline).

The powder and liquid ratio was as follows

I. ZOE	Zinc oxide	Eugenol
	1 Scoop	7 Drops
	0.2 g	0.07 cm^3
II. Vaseline	Commercial product	

According to the minimum inhibitory concentration obtained MIC of *M. citrifolia* was 6.25%, *A. vera* was 6.25, 12.5, and 25% and neem was 6.25 and 12.5%. The zinc oxide powder was mixed with MIC percentage value of herbal extract.

Mixing of powder and liquid was done on a presterilized glass slab. Mixing was done using a cement spatula at room temperature. Freeze-dried, pure strains of the three test microorganisms which are mostly reported to inhabit nonvital root canals of primary teeth were employed in this inhibition experiment. Sensitivity testing was done using standard agar diffusion method.[25] The media used for broth cultures was Brain Heart Infusion (BHI) broth for *S. aureus* and *P. aeruginosa*. A pure culture of *C. albicans* was used. It was inoculated on Sabouraud dextrose agar medium, and it was further incubated at 37°C overnight. It was adjusted to an optical density of one with sterile BHI broth. MuellerHinton agar plates were used for *S. aureus* and *P. aeruginosa*. Three wells were prepared on the three agar plates with a sterile agar puncher. These wells were 3 mm in depth and in diameter. Two wells were also prepared of similar dimensions on other three agar plates. The five wells which were made on the agar plates were completely filled with the test material and control materials. The test materials used were ZOE, zinc oxide powder mixed with *M. citrifolia* extract, zinc oxide powder mixed with *A. vera* extract, zinc oxide powder mixed with neem extract and the control material was Vaseline. The agar plates which were used in this study were preincubated for 1 hour at room temperature in order to allow diffusion of the materials through the agar. Then this plate were incubated at 37°C for 24 to 48 hours. The diameters of the zones of microbial inhibition was measured in millimeters around each test material at the end of 24 hours for *S. aureus*[1,26] and *P. aeruginosa*[1,26] and at the end of 24 and 48 hours for *C. albicans*.[26] The procedure was repeated thrice for each strain and two observers measured the zones. The mean zone of inhibition for each material microbial strain combination was calculated.

Results

The present study was done in order to evaluate and compare antimicrobial efficacy of ZOE with zinc oxide powder mixed with *M. citrifolia* extract, *A. vera* extract and Neem extract , respectively as a primary root canal filling material which can be used in primary teeth against *S. aureus*, *P. aeruginosa,* and *C. albicans* strains.

Table 1 and Figure 1 show intermaterial comparison of antimicrobial efficacy of test material against *S. aureus* at the end of 24 hours. A statistically significant difference was found between ZOE and zinc oxide + *M. citrifolia*, ZOE and zinc oxide + *A. vera*, ZOE and zinc oxide + neem, ZOE and petroleum jelly (vaseline), zinc oxide + *M. citrifolia* and petroleum jelly (Vaseline), zinc oxide + *A. vera* and petroleum jelly (Vaseline), zinc oxide + neem and petroleum jelly (Vaseline) (p value = 0.0001).

Table 2 and Figure 2 show intermaterial comparison of antimicrobial efficacy of test material against *P. aeruginosa* at the end of 24 hours. A statistically significant difference was found between ZOE and zinc oxide + *M. citrifolia*, ZOE and petroleum jelly (Vaseline), zinc oxide + *M. citrifolia* and zinc oxide + *A. vera*, zinc oxide + *M. citrifolia* and zinc oxide + neem, zinc oxide + *M. citrifolia* and petroleum jelly (Vaseline), zinc oxide + *A. vera* and petroleum jelly (Vaseline), zinc oxide + neem, and petroleum jelly (Vaseline) (p value = 0.0001) and ZOE and zinc oxide + *A. vera* (p value = 0.046).

