



# COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF 5% ALLIUM SATIVUM, 5% SODIUM HYPOCHLORITE AND MTA AS PULPOTOMY MEDICAMENTS IN PRIMARY TEETH: AN IN VITRO STUDY.”

<sup>1</sup>Dr. Jalpa Solanki, <sup>2</sup>Dr. Vikram Jhamb, <sup>3</sup>Dr. Shitalkiran D.P., <sup>4</sup>Dr. Shalin Shah. <sup>5</sup>Dr. Malay Trivedi

<sup>1</sup>Post Graduate student, <sup>2</sup>Reader, <sup>3</sup>Professor and Head, <sup>4</sup>Senior lecturer, <sup>5</sup> Senior Lecturer

<sup>1</sup>Department of Pediatric and Preventive Dentistry,

<sup>1</sup>College of Dental Science, Amargadh, Bhavnagar, India

**Abstract :** **Aim:** The Aim of the study was to compare and evaluate the antimicrobial activity of Allium sativum, mineral trioxide aggregate (MTA) and Sodium hypochlorite against E. faecalis and S. aureus as a pulpotomy medicament in primary teeth. **Objective:** To compare and evaluate the antibacterial activity of herbal plant extract allium sativum oil (garlic oil), mineral trioxide aggregate (MTA) and sodium hypochlorite (Naocl) against E. faecalis and S. aureus as a pulpotomy medicament in primary teeth. **Material and method:** Total 120 samples were taken and divided into three groups. Group – 1 was taken as a control group with 24 samples in which 5% Sodium hypochlorite was used. Group - 2 was taken as experimental group with 72 samples which were further subdivided into 3 groups of 24 samples each in which 5%, 50%, 80% Allium sativum oil was used and group – 3 was taken as experimental group with 24 samples in which Mineral trioxide aggravates (MTA Angelus) was used, as pulpotomy medicament in primary teeth using an agar diffusion method. Antimicrobial activities of the 5% Sodium hypochlorite, 5%, 50%, 80% Allium sativum oil and Mineral trioxide aggravates was evaluated against staphylococcus aureus and Enterococcus faecalis using agar diffusion method. All the microbial strains were grown at 37°C at 24 hours in Mueller-Hinton broth. The plates were kept at room temperature for 2 hours for the pre diffusion of materials and then Incubated at 37°C for 24 hours and 48 hours respectively. After that, inhibition zones were measured after 24 and 48 hours by using a digital calliper. Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the well. **Result:** Statistical analysis was done by one way analysis of variance to check the difference between the group and post hoc analysis was done by turkey's test. The result showed that the comparison of Staphylococcus aureus after 24 hours between the five groups was statistically significant in which p value was <0.001 and after 48 hours comparison was not Significant with a statistics p value of 0.207 and in Enterococcus Faecalis group comparison between five groups after 24 hours showed statistically Significant result with a test value of <0.001 and after 48 hours this difference was not statistically significant with a test value was 0.135. **Conclusion:** In the present study we observed that 50% and 80% allium sativum, MTA and 5% sodium hypochlorite have antimicrobial activity against Staphylococcus aureus and Enterococcus faecalis. However, the concentration of 5% allium sativum is not so effective on the Enterococcus faecalis. So garlic extract in the concentration of 50% and 80% could be an alternative to commercially available pulpotomy agent due to its cost effectiveness and minimum side effects.

**IndexTerms -** Pulpotomy, Allium Sativum, MTA

## INTRODUCTION

Pulpotomy is the recommended treatment procedure for primary teeth with exposed coronal pulps inflamed by bacteria due to caries, traumatic injury and any other iatrogenic cause.<sup>1</sup> Pulpotomy is done to remove infected coronal pulp tissue without removal of healthy radicular pulp, thereby preserving the vascularity and space for the succedaneous of teeth until physiologic exfoliation.<sup>2</sup>

Different pulpotomy medicaments used now a days in dentistry for the devitalisation, preservation and regeneration of the remaining radicular pulp tissue.<sup>2</sup> The ideal pulpotomy medicament would be bactericidal and biocompatible, it should be able to promote the healing of the remaining pulpal tissue and be resorbable with the physiological process of root resorption.<sup>3</sup>

There are different pulpotomy agents that are used with better clinical efficacy and without secondary effects are calcium hydroxide, ferric sulfate (FS), mineral trioxide aggregate (MTA), sodium hypochlorite, electrosurgery and laser therapies have been used for pulpotomies.

