

## **Evaluation of Essential Oil–Modified Zinc Oxide Pastes for Antifungal Activity against *Candida albicans***

**Dr. Meenakshi V. Gaikwad, Dr. Pritam S. Dandekar, Dr. Neeraj A. Kulkarni, Dr. Ashwini, S. Kharat\***

Professor, Dept. of Pedodontics & Preventive Dentistry, Bharati Vidyapeeth Dental College, Sangli, Maharashtra, India.

Professor and Head, Dept. of Periodontology, Bharati Vidyapeeth Dental College, Sangli, Maharashtra, India.\*

Professor and Head, Dept. of Orthodontics, DY Patil Dental School, Pune, India.\*

Professor, Dept. of Microbiology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India.\*

### **Abstract**

**Background:** Endodontic treatment of primary teeth is required to eliminate microorganisms from the infected root canals having complex root canal anatomy.

**Objectives:** To assess the antifungal effect of zinc oxide paste with tea tree oil, thyme oil, peppermint oil on *Candida albicans* and to compare it with zinc oxide eugenol paste.

**Materials and methods:** Zinc oxide pastes containing essential oils used in the present study were zinc oxide tea tree oil paste (ZOT); zinc oxide thyme oil paste (ZOT<sub>h</sub>); zinc oxide peppermint oil paste (ZOP) which were compared with zinc oxide eugenol paste (ZO). Antifungal effect in the form of zones of inhibition was evaluated by agar diffusion method. Sabouraud's dextrose agar medium was used for evaluation of antifungal effect of *Candida albicans*. Statistical analysis was done using one way ANOVA and Tukey's post-hoc test with significance at the level of 5%.

**Results:** Antifungal effect in the form of zones of inhibition against *Candida albicans* found in decreasing order was ZOT<sub>h</sub> paste ( $50.00 \pm 0.00$ ) > ZOP paste ( $45.66 \pm 0.51$ ) > ZOT paste ( $44.33 \pm 0.51$ ) > ZOE paste ( $16.83 \pm 0.91$ ) respectively with difference found to be statistically significant ( $0.0001$ ,  $S, p < 0.05$ ).

**Conclusion:** ZOT<sub>h</sub> paste can be successfully used for root canal filling of deciduous teeth against *Candida albicans*.

### **Keywords:**

ZOT<sub>h</sub> paste, ZOT paste, ZOP paste, ZOE paste, *Candida albicans*.

### **Introduction**

Primary goal of endodontic treatment is to eliminate the microorganisms from the infected root canal system<sup>1</sup>. Complexity of primary root canals prevent the normal instrumentation and irrigation difficult to remove these microorganisms including fungi i.e; *Candida albicans*.

The most commonly found fungus in the cases of persistent apical periodontitis as concluded from various studies is *Candida albicans* in the range of 7-18%<sup>2</sup>. It is considered that *C. albicans* is a part of normal flora of oral cavity. Therefore there are high chances that it can be present in the infected root canals<sup>3-5</sup>. Virulence factors of *C. albicans* makes it survive in persistent apical periodontitis. *C. albicans* has properties like hyphal formation and thigmotropism which allows it to penetrate deep into dentine<sup>6</sup>.

Zinc oxide has been said to have limited antimicrobial effect<sup>7</sup>. Also it has certain disadvantages like slow resorption of material as compared to normal physiologic root resorption<sup>8,9</sup>. Medicinal plants have been successfully used as natural antimicrobial agents as reported its usage in many fields also including dental field<sup>10</sup>. Essential oils, called as volatile oils, are the aromatic oils obtained from different parts of the plants like buds, seeds, flowers, fruits, leaves, twigs, herbs, bark, wood and roots<sup>11</sup>. The antimicrobial effect of essential oils is due to a number of small terpenoids and phenol compounds<sup>12</sup>. Essential oils are reported to have its use in aromatherapy<sup>13</sup>, fragrance industries<sup>11</sup> and food preservation<sup>14</sup>. Essential oils defend against pathogens<sup>15</sup> and they have been considered to be safe<sup>16</sup>.