Table 3 and Figure 3 show intermaterial comparison of antimicrobial efficacy of test material against *C. albicans* at the end of 24 hours. A statistically significant difference was found

Table 1: Intermaterial comparison of antimicrobial efficacy of test material against *S. aureus* at the end of 24 hours

Material		*Mean (I-J)*	*Std. error*	*p*	*95% Confidence interval*	
					Lower bound	*Upper bound*
Zinc oxide eugenol	Zinc oxide + *M. citrifolia*	4.33	0.48	**0.0001,S**	2.91	5.75
	Zinc oxide + *A. vera*	4.16	0.48	**0.0001,S**	2.74	5.58
	Zinc oxide + Neem	4.33	0.48	**0.0001,S**	2.91	5.75
	Petroleum jelly (Vaseline)	20.66	0.48	**0.0001,S**	19.24	22.08
Zinc oxide + *M. citrifolia*	Zinc oxide + *A. vera*	–0.16	0.48	0.997,NS	–1.58	1.25
	Zinc oxide + Neem	0.00	0.48	1.000,NS	–1.41	1.41
	Petroleum jelly (Vaseline)	16.33	0.48	**0.0001,S**	14.91	17.75
Zinc oxide + *A. vera*	Zinc oxide + Neem	0.16	0.48	0.997,NS	–1.25	1.58
	Petroleum jelly (Vaseline)	16.50	0.48	**0.0001,S**	15.08	17.91
Zinc oxide + neem	Petroleum jelly (Vaseline)	16.33	0.48	**0.0001,S**	14.91	17.75

Multiple comparisons: Tukey test.

Table 2: Intermaterial comparison of antimicrobial efficacy of test material against *P. aeruginosa* at the end of 24 hours

Material		*Mean difference (I-J)*	*Std. error*	*p*	*95% Confidence interval*	
					Lower bound	*Upper bound*
Zinc oxide eugenol	Zinc oxide+ *M. citrifolia*	–4.16	0.50	**0.0001,S**	–5.64	–2.68
	Zinc oxide+ *A. vera*	-1.50	0.50	**0.046,S**	–2.97	–0.02
	Zinc oxide + Neem	–0.33	0.50	0.963,NS	–1.81	1.14
	Petroleum jelly (Vaseline)	24.33	0.50	**0.0001,S**	22.85	25.81
Zinc oxide + *M. citrifolia*	Zinc oxide + *A. vera*	2.66	0.50	**0.0001,S**	1.18	4.14
	Zinc oxide + Neem	3.83	0.50	**0.0001,S**	2.35	5.31
	Petroleum jelly (Vaseline)	28.50	0.50	**0.0001,S**	27.02	29.97
Zinc oxide+ *A. vera*	Zinc oxide + Neem	1.16	0.50	0.173,NS	–0.31	2.64
	Petroleum jelly (Vaseline)	25.83	0.50	**0.0001,S**	24.35	27.31
Zinc oxide + neem	Petroleum jelly (Vaseline)	24.66	0.50	**0.0001,S**	23.18	26.14

Multiple comparisons: Tukey test.

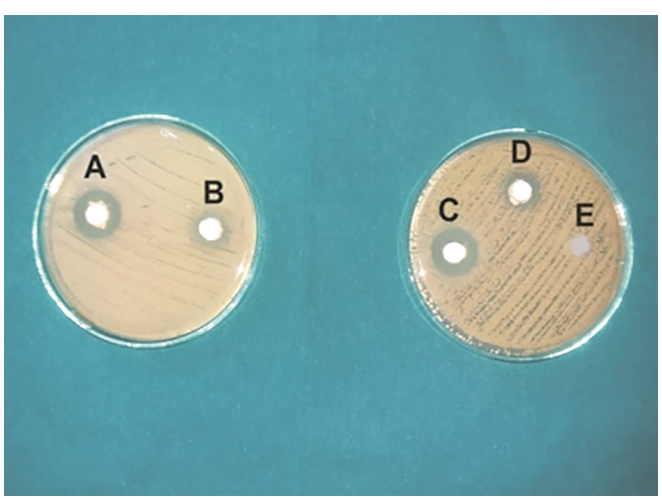

Fig. 1: Zone of inhibition of: A. Zinc oxide eugenol. B. Zinc oxide powder mixed with *M. citrifolia* extract. C. Zinc oxide powder mixed with *A. vera* extract. D. Zinc oxide powder mixed with neem extract. E. Vaseline against *S. aureus* after 24 hours.