Now a days, in dentistry Natural products and traditional medicines used as a pulpotomy medicament and in irrigation procedure due to their antibacterial activity. Modern medicine may not be the sole antidote for the ailments prevailing today. Therefore, people positively perceive 'back to nature' like phytotherapy as plant products are approaching now a days rich in pharmaceuticals. *Allium sativum*, known as garlic, which come from the family Amaryllidaceae.<sup>4, 5, 6</sup>

Garlic, has antibacterial properties against oral pathogens and can treat periodontal disease, dental caries and oral cancer.<sup>7</sup> In dentistry, use of *allium sativum* as a disinfective agent on dental plaque and calculus, which are the critical etiology for the gingivitis and periodontitis. Caries is one of the most common oral diseases widespread globally in every segment of population and it's development is influenced by dietary components which act on the etiological agents *streptococcus sobrinus* and *streptococcus mutans*.

The survey of the literature has revealed that no studies have been conducted on the anti-bacterial activity of 5%, 50% and 80% *allium Sativum* oil and compare the same with existing materials mineral trioxide aggregate (MTA) and sodium hypochlorite, against commonly found root canal pathogens like *E. Faecalis* and *S. aureus*.

## MATERIAL AND METHODOLOGY

This vitro study was done in Department of Microbiology in Government Medical College Bhavnagar, Gujarat, India. The study performed for the period of 2 years (from 31 December 2020 to 31 December 2022) and Ethical approval was obtained from institutional Ethics Committee (IEC) (ECR/1189/INST/GJ/2019) (REF NO. CODS/IEC/92/2020). Study permission was taken from the Department of Microbiology in Government Medical College, Bhavnagar, Gujarat, India.

### SAMPAL SIZE CALCULATION

The total sample size was determined with error of 5% with power 95%. Therefore, the minimum required sample size set at 120.

Inclusion criteria:

- 1) Freshly prepared garlic oil
- 2) 5%, 50% and 80% *allium Sativum* oil (garlic oil).

Exclusion criteria:

- 1) Agar plates with inconsistent cavities.
- 2) Unsterile agar media.

### PREPARATION OF ALLIUM SATIVUM OIL

- Fresh natural garlic seeds were brought from vegetable market and outer covering of garlic seeds were peeled and cleaned.
- After that Garlic seeds were blended with a sterilized mortar and pestle and garlic extract was prepared.
- Garlic extract was centrifuged at 12,000 rpm for 10 min and filter the 100% garlic extract and it was stored at - 20°C until use. Preparation of 5%, 50% & 80% *allium sativum* oil was done by diluting 0.05g, 0.5g, and 0.8g in 10ml distilled water, to make 5%, 50% & 80% garlic oil accordingly.

In this study total sample size were taken 120 and randomly divided in to three group as follows:

Group-1 taken as a control group with 24 samples in which 5% Sodium hypochlorite is used.

Group - 2 taken as experimental group with 72 samples in which 5%, 50%, 80% Allium sativum oil is used.

Group - 3 taken as experimental group with 24 samples in which Mineral trioxide aggravates (MTA Angelus) is used.

### STUDY DESIGN

In this study double-layered agar diffusion plates were taken, in which 10ml of the base layer was made of sterilized Mueller – Hinton (MH) agar and poured in 2 × 10 cm sterilized Petri plates. Uniform cavities (5mm diameter, 1mm for each) were punched at equidistant points in agar by means of a sterile copper coil after 24 hours [figure 1]. The medium was sterilized by autoclaving at 121°C for 15 min and left it to be cool.

The strains were obtained from Department of Microbiology, Government Medical Collage, Bhavnagar, Gujarat, India. After activation from stock culture, microorganism were maintained in MH broth until used. All the microbial strains were grown at 37°C for 24 hours in MH broth. Prepared cavities were fill immediately with 5% sodium hypochlorite, 5%, 50%, 80% Allium sativum oil and Mineral trioxide aggravates, and turbidity was measured on MC Farland scale and the seeded agar was added over the plates immediately after the insertion of freshly test materials, then were incubated at 37°C for 24 hours and 48 hours respectively. Plates were kept at room temperature for 2 hours for the pre diffusion of materials and antimicrobial activities of the 5% Sodium hypochlorite, 5%, 50%, 80% Allium sativum oil and Mineral trioxide aggravates was evaluated against staphylococcus aureus and Enterococcus faecalis by measuring inhibition zone at 24 and 48 hours using a digital calliper [figure 2, 3]. Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the well.