Considering the well documented benefits of tea tree oil, thyme oil and peppermint oil as available in the literature, present study was planned to find out the antifungal effect of zinc oxide based cement with tea tree oil, thyme oil, peppermint oil against *Candida albicans* and its comparison with zinc oxide eugenol.

## **Materials and methods**

Institutional ethical committee approval was taken before starting the study. Essential oils used in the study were tea tree oil, thyme oil, peppermint oil (Aromatantra, Mumbai).

Zinc oxide powder and eugenol of the department of Pedodontics and Preventive Dentistry was used (Prime Dental Products Pvt. Ltd., Thane). For all the materials studied, 1 scoop (0.2gm) of powder was taken and mixed with 7 drops of oil (0.07cc) on the dry and sterile glass slab using cement spatula to get the desired soft creamy consistency<sup>17</sup>.

Strain of *Candida albicans* used for the present study was obtained from the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. Strain of *Candida albicans* used for the study was ATCC 90028.

Sabouraud's dextrose Agar was used for evaluating the susceptibility of *Candida albicans*.

**Procedure:** Standard inoculum of *Candida albicans* was obtained by passing single colony of *Candida albicans* in nutrient broth. It was then incubated at 37°C for 4-6 hrs. turbidity was of broth was adjusted with McFarland 0.5 turbidity standard. Colonies of *Candida albicans* were picked up with the help of sterile cotton swab. By using lawn technique, microbial colonies were spread uniformly on Sabouraud's dextrose Agar. Holes of 6 mm diameter were punched at two equidistant points. Freshly mixed material was then filled in the punched holes. To ensure prediffusion of material in agar, plates were kept for 2 hrs. at room temperature. Whole experiment was six times repeated for each material. All the plates were kept in incubator at 37°C for 24 hrs. Diameter of zones of inhibition in millimeters around each test material was measured with the help of HiAntibiotic Zone Scale (HiMedia). Data of antibacterial activity was analyzed statistically using ANOVA and Tukey's post-hoc test at a significance level of 5% using the Graph Pad Prism 4 software.

## **Results**

Table 1 shows antifungal effect of ZOT, ZOT<sub>h</sub>, ZOP and ZOE in the form of zone of inhibition on *Candida albicans*. Diameters of zones of inhibition in mm for ZOT<sub>h</sub> paste were largest against *Candida albicans* i.e; 50.00±0.00. Zones of inhibition of ZOP paste against *Candida albicans* was 45.66±0.51. With ZOT paste, it was 44.33±0.51 and with ZOE paste it was 16.83±0.98 (Figure 1).

Table 2 shows Antifungal effect of ZOT, ZOT<sub>h</sub>, ZOP and ZOE in the form of zone of inhibition on *Candida albicans* by using One way ANOVA. Difference between and within groups was found to be statistically significant (p-value: 0.0001, p<0.05).

Table 3 shows antifungal effect of ZOT, ZOT<sub>h</sub>, ZOP and ZOE in the form of zone of inhibition on *Candida albicans* by using Multiple Comparison: Tukey Test. When antifungal effect of ZOT was compared with ZOT<sub>h</sub>, ZOP and ZOP paste, it was observed that difference was statistically significant (p-value: 0.0001, p<0.05).

Antifungal effect of ZOT<sub>h</sub> when compared with ZOP and ZOE paste, the difference was found to be statistically significant (p-value: 0.0001, p<0.05).

When ZOP was compared with ZOE paste for antifungal effect, the difference was found to be statistically significant (p-value: 0.246, p>0.05).

## **Discussion**

Root canal infections are polymicrobial in nature. *Candida albicans*, though are less in quantity of all the microorganisms, are found in infected root canals and are isolated from infected dental pulp<sup>18</sup>. Mechanical instrumentation and irrigation fail to remove this fungus from the complex structure of root canals of primary teeth.