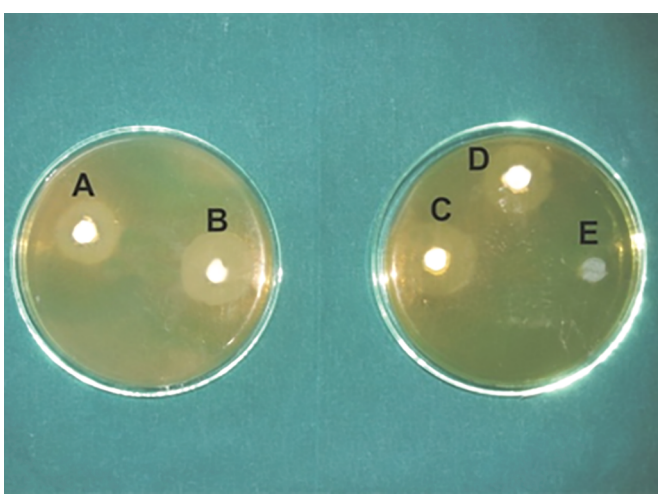

Fig. 2: Zone of inhibition of: A. Zinc oxide eugenol. B. Zinc oxide powder mixed with *M. citrifolia* extract. C. Zinc oxide powder mixed with *A. vera* extract. D. Zinc oxide powder mixed with neem extract. E. Vaseline against *P. aeruginosa* after 24 hours

Table 3: Intermaterial comparison of antimicrobial efficacy of test material against *C. albicans* at the end of 24 hrs.

Material		*Mean Difference (I-J)*	*Std. Error*	*p-value*	*95% Confidence Interval*	
					Lower Bound	*Upper Bound*
Zinc oxide eugenol	Zinc oxide + *M. citrifolia*	22.16	0.82	**0.0001,S**	19.73	24.60
	Zinc oxide + *A. vera*	19.50	0.82	**0.0001,S**	17.06	21.93
	Zinc oxide + Neem	20.50	0.82	**0.0001,S**	18.06	22.93
	Petroleum jelly (Vaseline)	32.16	0.82	**0.0001,S**	29.73	34.60
Zinc oxide + *M. citrifolia*	Zinc oxide + *A. vera*	-2.66	0.82	**0.027,S**	-5.10	-0.23
	Zinc oxide + Neem	-1.66	0.82	0.290,NS	-4.10	0.76
	Petroleum jelly (Vaseline)	10.00	0.825	**0.0001,S**	7.56	12.43
Zinc oxide + *A. vera*	Zinc oxide + Neem	1.00	0.82	0.748,NS	-1.43	3.43
	Petroleum jelly (Vaseline)	12.66	0.82	**0.0001,S**	10.23	15.10
Zinc oxide + Neem	Petroleum jelly (Vaseline)	11.66	0.82	**0.0001,S**	9.23	14.10

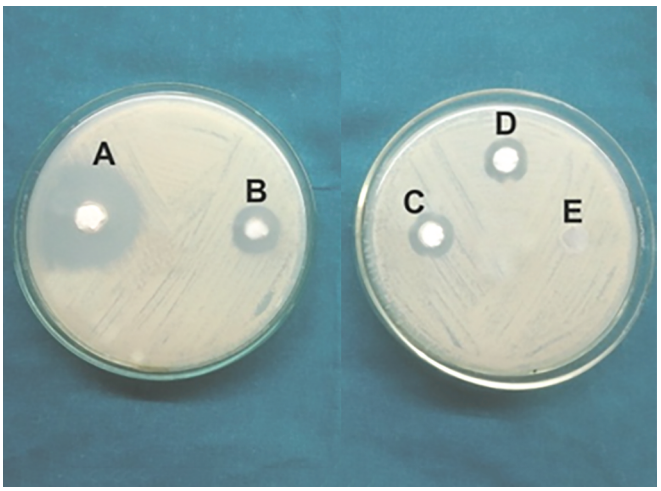

Fig. 3: Zone of inhibition of: A. Zinc oxide eugenol. B. Zinc oxide powder mixed with *M. citrifolia* extract. C. Zinc oxide powder mixed with *A. vera* extract. D. Zinc oxide powder mixed with neem extract. E. Vaseline against *C. albicans* after 24 hours

between ZOE and zinc oxide + *M. citrifolia*, ZOE and zinc oxide + *A. vera*, ZOE and zinc oxide + neem, ZOE and petroleum jelly (Vaseline), zinc oxide + *M. citrifolia* and petroleum jelly (Vaseline), zinc oxide + *A. vera* and petroleum jelly (Vaseline), zinc oxide + neem and petroleum jelly (Vaseline) (p value = 0.0001) and zinc oxide + *M. citrifolia* and zinc oxide + *A. vera* (p value = 0.027)

Table 4 and Figure 4 show intermaterial comparison of antimicrobial efficacy of test material against *C. albicans* at the end of 48 hours. A statistically significant difference was found between ZOE and zinc oxide + *M. citrifolia*, ZOE and zinc oxide + *A. vera*, ZOE and zinc oxide + neem, ZOE and petroleum jelly (Vaseline), zinc oxide + *M. citrifolia* and petroleum jelly (Vaseline), zinc oxide + *A. vera* and petroleum jelly (Vaseline), zinc oxide + neem and petroleum jelly (Vaseline) (p value = 0.0001) and zinc oxide + *M. citrifolia* and zinc oxide + *A. vera* (p value = 0.007).