### Statistical analysis

Statistical analysis was done by one way analysis of variance was used to check the antimicrobial efficacy between groups. Post hoc analysis was done by tukey's for the intergroup comparison of antimicrobial efficacy. Data was analysed by the IBM SPSS statistics for windows, version 23. A P- value of less than 0.05 was considered as statistically significant.

### RESULTS AND DISCUSSION

[ Table 1 and Graph 1 ] showed after 24 hours comparison of Staphylococcus aureus between the five groups using one way ANOVA test showed that the mean values of inhibition zone were 80% Allium Sativum was the highest followed by MTA, 5% Sodium hypochlorite, 50% Allium Sativum and least in 5% Allium Sativum. This comparison was Significant with a statistics of 80.92 and p value of <0.001.

After 48 hours the comparison of Staphylococcus aureus between the five groups showed mean values of inhibition zone with 80% Allium Sativum was the highest followed by MTA, 50% Allium Sativum, 5% Sodium hypochlorite and least in 5% Allium Sativum. This comparison was Significant with a statistics of 87.783 and p value of <0.001.

[Table 2, Graph 2] Comparison of Staphylococcus aureus between the five groups showed that the mean values of inhibition zone with 80% Allium Sativum was the highest followed by MTA, 50% Allium Sativum, 5% Sodium hypochlorite and least in 5% Allium Sativum. This comparison was Significant with a statistics of 1.526 and p value of 0.207.

[Table 3 and Graph 3] After 24 hours the Comparison of Enterococcus Faecalis at 24 hours using one way ANOVA test showed that the mean value of inhibition zone with MTA (14.691667) was highest followed by 5% Sodium hypochlorite (11.566667), 80% Allium Sativum (9.391667), 50% (5.15) Allium Sativum and least in 5% Allium Sativum. This difference was statistically Significant with a test value of 83.696 and p value of <0.001.

After 48 hours the Comparison of Enterococcus Faecalis using one way ANOVA test showed that the mean value of inhibition zone with MTA (15.916667) was highest followed by 5% Sodium hypochlorite (12.566667),80% Allium Sativum (10.541667), 50% (5.9) Allium Sativum and least in 5% Allium Sativum. This difference was statistically Significant with a test value of 23.532 and p value of <0.001.

[Table 4 and Graph 4] The Comparison of Enterococcus Faecalis between groups using one way ANOVA test showed that the mean value of inhibition zone with MTA (1.225) is highest followed by 80%



Allium Sativum (1.15), 5% Sodium hypochlorite (1), 50% (0.75) Allium Sativum and least in 5% Allium Sativum. This difference was statistically not Significant with a test value of 1.951 and p value of 0.135.

#### Discussion:

Pulpotomy is the most common treatment for primary teeth with coronal pulp involvement due to caries. This treatment helps to maintain the integrity and function of the dental arch.<sup>8</sup> It makes sense to substitute a safe material for formocresol, a frequent devitalizing substance used in pulpotomy, because of its unfavourable side effects such cytotoxicity, mutagenicity and carcinogenicity.<sup>9</sup>

This study was done to evaluate and compare the antimicrobial efficacy of different pulpotomy material like Allium sativum in concentration of 5%, 50% and 80%, 5% sodium hypochlorite and MTA against Staphylococcus aureus and Enterococcus faecalis in primary teeth by using disk diffusion method.

Allium sativum oil was discovered to have strong anti-inflammatory and analgesic characteristics and it is used as traditional medicine to cure toothache without causing any negative side effects. The analgesic effect might be due to ajoene and diallyl sulphide which inhibit prostaglandin. The anti-inflammatory effect of Allium sativum has been reported by several investigators.<sup>10</sup> Allium sativum extract inhibits the growth of numerous pathogenic bacteria, viruses and fungi. It has been documented that garlic extract has an inhibiting effect on Streptococcus mutans strains that isolated from human carious teeth.<sup>11</sup>

A Saeed (2007)<sup>12</sup> concluded that Calcium enriched mixture cement has an effective antibacterial properties that are comparable to calcium hydroxide and much superior to MTA group. As similar study in which antimicrobial activity of MTA was reported by Torabinejad (1995)<sup>13</sup> who discovered its effectiveness against a few facultative bacteria; no activity was discovered for E. faecalis, S. aureus, B. subtilis, or E. coli or against anaerobic bacteria. Estrela (1995)<sup>14</sup> demonstrated that MTA did not reveal any antimicrobial activity against S. aureus, E. faecalis, P. aeruginosa, B. subtilis and C. albicans. Our study was contrast to this study that MTA gives the superficial effect against the E. faecalis and S. aureus at 24 and 48 hours period time. This difference is statistically Significant with a test value of 83.696 and p value of <0.001.

