

An In Vitro Study On The Antimicrobial Efficacy Of Triticum Aestivum Extract On Enterococcus Faecalis And Candida Albicans

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Abstract

Aim: Evaluated Antimicrobial action of *Triticum aestivum* extract against *Enterococcus faecalis* and *Candida albicans*.

Objective: Investigated whether *Triticum aestivum* can be used as an alternative phyto-antimicrobial agent during root canal therapy.

Materials and Methods: Fifty milligram of the wheat grass (*T. aestivum*) powdered sample was weighed and added to Soxhlet apparatus for purification of extract. The filtrate was collected and poured into wide glass petriplate and kept for drying in hot air oven at 400°C. 100 µl of *E. faecalis* and *C. albicans* suspensions were taken from prepared cultures and inoculated in 130 mm culture plates with previously set layers of Mueller Hinton and Sabouraud dextrose agar respectively. Inoculations were performed using sterile spreader across the media. After that, 3 uniform wells of 6 mm diameter were made in the agar plates containing cultures of *E. faecalis* and *C. albicans*. 50 µl of *Triticum aestivum* extract and Control groups were added to the respective wells on each plate and were incubated at 37°C for 24 hours in an incubator.

Results: After incubation period, the plates were removed and the diameters of zones of inhibition of microbial growth attained by all three groups against the two tested organisms are recorded in millimeters. The results obtained conveyed that all the samples showed zones of inhibition of growth of microbes. Formocresol showed the highest inhibitory zone diameter (IZD) followed by *Triticum aestivum* extract. This study also shows that *Triticum aestivum* extract showed a higher inhibition zone diameter on comparing Calcium Hydroxide. Statistical significance are measured by using one-way ANOVA followed by Post Tukey B hoc test. P values <0.001 are considered statistically significant.

Keywords

Triticum aestivum, *E. faecalis*, *C. albicans*, zones of inhibition, one-way ANOVA, Post Tukey B hoc test.

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I. Introduction

Bacteria and their products play an essential role in the pathogenesis of pulpo-periapical diseases.¹ A long-standing endodontic infection permits bacteria to propagate to the entire root canal system, including ramifications, isthmuses, apical deltas and dentinal tubules. In contemporary endodontics, chemomechanical preparation associated with antiseptic medication has been recommended for infection control.¹ Despite this, residual microorganisms may persist in root canals.¹

The main objective of endodontic treatment is to eliminate bacteria from the root canal system and to prevent them from infecting or re-infecting the root canal or the peri-apical tissues.⁴ *Enterococcus faecalis* is one of the most prominent bacterial species isolated from root canals of treatment failed teeth.⁴ Studies have shown *E. faecalis* in 30%–89% of teeth with post endodontic treatment failures, mostly as monoculture.⁵ *Candida albicans* have been associated with secondary or persistent endodontic infections.³

E. faecalis are gram positive cocci and facultative anaerobes.⁴ They are normal intestinal organisms and may inhabit the oral cavity and gingival sulcus.⁴ The most commonly isolated species from the secondary

infection of the root canal system is *Enterococcus faecalis* and is reported to be present in more than 60% of the re-infection cases.⁷ Studies have suggested that resistance incorporated in *E. faecalis* against commonly used intracanal medicaments is because of its ability to form biofilm.¹ Biofilm is a complex extracellular polymeric matrix that protects the bacteria against nutrient deprived and other unfavorable conditions, such as high alkaline and salt concentrations, created by intracanal medicaments.²

Culture studies have indicated the presence of multiple flora in secondary infection of the root canal system rather than single microorganism.⁸ Apart from *E. faecalis*, the other commonly isolated microorganism is *Candida* species.³ Among the *Candida* species, *Candida albicans* is the most commonly isolated species in secondary root canal infection. However, because of the increasing evidence suggestive of biofilm-mediated resistance of the microflora toward the commonly used intracanal medicaments, a greater emphasis is now being laid in developing materials that can disrupt the biofilm and eliminate the microorganisms from the root canal system.³