The measurements of zones of inhibition of all test materials against *S. aureus*, *P. aeruginosa,* and *C. albicans* were ranked arbitrarily into the following four categories according to the proportional distribution of the dataset (Table 5).[1]

Table 6 and Figure 5 show the inhibition results of all five test filling materials against *S. aureus*, *P. aeruginosa,* and *C. albicans* bacterial strains according to the ranking scale.

Statistical Analysis

Statistical analysis was done by using descriptive and inferential statistics using Student's paired *t* test, one-way ANOVA and multiple comparison : Tukey test and software used in the analysis were SPSS 22.0 version and GraphPad Prism 6.0 version and $p < 0.05$ is considered as level of significance.

Discussion

Premature loss of pulpally involved primary teeth most of the times remains a common problem.[1] Most important cause of pulpal and periradicular pathologies include microorganisms and their by-products.[6] Most common forms of dental treatment specifically designed to retain the primary tooth as a functional unit in the dental arch is pulpectomy.[1] There are different root canal filling materials which are available for primary teeth whose significant objective is to disinfect the entire root canal system for proper root canal treatment. Generally the most commonly used materials for root canal treatment in primary teeth are ZOE and calcium hydroxide iodoform paste, but due their disadvantages they are not considered as ideal root canal filling material.[27] Phytodentistry is generally considered as an emerging branch in dentistry. It generally implies the use of medicinal plants and also their products for treating disease directly or indirectly.[28]

Hegde et al.[29] found that ZOE have shown a strong inhibition of Gram-positive microorganisms and also the fungi which is similar to our study where ZOE shows strong inhibitory zones against Gram positive organism and fungi. Cox **and** Hembree[30] found that zinc oxide when used alone has absence of any inhibitory effect on any of the test organisms and the authors concluded from their study that the antimicrobial activity of ZOE may be due to the free eugenol which is generally released from the set materials. But Spencer et al.[31] concluded in their study that zinc oxide when used without eugenol does have an antimicrobial action, which was proved in our study where zinc oxide powder mixed with *M. citrifolia*, *A. vera,* and neem extract against *S. aureus*, *P. aeruginosa,* and *C. albicans* showed inhibitory effect.

Hegde et al.[29] in their study they found that zinc oxide powder mixed with $Ca(OH)_2$ showed that there is no inhibition of *S. aureus*, *E. faecalis*, *P. aeruginosa,* and *C. albicans,* whereas in present study zinc oxide powder mixed with *M. citrifolia* extract, zinc oxide powder mixed with *A. vera* extract, zinc oxide powder mixed with neem extract showed medium inhibition zones against *S. aureus*, strong inhibition zones against *P. aeruginosa* and weak and medium

Table 4: Intermaterial comparison of antimicrobial efficacy of test material against *C. albicans* at the end of 48 hrs.

Material		*Mean Difference (I-J)*	*Std. Error*	*p-value*	*95% Confidence Interval*	
					Lower Bound	*Upper Bound*
Zinc oxide eugenol	Zinc oxide + *M. citrifolia*	21.83	0.87	**0.0001,S**	19.25	24.412
	Zinc oxide + A. vera	18.50	0.87	**0.0001,S**	15.92	21.07
	Zinc oxide + Neem	19.50	0.87	**0.0001,S**	16.92	22.07
	Petroleum jelly (Vaseline)	32.00	0.87	**0.0001,S**	29.42	34.57
Zinc oxide + *M. citrifolia*	Zinc oxide + *A. vera*	-3.33	0.87	**0.007,S**	-5.91	-0.75
	Zinc oxide + Neem	-2.33	0.87	0.090,NS	-4.91	0.24
	Petroleum jelly (Vaseline)	10.16	0.87	**0.0001,S**	7.58	12.74
Zinc oxide + *A. vera*	Zinc oxide + Neem	1.00	0.87	0.785,NS	-1.57	3.57
	Petroleum jelly (Vaseline)	13.50	0.87	**0.0001,S**	10.92	16.07
Zinc oxide + Neem	Petroleum jelly (Vaseline)	12.50	0.87	**0.0001,S**	9.92	15.07