Devaraju R (2022)<sup>15</sup> done a study in which the mean zone of inhibition produced was highest for 100% A. sativum (13 ± 0.50 mm), 50% A. sativum (11 ± 0.1mm) and 25% A. sativum (9 ± 0.1mm). The recorded antimicrobial activity of A. sativum was less when compared to formocresol which was used as a positive hours. In our study mean zone of inhibition produced was highest against S. aureus by 80% A. sativum followed by the 50% and 5% A. sativum inhibition zone were 12.63mm, 10.11mm, 2.091mm respectively and against E. Faecalis inhibition zone of 80% , 50% and 5 % A. sativum inhibition zones were 9.39mm, 5.15mm, 0.00mm respectively. This finding is similar with study done by Shukry Gamal Mohammad (2020)<sup>16</sup> in which better results were obtained when A. sativum oil was used as a pulpotomy medicament in primary teeth. According to Kallel (2014)<sup>17</sup> A. Sativum ethanol extract had moderate antibacterial activity against B. subtilis and S. aureus, which were 10–15mm and low-level activity against B. thuringiensis and P. aeruginosa, which were <10 mm.

In present study we compared the antimicrobial efficacy of 5% , 50% and 80% allium sativum, MTA and 5% sodium hypochlorite in this disk diffusion test, after the 24 hours higher inhibition zone was seen in 80% allium sativum as compared to MTA, 5% sodium hypochlorite and 5% , 50% allium sativum. However, it was not in killing all bacteria in the inhibition zone as compared to the MTA and 5% sodium hypochlorite.

After 48 hours higher inhibition zone was seen in the 80% allium sativum followed by the MTA, 50% allium sativum, 5% sodium hypochlorite and 5% allium sativum, it means the 50% and 80% allium sativum can be used as a pulpotomy agent.

The 50% and 80% allium sativum as a pulpotomy agent seen to be more effective against Staphylococcus aureus after 48 hours but less effective on Enterococcus faecalis and MTA found to be more effective against Enterococcus faecalis after 48 hours disk diffusion test.



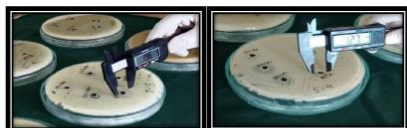
[Figure N] Prepared Figure 1 sterilized copper coil in agar plates in *Staphylococcus aureus* group samples



[Figure S] Inhibition zone of 5% Sodium hypochlorite and 50 % Allium Sativum oil after 24 hours in *Staphylococcus aureus* group samples



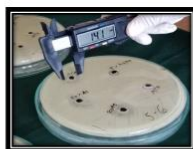
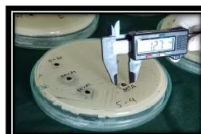
[Figure X] Inhibition zone of 5% Sodium hypochlorite and 50 % Allium Sativum oil after 48 hours in *Staphylococcus aureus* group samples



[Figure T] Inhibition zone of 80 % Allium Sativum oil and MTA after 24 hours in *Staphylococcus aureus* group samples



[Figure Y] Inhibition zone of 80 % Allium Sativum oil and MTA after 48 hours in *Staphylococcus aureus* group samples

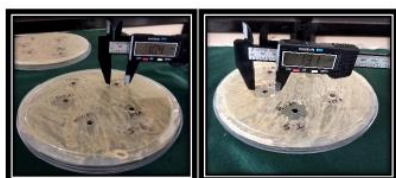


[Figure U] Inhibition zone of 5% Allium Sativum after 24 hours in *Staphylococcus aureus* group samples

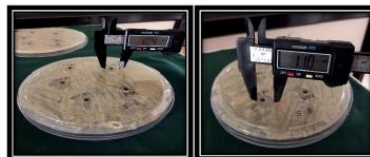


[Figure Z] Inhibition zone of 5% Allium Sativum after 24 hours in *Staphylococcus aureus* group samples

[Figure 2] After 24 and 48 hours incubation zone of 5%, 50%, 80% allium sativum, MTA and 5% sodium hypochlorite in *Staphylococcus aureus* group



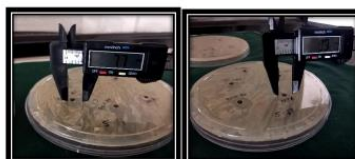
[Figure h] Inhibition zone of 5% Sodium hypochlorite and 50 % Allium Sativum oil after 24 hours in *Enterococcus faecalis* group samples



