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Evaluating the Effect of Adding *Calendula officinalis* on Anti-microbial Strength of Glass Ionomer Cement: An Invitro Study

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ABSTRACT
Band materials are fixed to tooth surfaces using a variety of luting cements. One common unfavorable property present in most of the cements is more dissolubility in liquid intakes and inadequate bond strength, are major factors for demineralization beneath bands. Glass ionomer cement used on regular basis has antibacterial property, but it is very minimal and covers a narrow range of micro-organisms and has a low bactericidal potential. Therefore, in most of the cases it has been noticed that GIC alone is not capable of preventing plaque accumulation and microbial development of periodontal disease and caries in majority of patients. Hence, it becomes really very important to have a complete knowledge about the influence of the cement that is used in orthodontics on the various microorganisms (bacteria, etc.). This may include impact on bacterial existence, adhesion and biofilm formation. So, it is necessary to check the interaction of orthodontic cement with these bacteria along with their effect on bacterial viability, adhesion and biofilm formation. The present analysis focuses on how to check antimicrobial features in glass ionomer cement by incorporating *Calendula officinalis.*

Keywords: *Calendula officinalis,* glass ionomer cement, antimicrobial.

Introduction:
Plaque accumulation is major problem which a practitioner faces during the orthodontic treatment, mainly nearby the brackets as well as coronal edges of bands as patient experiences certain discomfort as far as cleanliness in these areas is concerned [1]. Accumulation of debris and food material around brackets and bands leads to development of ecosystem very favorable for the development of myriad of problems like enamel demineralization, caries and hyper plastic gingivitis. Band materials are fixed to tooth surfaces using variety of luting cements. One common unfavorable property present in most of the cements is more dissolubility in liquid intakes and inadequate bond strength, are major factors for demineralization beneath bands [2-4].Among most commonly used dental cements, GIC is most preferable cement for band cementation. One of the main properties of GIC cement is absorption of fluoride & constant release, which provides certain anti-bacterial activities [5-7].
Although Glass ionomer cement used on regular basis has antibacterial property, but it is very minimal and covers narrow range of micro-organisms and has a low bactericidal potential. Therefore, in most of the cases it has been noticed that GIC alone is not capable of preventing plaque accumulation and microbial development of periodontal disease and caries in majority of patients [6-8].*Streptococcus mutans* is the major fertile organism involved in caries development.
Hence, it becomes really very important to have a complete knowledge about the
influence of the cement that is used in orthodontics on the various microorganisms (bacteria, etc.). This may include impact on bacterial existence, adhesion and biofilm formation.
So, it is necessary to check the interaction of orthodontic cement with these bacteria along with their effect on bacterial viability, adhesion and biofilm formation [9, 10].

Calendula officinalis
Family of Plant- Asteraceae
Duration of its peak growth- May to October
Part of Plant used- Flowers
Significance from the point of Dentistry- Mouthwash having *Calendula officinalis* are remarkable in controlling gingival bleeding.

OBJECTIVES
The present analysis focuses on how to check antimicrobial features in glass ionomer cement by incorporating *Calendula officinalis.*

OBJECTIVES OF THE STUDY
This invitro study will be conducted to check antimicrobial properties of glass ionomer cement in following groups-
Group (A) Banded teeth, treated using *Calendula officinalis.*10%, Group (B) Banded teeth using *Calendula officinalis.*25%, Group (C) Banded teeth using *Calendula officinalis.*50% and Group (D) GIC only (Control group).

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Material and Method

This study was conducted to investigate whether adding *Calendula officinalis* might influence the Shear-peel band strength of the Glass Ionomer Cement (GIC) utilized for cementation of denture bands.
Anti-bacterial tests were conducted in Mahatma Gandhi Medical college (microbiology lab), Jaipur.

Total 10 samples of each concentration were prepared.

1) Group A 10% *Calendula officinalis* in GIC
2) Group B 25% *Calendula officinalis* in GIC
3) Group C 50% *Calendula officinalis* in GIC
4) Group D 0% *Calendula officinalis* in GIC

Total 40 samples were premolar were collected which were recently extracted human teeth with periodontal involvement or extracted for therapeutic purpose in the oral surgery department, Mahatma Gandhi Dental College and Hospital Jaipur.