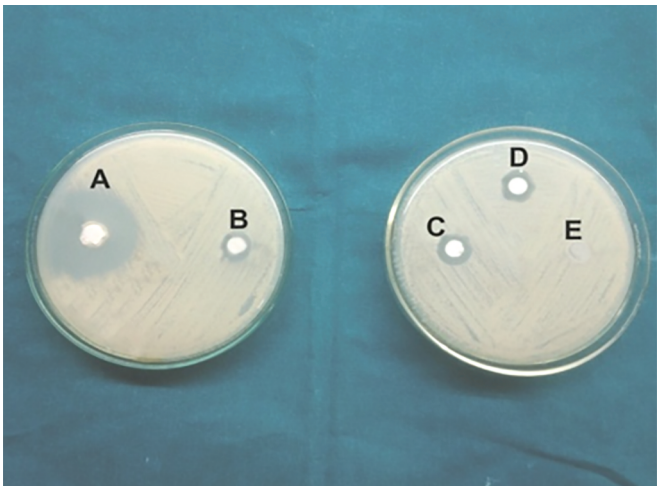

Fig. 4: Zone of inhibition of: A. Zinc oxide eugenol. B. Zinc oxide powder mixed with *M. citrifolia* extract. C. Zinc oxide powder mixed with *A. vera* extract. D. Zinc oxide powder mixed with neem extract. E. Vaseline against *C. albicans* after 48 hours

Table 5: Ranking scheme for microbial inhibition

Rank	*Range of zone diameter (mm)*
No	0
Weak	0.1–11.5
Medium	11.5–19.7

inhibition zones against *C. albicans*. Bohora et al.[32] have concluded in their study on comparison of the antibacterial efficiency of neem leaf extract along with 2% sodium hypochlorite against *E. faecalis, C. albicans,* and mixed culture that neem leaf extract has a major antimicrobial effect against *C. albicans*. This is similar with our study where zinc oxide powder mixed with neem showed medium zone of inhibition against *C. albicans*.

Kriplani et al.[27] found that *A. vera* showed strong inhibitory effect against *P. aeruginosa* microorganisms and showed medium inhibitory effect against *S. aureus* test microorganisms and zinc oxide powder mixed with *A. vera* showed medium inhibitory effect against both the groups of test microorganisms, i.e., *S. aureus* and *P. aeruginosa* which is similar to our study where we found medium inhibitory zones of zinc oxide powder mixed with *A. vera* against *S. aureus* but contradictory to the fact where we found strong inhibitory zone of zinc oxide powder mixed with *A. vera* against *P. aeruginosa*.

Sureshchandra et al.[28] found in an *in vitro* study that the average zone of inhibition with chloroform extract of *A. vera* against *C. albicans* was found to be 14 mm, which was near to the value of our study where zinc oxide powder mixed with *A. vera* extract against *C. albicans* showed mean zone of inhibition of 12.66 and 13.5 mm at the end of 24 and 48 hours , respectively.

Murray et al.[33] from their study concluded that *M. citrifolia* has similar intracanal irrigating properties as that of NaOCl along with Ethylenediamine tetraacetic acid (EDTA). Various studies done previously have claimed that the *M. citrifolia* fruit extract shows antifungal effects on *C. albicans*[9] which is similar to our study where zinc oxide powder mixed with *M. citrifolia* showed weak zones of inhibition against *C. albicans*.

In our study ZOE showed strong inhibitory effect followed by zinc oxide + *A. vera*, zinc oxide + *M. citrifolia,* and zinc oxide + neem in descending order against *S. aureus*. For *P. aeruginosa,* zinc oxide + *M. citrifolia* showed strong inhibitory effect followed by zinc oxide + *A. vera*, zinc oxide + neem and ZOE in descending order. For *C. albicans,* ZOE showed strong inhibitory effect followed by zinc oxide + *A. vera*, zinc oxide + neem, and zinc oxide + *M. citrifolia* in descending order. Petroleum jelly (Vaseline) was used as control agent which showed no zone of inhibition. Additional, *in vivo* studies are required in order to state the specific antimicrobial activity and also the advantages and disadvantages of any of the test filling materials.