C. albicans is a polymorphic fungus that exists in blastomere form, germ tube form, hyphae form and chlamyospore form depending on environmental conditions³. They can adapt to extreme range of pH, low oxygen and nutritional environment. They are more commonly associated with persistent cases of apical periodontitis. They have a number of virulence factors required for tissue penetration. It was hypothesized that *C. albicans* may survive and infect the periradicular tissues.⁷

Antimicrobial peptides (AMPs) are an ancient mechanism of defense against pathogens in prokaryotes and eucaryotes (Castro, Fontes, 2005; Manners, 2007; Farrokhi et al., 2008; Sels et al., 2008; Tavares et al., 2008; Ajesh, Sreejith, 2009; Benko_Iseppon et al., 2010; da Rocha Pitta et al., 2010; Kido et al., 2010; Padovan et al., 2010).²

The concept of participation of AMPs in plant defense is based on experimental facts, such as antimicrobial activity in vitro, upregulation of the corresponding genes in response to biotic and abiotic stress, and an increase in pathogen resistance of transgenic plants constitutively expressing AMPs (Broekaert et al., 1997; Garcia_Olmedo et al., 1998, 2001).²

The precise mechanisms of pathogen inhibition by plant AMPs are not yet clear. Most of the data available suggest that they cause injury to a pathogen's membrane, formation of pores, and damage to barrier function. There are also data showing that some AMP's hit intracellular targets in microorganisms (Lobo et al., 2007; van der Weerden et al., 2008).²

Wheat grass, *Triticum aestivum* has a long history and is widely used as a health food supplement. The plant *Triticum aestivum* belonging to family *Gramineae*² is an easily grown plant, the young stems had proven effect for the treatment of biliousness, intoxication, remove skin blemishes. The fruit has antipyretic, antihydrotic and sedative.² *T. aestivum* have been also used against cough, sore throat, malaise, spasmic pain and abdominal coldness, constipation and. The plant is also known to have anticancer and anti-microbial properties.² It is found to be used as a treatment for minor ailments and serious life threatening issues. Another interesting quality is the antioxidant potential of wheat grass extract in particular its superoxide dismutase (SOD) content. This enzyme has gained much more attention in recent years with regard to its ability to inhibit cell mutation. The therapeutic activity of wheat grass is due to the presence of the nutritional content like chlorophyll, vitamin A, vitamin C and vitamin and iron biflavonoids mineral like calcium and magnesium.²

It consists of sixteen amino acids of which eight are essential. Although the therapeutic activity of wheat grass has approved for four decades before but it was not in use before because of the lack of powerful clinical data to support the therapeutic activity.²

According to Ladusingh Rajpurohit, stable *T. aestivum* (wheat grass) exhibited maximum antimicrobial activity against *S. mutans* 1.25% and *Lactobacillus* spp 5%¹.

Despite the diversity of amino acid sequences, Anti Microbial Peptides share some physical and chemical properties, such as low molecular weight, positive charge of molecules and amphiphilic structure. These properties enable AMPs to interact with cell walls and membranes of microorganisms and damage their permeability, thus demonstrating antimicrobial activity.²

Triticum aestivum was found to consist of four structurally different glycine rich peptides that did not contain cysteine residues in their primary structures. Apart from a large number of glycine residues, these peptides were characterized by the presence of oligopeptide repeats, which differed between subfamilies, such as GnYPGN in peptides TkAMPG1 and TkAMPG2 or GnYP in peptides TkAMPG3 and TkAMPG4, where n is a variable number.² Analysis of the antifungal activity of these peptides against several plant pathogenic fungi demonstrated that, at a concentration of 100–150 µg/mL, glycine rich peptides caused morphological changes in fungi.²

'Defensins' have been found in all living organisms and, therefore, are considered to be the most ancient AMP family. Seeds of *T. kiharae* were found to contain 11 defensins, which were divided into three structural groups on the basis of Nterminal sequence homology: TkAMPD (group I); TkAMP γ 1, TkAMP γ 2, and TkAMP γ 3 (group II); and TkAMP ω 2 and AMP ω 3 (group III).²

Thus, the purpose of this study was to evaluate invitro the action of *Triticum aestivum* extract on *Enterococcus faecalis* and *Candida albicans*.