[Figure l] Inhibition zone of 5% Sodium hypochlorite and 50 % Allium Sativum oil after 48 hours in *Enterococcus faecalis* group samples



[Figure i] Inhibition zone of 80 % Allium Sativum oil and MTA after 24 hours in *Enterococcus faecalis* group samples



[Figure m] Inhibition zone of 80 % Allium Sativum oil and MTA after 48 hours in *Enterococcus faecalis* group samples

[Figure 3] After 48 hours incubation zone of 5%, 50%, 80% allium sativum, MTA and 5% sodium hypochlorite in Enterococcus faecalis group

### ONE WAY ANOVA

	GROUPS	N	Mean	Std. Deviation	Statistic/F	P VALUE
S. aureus 24 hours	5% ALLIUM SATIVUM	12	2.091667	1.5222342	80.92	<u>&lt;0.001</u>
	50% ALLIUM SATIVUM	12	10.116667	1.6513539		
	80% ALLIUM SATIVUM	12	12.633333	2.33329		
	MTA	12	10.391667	0.5696224		
	5% SODIUM HYPOCHLORITE	12	10.383333	0.6264377		
	Total	60	9.123333	3.937278		
S. aureus 48 hours	5% ALLIUM SATIVUM	12	3.058333	1.5424056	87.783	<u>&lt;0.001</u>
	50% ALLIUM SATIVUM	12	11.766667	1.9392282		
	80% ALLIUM SATIVUM	12	13.983333	2.3036861		
	MTA	12	11.891667	0.8050503		
	5% SODIUM HYPOCHLORITE	12	11.65	0.5213619		
	Total	60	10.47	4.1261588		
S. Aureus Difference	5% ALLIUM SATIVUM	12	0.966667	0.7969639	1.526	0.207
	50% ALLIUM SATIVUM	12	1.65	0.7166843		
	80% ALLIUM SATIVUM	12	1.35	0.6274045		
	MTA	12	1.5	0.7675226		
	5% SODIUM HYPOCHLORITE	12	1.266667	0.6971805		
	Total	60	1.346667	0.7363369		

[Table – 1] Comparison of S. aureus 24 and 48 hours inhibition zone between the five groups. After 24 hours staphylococcus aureus group inhibition zone statistically Significant with p value of <0.001 and After 48 hours staphylococcus aureus group inhibition zone statistically significant with p value of <0.001