Determination of size of the sample-

Numerical values involved were:
Mean Deviation
Standard Deviation

Formula used in Calculation-

$$n = \frac{2\,(Z_\alpha + Z_\beta)^2\ [s]^2}{d^2}$$

Here, Z_α = Z Variate of α-error (It's a constant having value = 1.96)
Z_β = Z variate of β-error (Another constant which is equal to 0.84)

Approximately the following estimates were obtained-

1. Powder to be Eighty percent
2. Only 5 % of the Type I Error
3. Percentage of Type II error came out to be 20
4. There was an accurate difference of 0.15 unite among all of the groups.
5. 0.12 was the value for Pooled standard deviation.

Now, when all the above obtained values were substituted in the above formula, then the results obtained were as shown under-

$$n = \frac{2\,(2.8)^2\ [0.12]^2}{(0.15)^2}$$

n = 10.03

Around **10 samples per group** should complete this study.
It is advisable to make more number of samples so that even after some errors in processing, we would be able to achieve the required minimum sample size. Also to reduce the total cost, you may consider removal of groups rather than reducing the sample size per group
Different materials used during the conduction of the anti-microbial test:

1) ***Streptococcus mutans*** **(Artificial Saliva**) *S. mutans* the main or the primary organism that was found responsible for the demineralization of the enamel part of the tooth. Therefore, in order to find out the anti-bacterial activity, these were used.
2) **Calendula officinalis** ***(medicinal plant abstract)*****-**Therapeutic preparations have been made for a long time from the floral parts of this plant. Gingival bleeding gets reduced when mouth washes containing *C.officinalis* is used.
3) **Brain Heart Infusion broth (Media)-** The much awaited anti-bacterial calculations were done with the incorporation of the Agar Diffusion Test. At the same time, another effective procedure i.e. the Broth-Dilution Technique was involved for knowing how *S. mutans* would grow.

Glass Ionomer cement (GC Gold)- The GIC's are the main substances utilized in restoring a tooth, as orthodontic adhesive, intermediate adhesive. ADA SPECIFICATION NUMBER: 96.

CRITERIA FOR SAMPLE SELECTION

Inclusion Standards

Teeth with no cracks
Teeth with no surface defects
Teeth with no decay or restoration

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Exclusion Measures
Teeth with fluorosis
Teeth with enamel Hypoplasia
Fractured teeth

Composition of GIC
1. A homogenous mixture composed of –
a. Calcium fluroaluminosilicate glass
b. Sand 41.9%
c. $Al_2O_3$28.6%
d. Aluminium Fluoridee 1.6%
e. Sodium fluoride 9.3%
f. $Al_2(PO)_4$ 3.8%

The fluoride part played the vital role of ceramic flux whereas radio opacity was given by Strontium, Barium or Zinc oxide.
2. Liquid portion comprising of-
a. Copolymer of -Polyacrylic acid itaconic acid maleic acid tartaric acid

Purpose- Enhancing work time and usage properties
b. Water which plays a universal role of acting as a medium for a variety of reactions and even helps in providing moisture to the various products formed in the process.

Plant extraction used- *Calendula officinalis*
Family of Plant- Asteraceae
Duration of its peak growth- May to October
Part of Plant used- Flowers
Significance from the point of Dentistry- Mouthwash having *Calendula officinalis* are quite effective in controlling gingival bleeding.

Four categories created were-
1. **T-10** (Ten percent of the sample of complete weight of powder plus ratio of Glass Ionomer Cement taken)
2. **T-25** (Quarter percent of sample of total weight of homogenous powder and liquid ration of GIC mixed with it)
3. **T-50** (Half of *C. officinalis* of the entire weight of powder added to the desired ratio of Glass Ionomer cement.)
4. T-0 (Nil amounts of *Calendula officinalis). This one was referred to as the Control Group.*

Minimal Inhibitory Concentrations (MICs).
In order to estimate the anti-bacterial capacity of the GIC with *Calendula officinalis*, procedure adopted was the Broth Dilution Method that has been incorporated by Andrews. This helped a lot in the determination of the same. In this method, where the microorganism used was inhibited, the interpretation of the results were as under-
The MIC value of Glass Ionomer Cement, *Calendula officinalis*, and GIC with *Calendula officinalis* (for various concentration chosen) for *S mutans* were supposed to be of minimum concentration.
The GIC samples having various concentrations of *Calendula officinalis* were made by addition of one unit of powdered GIC to one drop of liquid having the various concentrations, one at a time. Then they were allowed to mix thoroughly and dried. Finally, they were placed in groups along with the 3 variable concentrations of the Glass Ionomer Cement with *Calendula officinalis* . For all four concentrations (0%, 10%, 25% & 50%), test were conducted in 10 test tubes for each concentration.