AIM OF THE STUDY :

Evaluated antimicrobial action of *Triticum aestivum* extract against *Enterococcus faecalis* and *Candida albicans*.

OBJECTIVE OF THE STUDY

Investigated whether *Triticum aestivum* extract can be used as an alternative phyto-antimicrobial agent during root canal therapy.

II. Materials Required

Materials :

Triticum aestivum extract

Calcium Hydroxide (DEEPTI DENTAL , Maharastra, INDIA)

Formocresol (PHARMADENT REMEDIES , Gujarat , INDIA)

Microbial strain and medium :

Candida albicans (MTCC 10231) - Sabouraud dextrose agar

Enterococcus faecalis (MTCC 2921) - Mueller Hinton Brain heart infusion broth

METHODOLOGY :

Method of collection of data :

Group 1 – *Triticum aestivum* extract

Group 2 – Calcium Hydroxide (Control 1)

Group 3 – Formocresol (Control 2)

Preparation of extracts :

The *Triticum aestivum* (wheat crop) was cultivated and wheat grass was removed from the crop and was shade dried for 5-7 days and grinded into powder. Fifty milligram of the wheat grass (*T. aestivum*) powdered sample was weighed and added to soxhlet apparatus for purification of extract . The filtrate was collected and poured into wide glass petriplate and kept for drying in hot air oven at 400°C.

Agar diffusion test :

100 µl each of *E.faecalis* and *C.albicans* suspensions are taken from prepared cultures and inoculated in 130 mm culture plates with previously set layers of Mueller Hinton and Sabouraud dextrose agar respectively. Inoculations are performed using sterile spreader across the media. After that, 3 uniform wells of 6 mm diameter are made in the agar plates containing cultures of *E. faecalis* and *C.albicans* . 50µl of *Triticum aestivum* extract and the Control groups are added to the respective wells on each plate and are incubated at 37°C for 24 hours in an incubator. After incubation period, the plates are removed and the diameters of zones of inhibition of microbial growth attained by all three groups against the two tested organisms are recorded in millimeters.

III. Results

The results obtained conveyed that all the samples showed zones of inhibition of growth of microbes. Formocresol showed the highest inhibition zone diameter (IZD) followed by *Triticum aestivum* extract. This study also shows that *Triticum aestivum* extract showed a higher inhibition zone diameter on comparing Calcium Hydroxide.

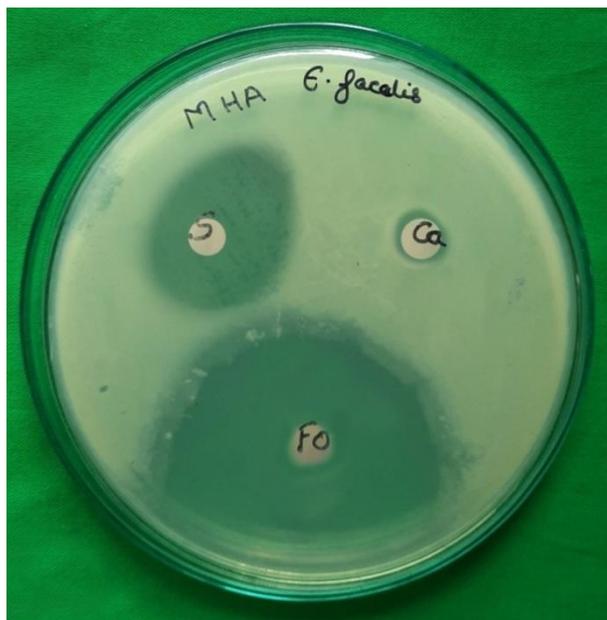


Fig 1: Mean Zone of Inhibition (in mm) between the samples for E. faecalis Organism