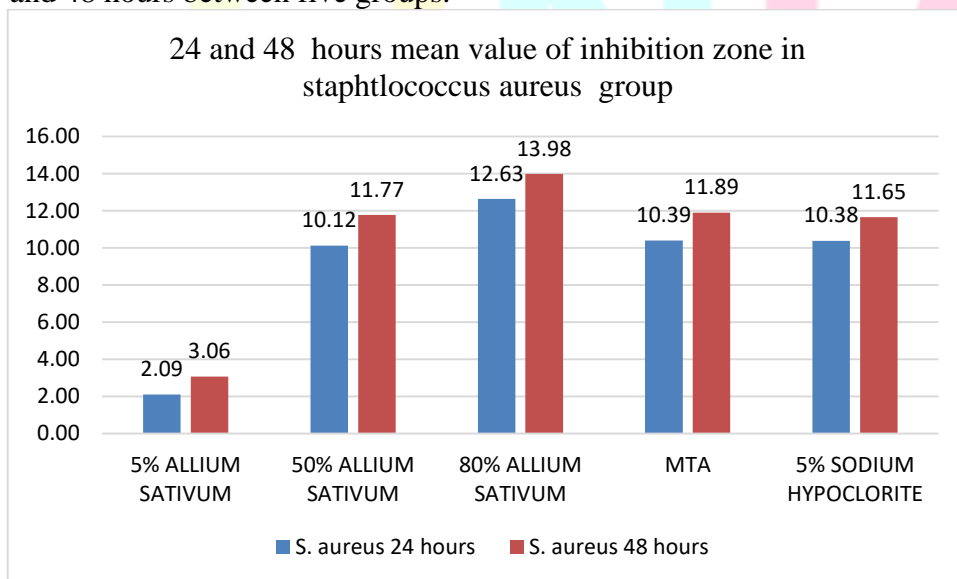
### POSTHOC TUKEY TEST

VARIABLE	COMPARISON OF	COMPARISON WITH	MEAN DIFFERENCE	STANDARD ERROR	P VALUE
S. aureus 24 hours	5% ALLIUM SATIVUM	50% ALLIUM SATIVUM	-8.0250000*	0.611155	0.7530
		80% ALLIUM SATIVUM	-10.5416667*	0.611155	<u>&lt;0.001</u>
		MTA	-8.3000000*	0.611155	<u>&lt;0.001</u>
	50% ALLIUM SATIVUM	5% SODIUM HYPOCHLORITE	-8.2916667*	0.611155	<u>&lt;0.001</u>
		80% ALLIUM SATIVUM	-2.5166667*	0.611155	<u>0.0010</u>
		MTA	-0.275	0.611155	0.9910
		5% SODIUM HYPOCHLORITE	-0.26667	0.611155	0.9920

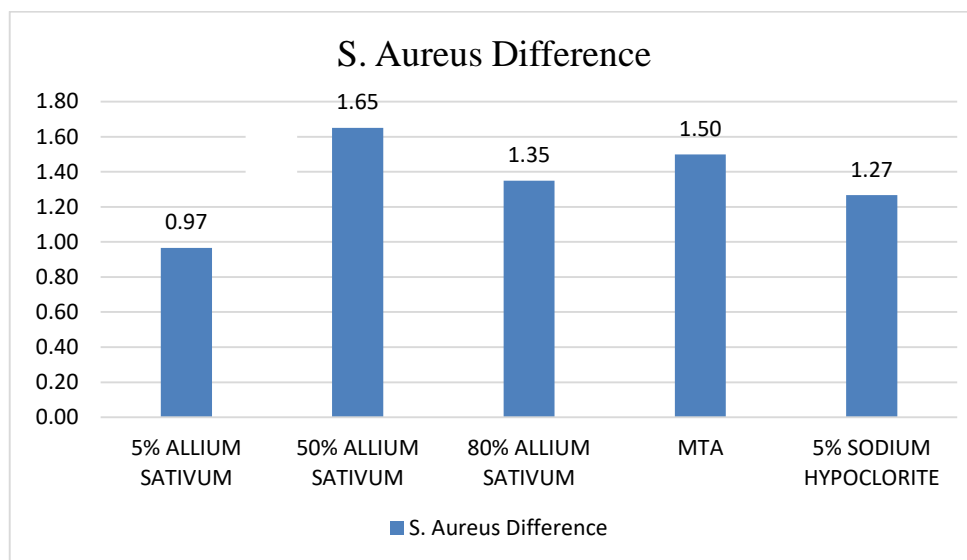


	80% ALLIUM SATIVUM	MTA	2.2416667*	0.611155	<u>0.0050</u>
		5% SODIUM HYPOCLORITE	2.2500000*	0.611155	<u>0.0050</u>
	MTA	5% SODIUM HYPOCLORITE	0.008333	0.611155	1.0000
	S. aureus 48 hours	5% ALLIUM SATIVUM	50% ALLIUM SATIVUM	-	0.642041
80% ALLIUM SATIVUM			8.7083333*	0.642041	<u>&lt;0.001</u>
MTA			-	0.642041	<u>&lt;0.001</u>
5% SODIUM HYPOCLORITE			8.8333333*	0.642041	<u>&lt;0.001</u>
50% ALLIUM SATIVUM		80% ALLIUM SATIVUM	-	0.642041	<u>0.0090</u>
		MTA	2.2166667*	0.642041	1.0000
		5% SODIUM HYPOCLORITE	-0.125	0.642041	1.0000
80% ALLIUM SATIVUM		MTA	0.116667	0.642041	1.0000
		5% SODIUM HYPOCLORITE	2.0916667*	0.642041	<u>0.0160</u>
MTA		5% SODIUM HYPOCLORITE	2.3333333*	0.642041	<u>0.0050</u>
		5% SODIUM HYPOCLORITE	0.241667	0.642041	0.9960
S. Aureus Difference		5% ALLIUM SATIVUM	50% ALLIUM SATIVUM	-0.68333	0.295385
	80% ALLIUM SATIVUM		-0.38333	0.295385	0.6940
	MTA		-0.53333	0.295385	0.3810
	5% SODIUM HYPOCLORITE		-0.3	0.295385	0.8470
	50% ALLIUM SATIVUM	80% ALLIUM SATIVUM	0.3	0.295385	0.8470
		MTA	0.15	0.295385	0.9860
		5% SODIUM HYPOCLORITE	0.383333	0.295385	0.6940
	80% ALLIUM SATIVUM	MTA	-0.15	0.295385	0.9860
		5% SODIUM HYPOCLORITE	0.083333	0.295385	0.9990
	MTA	5% SODIUM HYPOCLORITE	0.233333	0.295385	0.9320
		5% SODIUM HYPOCLORITE			

[Table – 2] Comparison of mean difference in inhibition zone of staphylococcus aureus group sample after 24 and 48 hours between five groups.