OVERALL DISTRIBUTION OF STUDY SAMPLES
Table l -1 shows the distribution of samples across the groups. In each of the four groups 10 sample were taken making a total sample of 40.

	N	Percentage
Group A	10	25%
Group B	10	25%
Group C	10	25%
Group D	10	25%

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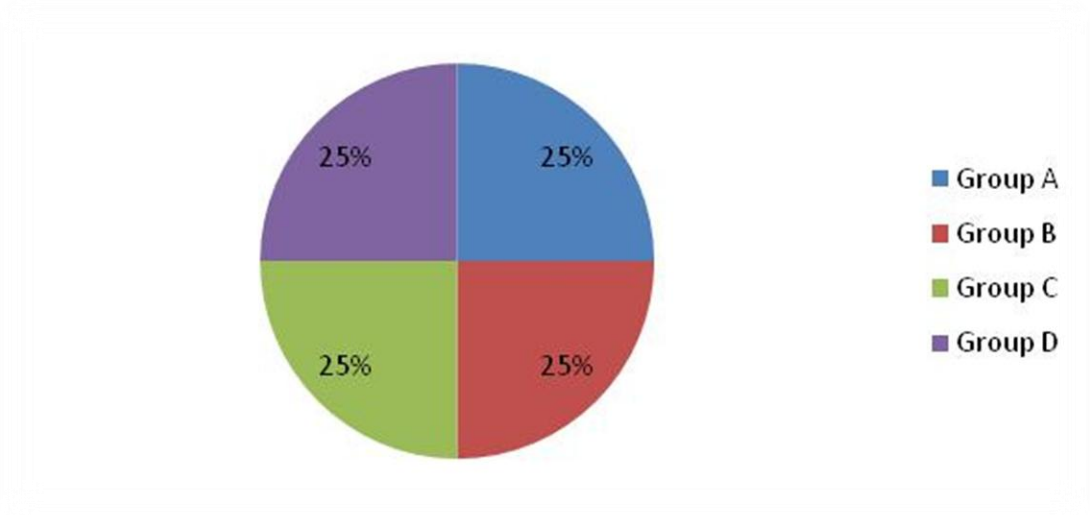
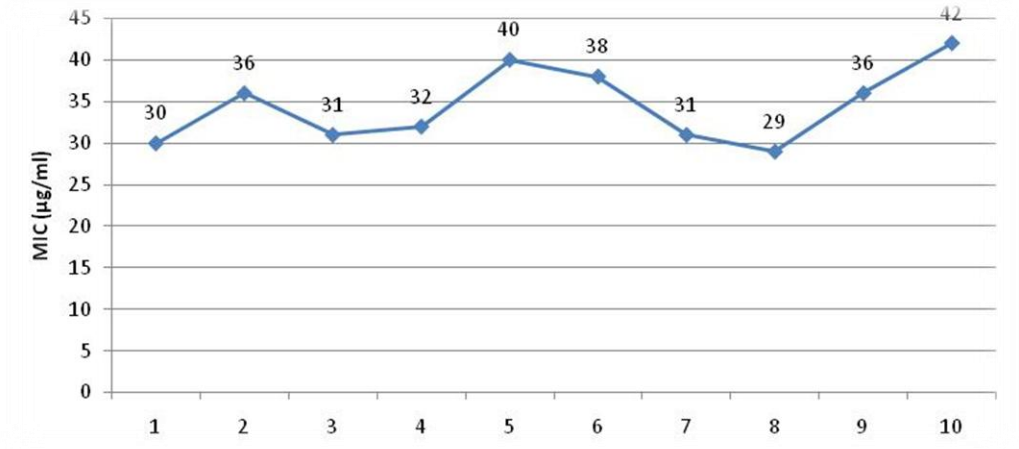


DISTRIBUTION OF MICROBIAL MIC IN STUDY SAMPLES IN GROUP (A)

S,NO	MIC (µg/ml)
1	30
2	36
3	31
4	32
5	40
6	38
7	31
8	29
9	36
10	42



DISTRIBUTION OF MICROBIAL MIC IN STUDY SAMPLES IN GROUP (B)