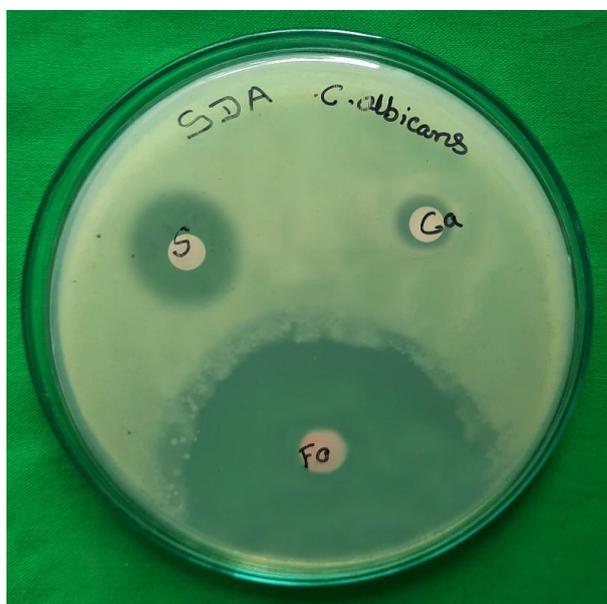


Fig 2: Mean Zone of Inhibition (in mm) between the samples for C.albicans Organism

E.faecalis	N	IZD (mm)				ANOVA	p
		Min	Max	Mean	SD		
Group I	5	19	23	21.60	1.67	667.65	0.001**
Group II	5	8	10	9.00	1.00		
Group III	5	46	52	49.40	2.41		

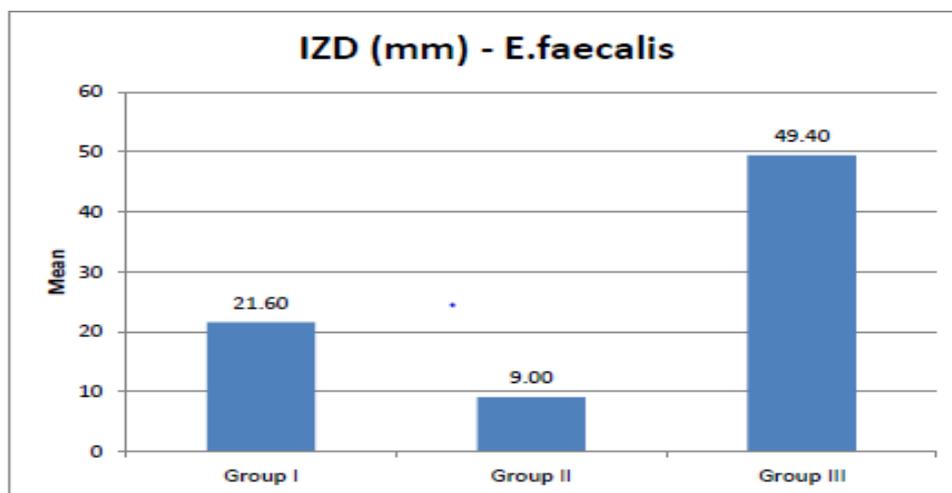
** Highly significant

Table 1: Comparison of mean Inhibitory Zone Diameter (in mm) between the samples using Oneway ANOVA tests for *Enterococcus faecalis* organism

Tukey B Post Hoc Tests - IZD (mm)

Group	N	Subset for alpha = .05		
		1	2	3
Group II	5	9.00		
Group I	5		21.60	
Group III	5			49.40

Table 2: Comparison of mean Inhibitory Zone Diameter (in mm) between the samples using Tukey B Post Hoc Tests for *Enterococcus faecalis* organism



Graph 1: Comparison of mean Inhibitory Zone Diameter (in mm) between the samples for *E. faecalis* Organism

C.albicans	N	IZD (mm)				ANOVA	p
		Min	Max	Mean	SD		
Group I	5	17	21	19.20	1.48	2164.72	0.001**
Group II	5	9	11	10.00	0.71		
Group III	5	57	60	59.00	1.41		