[Graph 1] Mean value of 24 and 48 hours inhibition zone in staphylococcus aureus group



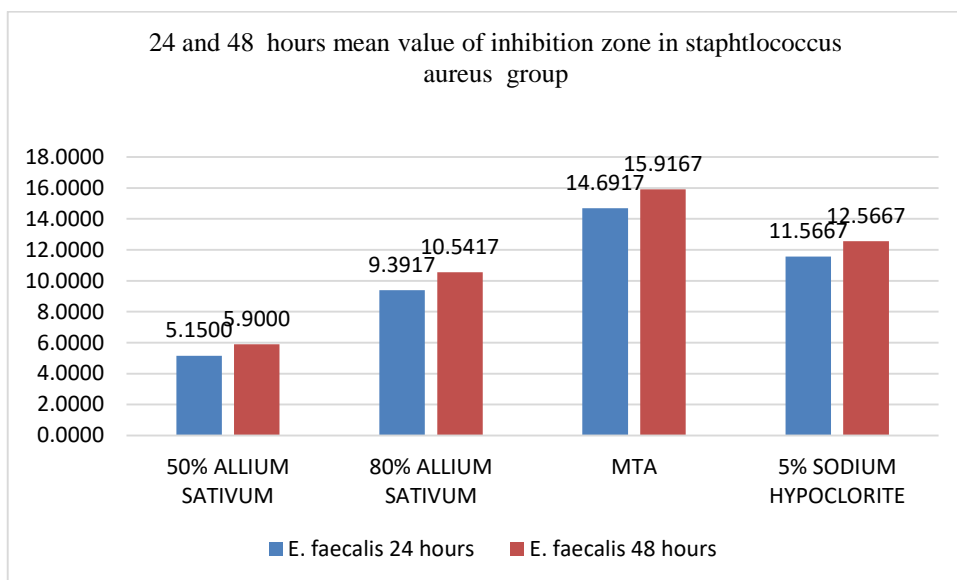
[Graph 2] mean different of inhibition zone in staphylococcus aureus group between all five groups 5% allium sativum , 50% allium sativum, 80% allium sativum, MTA and 5 % sodium hypochlorite

**ONE WAY ANOVA**

		N	Mean	Std. Deviation	Statistics/ mean squares	df2(welch) / F(Anova)	p value
E. faecalis 24 hours	50% ALLIUM SATIVUM	12	5.15	1.384656	192.795	83.696	<u>&lt;0.001</u>
	80% ALLIUM SATIVUM	12	9.391667	2.043374			
	MTA	12	14.69167	0.819599			
	5% SODIUM HYPOCHLORITE	12	11.56667	1.565151			
	Total	48	10.2	3.802966			
E. faecalis 48 hours	50% ALLIUM SATIVUM	12	5.9	1.206045	186.609	23.532	<u>&lt;0.001</u>
	80% ALLIUM SATIVUM	12	10.54167	1.888462			
	MTA	12	15.91667	0.798673			
	5% SODIUM HYPOCHLORITE	12	12.56667	1.230176			
	Total	48	11.23125	3.88807			
E. faecalis difference	50% ALLIUM SATIVUM	12	0.75	0.368042	0.527	1.951	0.135
	80% ALLIUM SATIVUM	12	1.15	0.697398			
	MTA	12	1.225	0.50834			
	5% SODIUM HYPOCHLORITE	12	1	0.447214			
	Total	48	1.03125	0.53521			

[Table – 3]Comparison of E. FAECALIS 24 and 48 hours inhibition zone between the five groups. After 24 hours enterococcus faecalis group inhibition zone statistically Significant with p value of <0.001 and After 48 hours inhibition zone statistically significant with p value of <0.001