S,NO	MIC (µg/ml)
1	30
2	20
3	32
4	18
5	25
6	23
7	28
8	26

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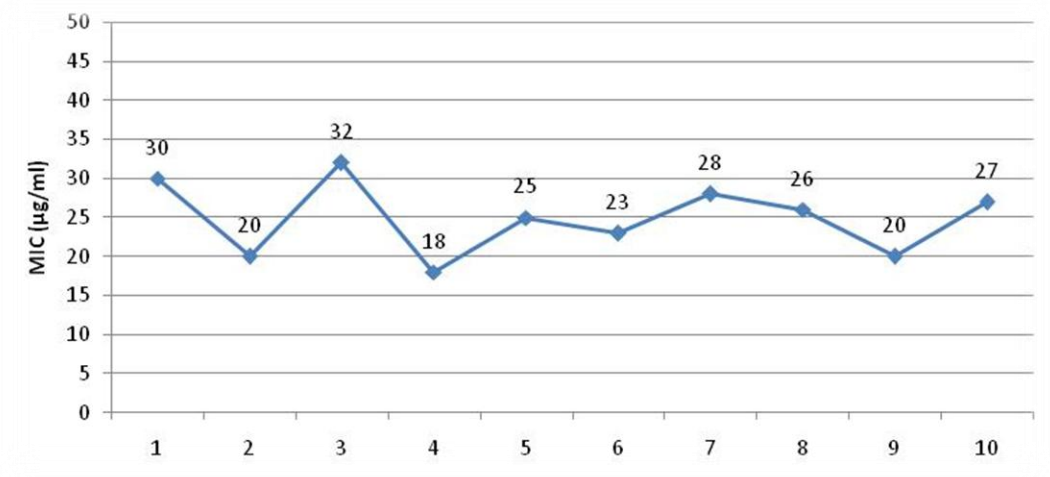
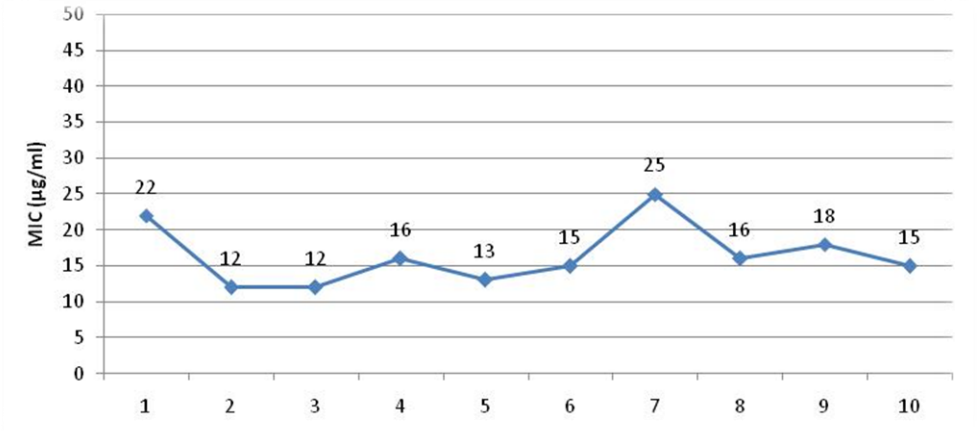
9	20
10	27



DISTRIBUTION OF MICROBIAL MIC IN STUDY SAMPLES IN GROUP (C)

S,NO	MIC (µg/ml)
1	22
2	12
3	12
4	16
5	13
6	15
7	25
8	16
9	18
10	15



DISTRIBUTION OF MICROBIAL MIC IN STUDY SAMPLES IN GROUP (D)

