Evaluating the antimicrobial efficacy and Compressive strength of dry sage leaves modified GIC

Type of Manuscript: Original Study

Running Title: Antimicrobial efficacy and physical strength of dry sage leaves modified GIC

Authors:

Barani Shankar
Department Of Pedodontics,
Saveetha Dental college and Hospitals,
Saveetha Institute Of Medical And Technical Science
Saveetha University,,Poonamallee High Road
Chennai,Tamil Nadu, India.
E mail :151801076.sdc@saveetha.com

Jessy P
Assistant Professor,
Department Of Pedodontics,
Saveetha Dental college and Hospitals,
Saveetha Institute Of Medical And Technical Science
Saveetha University,,Poonamallee High Road
Chennai,Tamil Nadu, India.
E mail :drjessyp.sdc@saveetha.com

Arun Jaikumar Ram
Saveetha dental college,
Saveetha Institute Of Medical And Technical Science
Saveetha University,Saveetha Institute Of Medical And Technical Science
Saveetha University,,Poonamallee High Road
Chennai,Tamil Nadu, India
Email: 151801057.sdc@saveetha.com

Rajeshkumar Shanmugam,
Associate Professor,
Department of Pharmacology,
Saveetha Dental college and Hospitals
Saveetha Institute of Medical & Technical Sciences,
Saveetha university,
Chennai - 600077, Tamil Nadu, India
Email id ::rajeshkumars.sdc@saveetha.com

Corresponding author:
Jessy P
Assistant Professor,
Department Of Pedodontics,
Saveetha Dental college and Hospitals,
Saveetha Institute Of Medical And Technical Science
Saveetha University,,Poonamallee High Road
Chennai,Tamil Nadu, India.
E mail :drjessyp.sdc@saveetha.com

ABSTRACT:

Aim: The aim of the study was to evaluate the antimicrobial efficacy and Compressive strength of dry sage leaves modified GIC

Materials and methods: The dry sage leaves extract was prepared. The powder and liquid of conventional GIC was mixed after which the prepared extract was incorporated with three different concentrations. Standard strains S. mutans and Lactobacillus were used to test the antimicrobial efficacy of modified and unmodified (control)GIC.MIC assay was done where the first three wells were used with three different concentrations of modified GIC (2:1:1),(3:1:2),(3:2:1) respectively and the fourth well was kept as control (Conventional unmodified GIC).The incubation is done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h). Compressive strength was evaluated according to ISO 9917- 1:2007 using cylindrical molds. The maximum force applied when the specimen fractures was recorded to calculate the compressive strength values in MPa.

Result: From this study it is shown that as the concentration of the extract increases the antimicrobial activity also increases. From this it is inferred that dry sage leaves modified GIC showed better antimicrobial activity against S.mutans and conventional GIC showed better antimicrobial activity against lactobacillus and there was no significant change in terms of compressive strength.

Conclusion: Based on the results recorded in the present findings, it is concluded that sage leaves extract has a potential antimicrobial agent on human pathogenic microorganisms especially S.mutans, and hence, the herbal drug may serve as one of the potential antimicrobial agents.

Keywords: Dental material, glass ionomer cement, dry sage leaves,plant extract, antimicrobial,physical properties.

INTRODUCTION:

Recently Herbal extracts have gained more attention as the agents to be added with the oral care products and dental materials. Most of the herbal products have potent antimicrobial

activities which could lead to the production of safe, economical, and efficient alternative materials to manage dental caries(1). 80% of the population rely on the traditional plants according to the World Health Organization(2). Salvia officinalis, which is also called Sage, is a perennial evergreen plant native to the Mediterranean region.(1) and is also naturalized in Iran.(3) It has woody stems, grayish leaves with blue-to-purplish flowers. S. officinalis extract has antimicrobial, analgesic, anti-inflammatory and antioxidant properties.(1,4) The essential oil of S. officinalis consists of alpha- and beta-thujone, borneol, camphor, and cineole. S. officinalis tea is used to control sore throat, inflammatory oral lesions, and gingivitis.

Dental caries is still a major concern in public health management.(5) Establishment of a productive dental health philosophy, especially for the low socioeconomic levels of the society, where the lack of development endangers the dental health, even more, seems to be necessary.(6) Microbial nature of caries and the contemporary concept of treatment based on the medical model necessitate the consideration of chemical control plans along with the traditional mechanical means of caries control.(7)Glass ionomer cements are acid-base cements. The proper name for them, according to the International Organization for Standardization is “glass polyalkenoate cement”, but the term “glass ionomer” is recognized as an acceptable trivial name, and is widely used within the dental community (8). Over the past years, GICs have been the most commonly used water-based cements for final cementation of dental crowns, bridges, orthodontic brackets and atraumatic restorative treatment (9).GIC has the ability to bond adhesively to enamel and dentin, their biocompatibility and their ability to release fluoride ions over a prolonged period of time. Furthermore, GICs were shown to be rechargeable with fluoride ions (10,11)