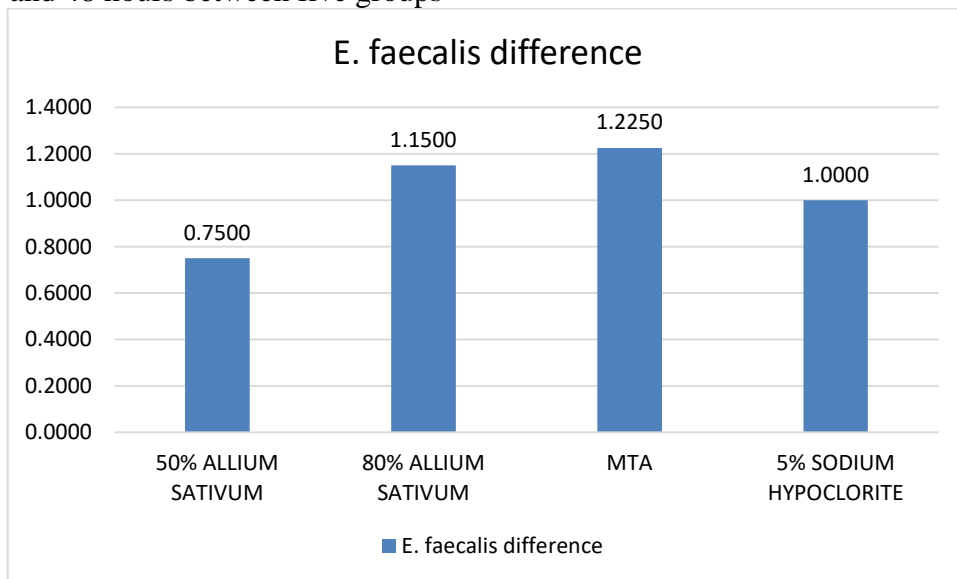
[Graph 3] Mean value of 24 and 48 hours inhibition zone in Enterococcus Faecalis group

**POSTHOC TUKEY TEST**

Dependent Variable	COMPARISON GROUP	COMPARED WITH	MEAN DIFFERENCE	Std. Error	P VALUE
E. faecalis 24 hours	50% ALLIUM SATIVUM	80% ALLIUM SATIVUM	-4.2416667*	0.619613	<0.001
		MTA	-9.5416667*	0.619613	<0.001
	80% ALLIUM SATIVUM	5% SODIUM HYPOCHLORITE	-6.4166667*	0.619613	<0.001
		MTA	-5.3000000*	0.619613	<0.001
	MTA	5% SODIUM HYPOCHLORITE	-2.1750000*	0.619613	0.006
		5% SODIUM HYPOCHLORITE	3.1250000*	0.619613	<0.001
E. faecalis 48 hours	50% ALLIUM SATIVUM	80% ALLIUM SATIVUM	-4.6416667*	0.546658	<0.001
		MTA	-10.0166667*	0.546658	<0.001
	80% ALLIUM SATIVUM	5% SODIUM HYPOCHLORITE	-6.6666667*	0.546658	<0.001
		MTA	-5.3750000*	0.546658	<0.001
	MTA	5% SODIUM HYPOCHLORITE	-2.0250000*	0.546658	0.003
		5% SODIUM HYPOCHLORITE	3.3500000*	0.546658	<0.001
E. faecalis difference	50% ALLIUM SATIVUM	80% ALLIUM SATIVUM	-0.4	0.212154	0.249

	MTA	-0.475	0.212154	0.129
	5% SODIUM HYPOCHLORITE	-0.25	0.212154	0.643
80% ALLIUM SATIVUM	MTA	-0.075	0.212154	0.985
	5% SODIUM HYPOCHLORITE	0.15	0.212154	0.894
MTA	5% SODIUM HYPOCHLORITE	0.225	0.212154	0.715

[Table – 4] comparison of mean difference in inhibition zone of enterococcus faecalis group sample after 24 and 48 hours between five groups



[Graph 4] mean different of inhibition zone in enterococcus faecalis group between all five groups 5% allium sativum , 50% allium sativum, 80% allium sativum, MTA and 5 % sodium hypochlorite

**CONCLUSION**

In the present study we observed that 50% and 80% allium sativum, MTA and 5% sodium hypochlorite have antimicrobial activity against Staphylococcus aureus and Enterococcus faecalis. However, in the concentration of 5% allium sativum is not effective against the Enterococcus faecalis. So garlic extract in the concentration of 50% and 80% could be an alternative to commercially available pulpotomy agent due to its cost effectiveness and minimum side effects.

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The preferred spelling of the word “acknowledgment” in America is without an “e” after the “g”. Avoid the stilted expression, “One of us (R.B.G.) thanks...” Instead, try “R.B.G. thanks”. Put applicable sponsor acknowledgments here; DONOT place them on the first page of your paper or as a footnote.

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