It is widely known that the plant essential oils including tea tree oil, thyme oil, peppermint oil possess antimicrobial efficacy. Therefore in the present study antifungal effect of ZOT, ZOT<sub>h</sub> and ZOP paste was evaluated against *Candida albicans* and compared with the routinely used zinc oxide eugenol paste.

In the study of Amorim et al, (2006)<sup>19</sup>, the antimicrobial efficacy of root canal filling pastes used in pediatric dentistry i.e; Guedes-Pinto paste (GPP), calcium hydroxide paste (CHP), chloramphenicol + tetracycline + zinc oxide and eugenol paste (CTZP), zinc oxide-eugenol paste (OZEP) and Vitapex was evaluated against *S. aureus*, *P. aeruginosa*, *E. faecalis*, *B. subtilis* and *C. albicans*. It was concluded that all materials formed inhibition zones except Vitapex.

Cassanho et al, (2005)<sup>20</sup> had evaluated the antimicrobial activity of zinc oxide-eugenol cements and glass ionomer against *Candida albicans*. and concluded that ZOE cement was more effective in vitro against *Candida albicans* than GIC. But in the present study ZOE was least effective in showing antifungal effect against *C. albicans*.

Almeida et al, (2016)<sup>21</sup> had evaluated the anti-biofilm efficacy and anti-*Candida* effect of two essential oils from Cinnamon cassia (cinnamon) and *Cymbopogon winterianus* (citronella). It was concluded from the study that citronella and cinnamon essential oils have potential for daily anti-candidal denture cleansing. In the present study, plant essential oils used were tea tree oil, thyme oil and peppermint oil which showed good antifungal effect compared to zinc oxide eugenol paste against *Candida albicans* and can be used as root canal filling material for primary teeth effectively.

In the study of Egan et al, (2002)<sup>18</sup>, frequency of occurrence of yeasts in root canals was 10%. It was assumed in the same study that the presence of yeasts in root canals can be associated with its presence in saliva and role of yeasts in the initiation of periapical infection needs to be determined.

Study done by Baumgartner et al, (2000)<sup>22</sup> had evaluated the presence of *Candida albicans* from the aspirates of abscesses and cellulitis of endodontic origin and infected root canals by using PCR method concluded the study by finding the presence of *Candida albicans* in 5 of 24 samples which were taken from root canals i.e; 21%. They could not find the presence of *Candida albicans* in periradicular aspirates. Reason for this, given was that PCR being an extremely sensitive molecular method, may be used to identify *Candida albicans* in samples directly taken from the infections of endodontic origin.

Study of Kovac et al, (2013)<sup>5</sup> concluded that *Enterococcus faecalis* and *Candida albicans* can participate in root canal and periapical infections. Irrigating solutions and intracanal medicaments which effectively prevent endodontic therapy failures should be used. Unexpected finding observed in their study was the isolation of *Candida albicans* in the sample of apical periodontitis taken from a 9 year old child. So possibility of the presence of *Candida albicans* as a potential etiological agent in endodontic infections at young age can also be suspected.

In the present study, all the materials have shown antifungal effect, but the zones of inhibition obtained with ZOT<sub>h</sub> paste were highest i.e 50.00±0.00. Zones of inhibition obtained with ZOP paste were also higher i.e, 45.66±0.51 which were nearly comparable with the zones obtained with ZOT paste i.e; 44.33±0.51. Zinc oxide eugenol which is routinely used for root canal filling of primary teeth had shown minimal antifungal effect with zones of inhibition of 16.83±0.98. Hence zinc oxide based paste by using tea tree oil, thyme oil and peppermint oil can be the best option for evaluation of antifungal effect in pediatric root canal infections.