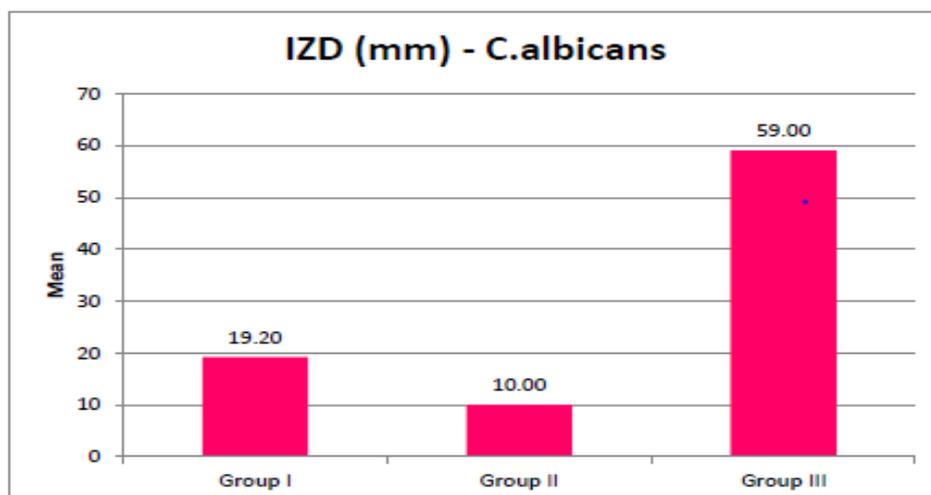
** Highly significant

Table 3: Comparison of mean Inhibitory Zone Diameter (in mm) between the samples using Oneway ANOVA tests for *Candidaalbicans* organism

Tukey B Post Hoc Tests - IZD (mm)

Group	N	Subset for alpha = .05		
		1	2	3
Group II	5	10.00		
Group I	5		19.20	
Group III	5			59.00

Table 4: Comparison of mean Inhibitory Zone Diameter (in mm) between the samples using Tukey B Post Hoc Tests tests for *Candidaalbicans* organism



Graph 2: Comparison of mean Inhibitory Zone Diameter (in mm) between the samples for C.albicans Organism

IV. Discussion

Numerous studies prove that microbes play a vital role in persistent radicular infections. The microorganisms have the potential to survive in harsh conditions even in the absence of nutrition, blood or oxygen.

E. faecalis is mainly associated with persistent periradicular lesions after root canal treatment. *E. faecalis* and yeast, mainly *C. albicans*, has been repeatedly identified as the species most commonly recovered from root canals undergoing retreatment, in cases of failed endodontic therapy and canals with persistent infections. *E. faecalis* are gram positive cocci and facultative anaerobes. They are normal intestinal organisms and may inhabit the oral cavity and gingival sulcus. When this bacterium is present in small numbers, it is easily eliminated; However, if it is in large numbers, there is difficult in eradication. (Ashraf et al., 2007) ³.

C.albicans, is a polymorphic fungus that exists in blastomeres, germ tubes, true hyphae, pseudohyphae and chlamydo spores depending on environmental conditions which helps in survival.⁽⁵⁾ They can adapt to extreme range of pH, low oxygen and nutritional environment. They are more commonly associated with persistent cases of apical periodontitis. They have a number of virulence factors required for tissue penetration. It was hypothesized that *C.albicans* may survive and infect the periradicular tissues. (Jay Kumar et al., 2015) ⁵.