Conclusion

The following conclusions were drawn from this study:

- The test root canal filling materials, i.e., ZOE, zinc oxide powder mixed with *M. citrifolia* extract, A. vera extract, and neem extract, respectively showed varied antimicrobial activity against the microorganisms tested, i.e., *S. aureus, P. aeruginosa*, and *C. albicans*.
- ZOE was found to have superior antimicrobial activity as compared to zinc oxide powder mixed with M. citrifolia extract, A. vera extract and neem extract , respectively against *S. aureus* and *C. albicans*.
- Zinc oxide powder mixed with M. citrifolia extract, A. vera extract, and neem extract, respectively showed superior antimicrobial activity as compared to ZOE against *P. aeruginosa*
- *M. citrifolia* extract, A. vera extract, and neem extract mixed with zinc oxide powder can be used as an potential root canal filling material

Table 6: Inhibition results of five test filling materials against *S. aureus, P. aeruginosa*, and *C. albicans* bacterial strains according to the ranking scale

Material	*Microorganism*			
	S. aureus	*P. aeruginosa*	*C. albicans*	
	24 hours	*24 hours*	*24 hours*	*48 hours*
Zinc oxide eugenol	20.66 (S)	24.33 (S)	32.16 (S)	32.00 (S)
Zinc oxide + *M. citrifolia*	16.33 (M)	28.50 (S)	10.00 (W)	10.16 (W)
Zinc oxide + *A. vera*	16.50 (M)	25.83 (S)	12.66 (M)	13.50 (M)
Zinc oxide + Neem	16.33 (M)	24.66 (S)	11.66 (M)	12.50 (M)
Petroleum jelly (Vaseline)	0.00 (No)	00.00 (No)	0.00 (No)	0.00 (No)

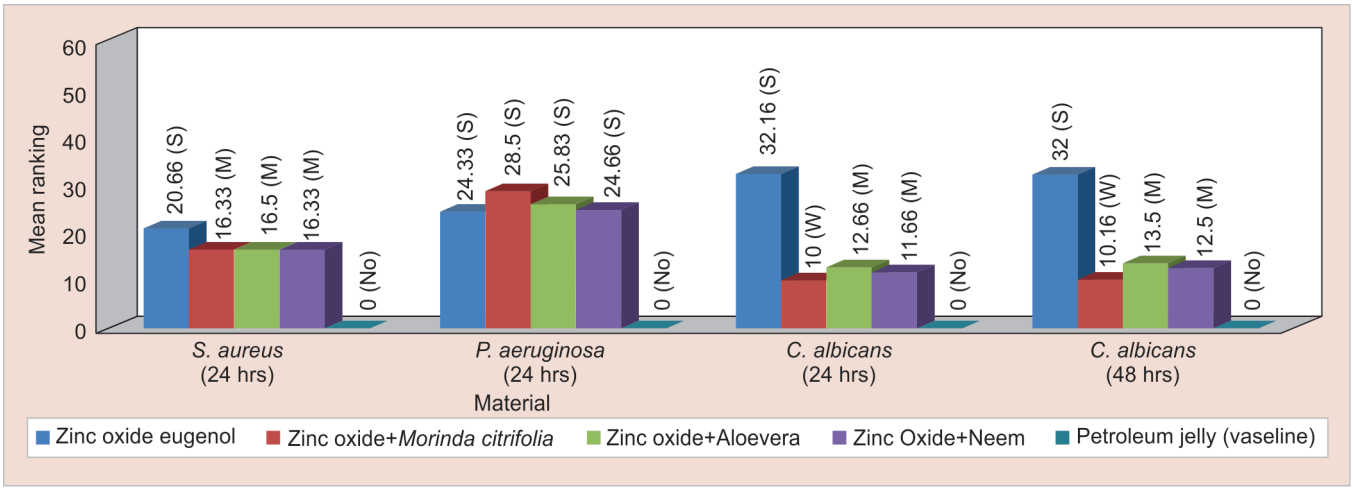

Fig. 5: Inhibition results of all five test filling materials against *S. aureus, P. aeruginosa*, and *C. albicans* bacterial strains according to the ranking scale.

Further clinical trials are required in order to know the specific antimicrobial efficacy of *M. citrifolia* extract, *A. vera* extract, and neem extract.

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