### **Conclusion**

Present study concludes that ZOT<sub>h</sub> paste was the best material which had shown larger zones of inhibition against *Candida albicans*. Other materials like ZOP paste and ZOTT paste also had shown better zones of inhibition against *Candida albicans*. Zinc oxide eugenol could not show antifungal effect against *Candida albicans* effectively. Antifungal effect against *Candida albicans* in decreasing order was ZOT<sub>h</sub>> ZOP> ZOT> ZOE. All these materials except zinc oxide eugenol can be successfully used as root canal filling material for primary teeth.

### **Acknowledgements**

Authors are thankful to the Head of the dept. of Microbiology for permission to work in their laboratory.

## References

1. Chua EG, Parolia A, Ahlawat P, Pau A, Amalraj FD. Antifungal effectiveness of various intracanal medicaments against *Candida albicans*: an ex-vivo study. *BMC Oral Health*. 2014;14:53.
2. Waltimo TM, Haapasalo M, Zehnder M, Meyer J. Clinical aspects related to endodontic yeast infections. *Endod Topics*. 2004;9:66–78. doi: 10.1111/j.1601-1546.2004.00086.x.
3. Najzar-Fleger D, Filipovic D, Prpic G, Kobler D. *Candida* in root canal in accordance with oral ecology. *Int Endod J*. 1992;25:40.
4. Zhang S, Wang QQ, Zhang CF, Soo I. Identification of dominant pathogens in periapical lesions associated with persistent apical periodontitis. *Chin J Dent Res Off J Sci Sect Chin Stomatol Assoc CSA*. 2010;13(2):115–21.
5. Kovac J, Kovac D, Slobodnikova L, Kotulova D. *Enterococcus faecalis* and *Candida albicans* in the dental root canal and periapical infections. *Bratisl Lekárske Listy*. 2013;114(12):716–20.
6. Ghogre P. Endodontic Mycology: A New Perspective of Root Canal Infection. *Research and Reviews: Journal of Dental Sciences* 2014; 2(1): 43-50.
7. Tchaou WS, Turng BF, Minah GE, Coll JA. Inhibition of pure cultures of oral bacteria by root canal filling materials. *Pediatr Dent*. 1996 Dec;18(7):444–9.
8. Mortazavi M, Mesbahi M. Comparison of zinc oxide and eugenol, and Vitapex for root canal treatment of necrotic primary teeth. *Int J Paediatr Dent Br Paedodontic Soc Int Assoc Dent Child*. 2004 Nov;14(6):417–24.
9. Ozalp N, Saroğlu I, Sönmez H. Evaluation of various root canal filling materials in primary molar pulpectomies: an in vivo study. *Am J Dent*. 2005 Dec;18(6):347–50.
10. Valera MC, Oliveira SA, Maekawa LE, Cardoso FG, Chung A, Silva SF, et al. Action of Chlorhexidine, Zingiber officinale, and Calcium Hydroxide on *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, and Endotoxin in the Root Canals. *J Contemp Dent Pract*. 2016;17(2):114–8.
11. Van de Braak SA, Leijten GC. Essential Oils and Oleoresins: A Survey in the Netherlands and Other Major Markets in the European Union. Rotterdam: CBI Centre for the Promotion of Imports from Developing Countries; 1999. p. 116.
12. Oussalah M, Caillet S, Saucier L, Lacroix M. Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. *Meat Sci*. 2006 Jun;73(2):236–44.
13. Buttner MP, Willeke K, Grinshpun SA. Sampling and analysis of airborne microorganisms. In manual of Environmental Microbiology. Edited by: Hurst CJ, Knudsen GR, McInerney MJ, Stezenbach LD, Walter MV. ASM Press 1996: Washington DC. Pp. 629-640.
14. Faid M, Bakhy K, Anshad M, Tantaoui-Elaraki A, Alomondpaste. Physiochemical and microbial characterizations and preservation with sorbic acid and cinnamon. *J. food Prod*. 1995; 58: 547-550.
15. Oxenham SK. Classification of an *Ocimum basilicum* germplasm collection and examination of the antifungal effects of the essential oil of basil. Ph.D. thesis 2003, Glasgow, UK, University of Glasgow.
16. Sawamura M. Aroma and functional properties of Japaneseyuzu (*Citrus junos* Tanaka) essential oil. *Aromatic Res* 2000;1:14–1.
17. Tchaou WS, Turng BF, Minah GE, Coll JA. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials. *Pediatr Dent*. 1995 Oct;17(5):351–5.
18. Egan MW, Spratt DA, Ng Y-L, Lam JM, Moles DR, Gulabivala K. Prevalence of yeasts in saliva and root canals of teeth associated with apical periodontitis. *Int Endod J*. 2002 Apr;35(4):321–9.
19. Amorim L de FG de, Toledo OA de, Estrela CR de A, Decurcio D de A, Estrela C. Antimicrobial analysis of different root canal filling pastes used in pediatric dentistry by two experimental methods. *Braz Dent J*. 2006;17(4):317–22.
20. Cassanho ACA, Fernandes AM, Oliveira LD de, Carvalho CAT, Jorge AOC, Koga-Ito CY. In vitro activity of zinc oxide-eugenol and glass ionomer cements on *Candida albicans*. *Braz Oral Res*. 2005 Jun;19(2):134–8.
21. Almeida L de FD de, Paula JF de, Almeida RVD de, Williams DW, Hebling J, Cavalcanti YW. Efficacy of citronella and cinnamon essential oils on *Candida albicans* biofilms. *Acta Odontol Scand*. 2016 Jul;74(5):393–8.
22. Baumgartner JC, Watts CM, Xia T. Occurrence of *Candida albicans* in infections of endodontic origin. *J Endod*. 2000 Dec;26(12):695–8.