Disinfection of the pulp is an important step during and after cleaning and shaping. Cleaning and shaping generally involves the use of endodontic instruments along with irrigants. However, in certain clinical conditions, the polymicrobial nature of the endodontic infection demands the use of an intracanal medicament in addition to the irrigants. (Grossman's endo practice-12th ed)

Fifty milligram of the wheat grass (*T. aestivum*) powdered sample was weighed and added to soxhlet apparatus for purification of extract. The filtrate was collected and poured into wide glass petriplate and kept for drying in hot air oven at 400°C. 3 uniform wells of 6 mm diameter are made in the agar plates containing cultures of *E. faecalis* and *C.albicans*. 50µl of *Triticum aestivum* extract and the Control groups are added to the respective wells on each plate and are incubated at 37°C for 24 hours in an incubator. After incubation period, the plates are removed and the diameters of zones of inhibition of microbial growth attained by all three groups against the two tested organisms are recorded in millimeters. The results obtained conveyed that all the samples showed zones of inhibition of growth of microbes. Formocresol showed the highest inhibitory zone diameter (IZD) followed by *Triticum aestivum* extract (Graph 1, Graph 2). This study also shows that *Triticum aestivum* extract showed a higher inhibition zone diameter on comparing Calcium Hydroxide.

The chemical composition of *Triticum aestivum* is highly complex, containing many nutrients and other biological active compounds, the proportion of which may vary considerably between strains and even between the plants of the same field. (Shirude AA et al., 2011)¹¹.

Phytochemical analysis of *T. aestivum* extracts have shown the presence of bioactive components like anti microbial peptides- defensins and glycine rich peptides, terpenoids, flavonoids, glycosides, alkaloids, tannins and saponins with neutral pH 7 contributing to its anti-microbial activity. (Ladusingh rajpurohit et al., 2015). ¹ According to Ladusingh Rajpurohit et al., 2015 he showed that stable *T. aestivum* (wheat grass) exhibited maximum anti-microbial activity against *S. mutans* 1.25% and *Lactobacillus* spp 5% ¹.

Formocresol, is been used as a medicament for pulpotomy procedures in primary teeth. It is a non specific bactericidal medicament that is effective against aerobic and anaerobic organisms. They are composed mainly of formaldehyde – 19% , cresol - 35% , water and glycerin – 46%

However, its toxic and mutagenic properties are of concern. It is a strong disinfectant which combines with albumin to form an insoluble, non decomposable substance. It was tested against living tissue – necrosis was followed by persistent inflammatory reaction was evident(Black, Grossman, Schilder & Amsterdam).Formocresol treated tissue produces a cell mediated immune response (Block & Associates)¹³.

Calcium Hydroxide was introduced by Herman in 1920. It is one of the commonly used Intracanal medicament. It is a broad spectrum anti microbial agent. It acts as a physical barrier for ingress of bacteria. Its antiseptic action probably relates to its high alkaline ph(12.5) and its leaching action on necrotic pulp tissue. The lethal effects on bacterial cell are probably due to damage to bacterial cytoplasmic membrane, Protein denaturation and damage to DNA(Raul A et al., 2014)¹⁴.

Calcium Hydroxide inhibits root resorption, stimulates periapical healing, encourage mineralization and is best used in ‘Weeping canals’ associated with large periapical lesion and in open apex cases.However the disadvantages of calcium hydroxide include difficulty to remove from root canals and residual $Ca(OH)_2$ + ZOE = Calcium Eugenolate, which is more soluble, less sealing, more film thickness higher water sorption value than the original sealer which could weaken the sealer in long term.(Anderson JO et al., 2002)¹⁴.

V. Conclusion

Within the limitations of the study, it can be concluded that *Triticum aestivum*(wheat grass) extract, has significant antimicrobial action against *Enterococcus faecalis* (21.60mm)^{Tab2} and *Candida albicans* (19.20 mm)^{Tab4} when compared with calcium hydroxide (9.0 mm and 10.0 mm)^{Tab2,4} but it is not greater than that of Formocresol (49.40 mm and 59.0 mm)^{Tab2,4}.

However *Triticum aestivum* can definitely be suggested as Intra canal medicament in weeping canals, periapical abscess and open apex cases and is contraindicated in patients with known “gluten allergy”. Where calcium hydroxide can be used as the intra canal medicament of choice.

Hence, it may be suggested that further research is needed to deeply investigate the antimicrobial action of *Triticum aestivum* on other numerous microbes prevailing in the infected root canal. Until then it can be used as an adjunct medicament in indicated cases of root canal therapy.

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CONFLICT OF INTEREST :

None.

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