S,NO	MIC (µg/ml)
1	40
2	44
3	42
4	34
5	52
6	62
7	48
8	38

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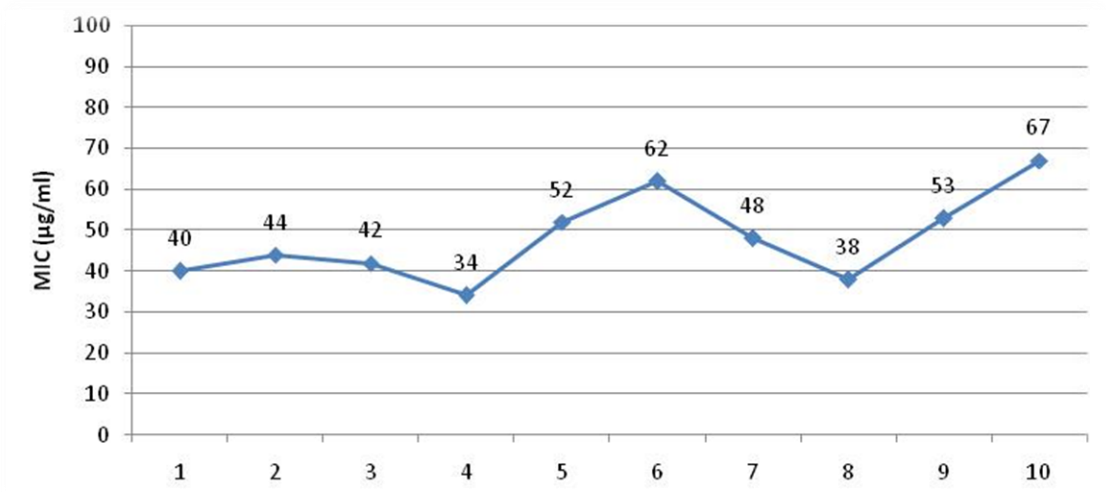
9	53
10	67

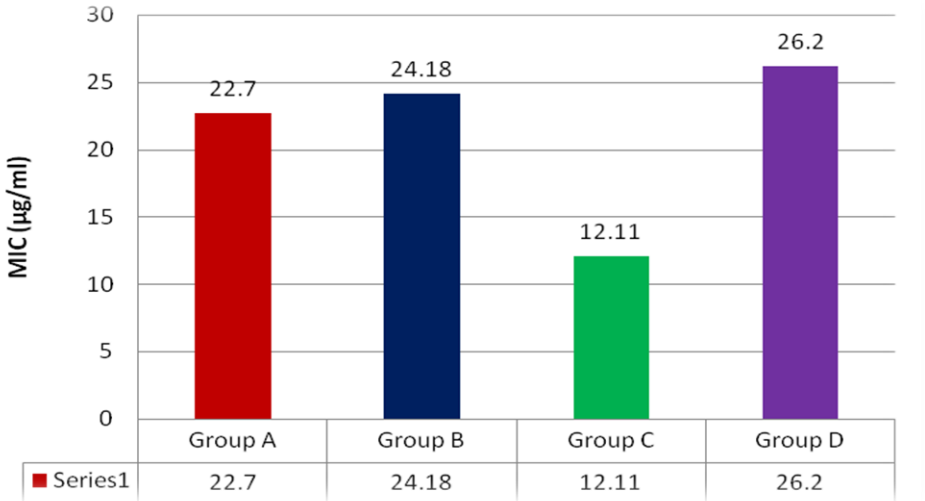


MEAN MICROBIAL MIC BETWEEN THE GROUPS

The mean MIC in the Group A (10%) was 34.50 with standard deviation of 4.527. The mean MIC in the Group B (25%) was 24.90with standard deviation of 4.605. The mean MIC in the Group C (50%) was 16.40with standard deviation of 4.247. The mean MIC in the Group D (0%) was 48.00with standard deviation of 10.593. The mean MIC was highest in the Group D followed by Group A, Group B and least in the Group C. The intergroup comparison of mean MIC between the four groups was statistically significant with p value of 0.085 when analyzed using the Kruskal Wallis test. The post intergroup comparison was done using the Mann Whitney U test. The intergroup comparison between each of the two groups was statistically significant with p value of more than 0.05 . The highest MIC values signifies the least antimicrobial quality

				95% Confidence Interval for Mean				
	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum	P value
Group A	34.50	4.527	1.431	31.261	37.738	29.00	42.00	0.001 (Sig)
Group B	24.90	4.605	1.456	21.605	28.194	18.00	32.00	
Group C	16.40	4.247	1.343	13.361	19.438	12.00	25.00	
Group D	48.00	10.593	3.349	40.421	55.578	34.00	67.00	