**Table 1**

**Antifungal effect of ZOT, ZOT<sub>h</sub>, ZOP and ZOE in the form of zone of inhibition on *Candida albicans***

Zinc oxide based based pastes with essential oils	N	Mean	Std. Deviation	Std.Error	95% Confidence Interval for Mean	
					Lower bound	Upper bound
ZOT	6	44.33	0.51	0.21	43.79	44.875
ZOT <sub>h</sub>	6	50.00	0.00	0.00	50.00	50.00
ZOP	6	45.66	0.51	0.21	45.12	46.20
ZOE	6	16.83	0.98	0.40	15.80	17.86

**Table 2**

**Antifungal effect of ZOT, ZOT<sub>h</sub>, ZOP and ZOE in the form of zone of inhibition on *Candida albicans* by using One way ANOVA**

Source of variation	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	4110.45	3	1370.15	3653.74	0.0001 S,p<0.05
Within Groups	7.50	20	0.37		
Total	4117.95	23			

S: Significant

**Table 3**

**Antifungal effect of ZOT, ZOT<sub>h</sub>, ZOP and ZOE in the form of zone of inhibition on *Candida albicans* by using Multiple Comparison: Tukey Test**

Zinc oxide based based pastes with essential oils		Mean Difference (I-J)	Std.Error	p-value	95% Confidence Interval	
					Lower bound	Upper bound
ZOT	ZOT <sub>h</sub>	-5.66	0.35	0.0001,S	-6.65	-4.67
	ZOP	-1.33	0.35	0.006,S	-2.32	-0.34
	ZOE	27.50	0.35	0.0001,S	26.51	28.48
ZOT <sub>h</sub>	ZOP	4.33	0.35	0.0001,S	3.34	5.32
	ZOE	33.16	0.35	0.0001,S	32.17	34.15
ZOP	ZOE	28.83	0.35	0.0001,S	27.84	29.82

S: Significant

**Figure 1: Zones of inhibition with ZOT, ZOT<sub>h</sub>, ZOP and ZOE pastes against *Candida albicans***