Kruskal Wallis test



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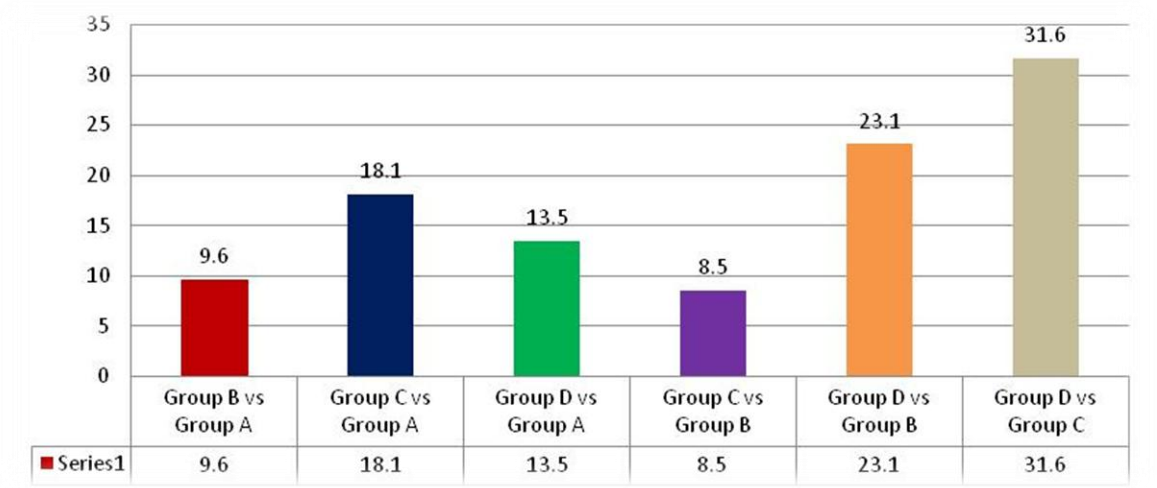
INTERGROUP COMPARISON OF MICROBIAL MIC BETWEEN THE GROUPS

Intergroup Comparison		Mean Diff	Std Error	P value	Significance
Group A	Group B	9.600	2.932	0.014	Significant
Group A	Group C	18.100	2.932	0.001	Significant
Group A	Group D	13.500	2.932	0.001	Significant
Group B	Group C	8.500	2.932	0.038	Significant
Group B	Group D	23.100	2.932	0.001	Significant
Group C	Group D	31.600	2.932	0.001	Significant

Mann Whitney U test



DISCUSSION

The various systems of Medicine that have gained popularity and importance in the recent years include Ayurveda, Siddha and Traditional Chinese Medicinal Systems. Researchers have proved that more than seven hundred herbal medicines and preparations whether in the form of Capsules, tablets or syrups are being used all over the world wherein a variety of more than one hundred plants are categorized as Medicinal Plants.

Calendula officinalis, is better known by the name **Pot Marigold**, is considered to be a valuable medicinal plant that has been an integral part of our rich cultural and traditional system of medicine. Several types of diseases have been reported to be cured by its involvement.

Fever and Cancer, are just a few of the many names that find their treatment in Ayurvedic System of Medicine using *Calendula.* A number of biological activities like, anti-bacterial, anti-inflammatory, diuretic, anti-spasmodic, anti-mutagenic

have been associated with several parts of *Calendula.* Not only these, *Calendula* is also remedial in Ophthalmological diseases, Dermatological issues, Gynecological problems and in some areas of burns.

Studies and reports claim that *Calendula officinalis* is a natural material with bactericidal activity and several studies have shown its effective use in the bacterial disease related treatments. Several studies have demonstrated *Calendula officinalis* effect against various bacteria and its anti- microbial activity on oral flora and microorganisms. So, the main idea behind this study was to evaluate the antibacterial and mechanical properties of GIC with *Calendula officinalis.*

The main part of the study was to check out the improvement in anti microbial efficacy on addition of Calendula to GIC. It was found out that anti microbial efficacy was improved with all concentrations of Calendula, highest effect being produced by 50% addition of Calendula. The Group with 10% Calendula also produced significant improvement in anti microbial property as compared to the Control group.

Conclusion:

Plaque accumulation around the bands pose serious threat with regards to development of dental caries and marginal gingivitis. So the efforts have continually been made to improve the anti microbial property of luting cements by addition of certain chemicals.

The main part of the study tested the Anti microbial efficacy of 3 groups with differing concentrations of Calendula compared with Control. Antibacterial action of Group C(50%) was found to be best but there was significant improvement even on addition of 10% *Calendula officinalis.*

Although the results of our study seem promising with regards to significant improvement in terms of anti microbial efficacy improvement and insignificant reduction in SPBS, but in Vitro study with a larger sample size and clinical studies

using the products in patients will go a long way in understanding the true efficacy of using Calendula as a constituent of Luting Caments.

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