Innovations in dental material science are come to the point of developing smart materials.(12) glass-ionomer cement, a tooth-colored acid-base material, with the capability of fluoride release in an aqueous environment is the first of this category.(12) It can be used as base, liner, or direct restorative material and is also considered as the material of choice in atraumatic restorative technique. The ART is a minimal intervention approach, particularly beneficial for pediatric and elderly patients as well as those with dental anxiety or learning difficulties.(13)Consequently, cariogenic bacteria can survive incarceration under GIC restoration and remain viable for up to 2 years resulting in secondary caries.(14) Literature has been evidence to the fact that fluoride released from GICs is not sufficiently potent to combat the effects of bacterial destruction over an extensive duration of time.(15) Moreover, in spite of the fact that studies have shown that GICs release ~10 ppm of fluoride during the first 48 hrs following insertion into the cavity, this is still regarded low for achieving the desired antibacterial effects (16)In an attempt to develop a GIC with direct antimicrobial properties, this study is conducted to investigate any possible inhibitory effects sage modified GIC may have on S. mutans and Lactobacillus as the main bacteria involved in caries initiation and progression process.

MATERIALS AND METHODS

Preparation of plant extract:

The dry sage leaves were dried for 5 days. The glasswares were properly washed, rinsed with distilled water and dried in a hot air oven at 70 degree celsius before use. In the beaker, 1g of leaves were measured and added to 100mL of distilled water. The mixture was boiled using a heating mantle at 60-70 degree celsius for 15 minutes . The solution is filtered using Whatman No: 1 filter paper and the obtained 80 mL filtrate is collected in a separate conical flask. This filtered extract was further condensed to 5 mL at 60-70 degree celsius.

Grouping : The type II GIC (GC corporation) was used in the present study.

Groups	**Description**
I	P^{GIC}: E: L^{GIC} = 2:1:1
II	P^{GIC}: E: L^{GIC} = 3:1:2
III	P^{GIC}: E: L^{GIC} = 3:2:1
IV	Control group –conventional GIC

Bacterial Strain and Inoculum Preparation:

Streptococcus mutans and Lactobacillus acidophilus bacterial strains were obtained from the Department Of microbiology, Saveetha Dental college and Hospitals. A sterile complete loop of each pure culture was taken, and the facultative strains of S. mutans and Lactobacillus acidophilus were fully grown on Mueller Hinton Agar. The microorganisms were subcultured in appropriate culture media and it was inoculated individually in tubes containing 5 mL of sterile Mueller Hinton broth and incubated at 37 degree celsius for 24 h. The suspension was then adjusted to 0.5 Mcfarland scale = 1.5 × 108 colony-forming unit (CFU).

Specimen preparation for antimicrobial testing :

After mixing the powder and liquid of conventional GIC, the plant extract was incorporated. The final obtained cement was placed into cylindrical molds measuring diameter of 6 mm and 2 mm in thickness and the prepared specimens were carried to the cylindrical wells in less than 1 minute using the sterile cement carrier, and the upper surface of the cement layer was pressed to the equal level using sterile glass slide. After setting off the cement, the disk-shaped specimens were removed from the mold. The precise specimen was measured using calipers and recorded. For

monitoring the antibacterial effects of the tested groups,Six specimens were prepared in each group, three for s.mutans and another three specimens for lactobacillus.

MIC Assay: Standard strains S. mutans and Lactobacillus were used to test the antimicrobial efficacy of modified and unmodified GIC.

1. MHA broth was prepared, sterilized and 200 µL was added to all four wells.

2. Bacterial suspensions about 50 µL (S. mutans and Lactobacillus acidophilus) were added to all 4 wells in the range of 5×105 CFU/ml.

3. The first three wells contain three different concentrations of GIC (1:1),(1:2),(2:1) and the fourth well is considered as the control (Conventional GIC).

4. The incubation is done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h).

5. Using an ELISA reader, the percentage of dead cells is calculated at a wavelength of 540 nm at regular time intervals.

Specimen preparation for compressive strength evaluation:

Compressive strength was evaluated according to ISO 9917- 1:2007 using cylindrical molds (4.0 mm diameter × 6.0 mm height). Six specimens were prepared for each group. Then materials were placed into the mold and leveled to obtain a smooth surface. One hour later specimens were removed from the mold, and stored in deionised water for 24 h. Malformed specimens or those with voids were discarded. The diameter of each specimen was checked using a digital micrometer gauge. The specimens were then placed in vertical position in a Zwick universal testing machine (Zwick Zmart Pro, ZwickRoell GmbH & Co. KG, Ulm, Germany). Compressive load was applied on the long axis of the specimens at a crosshead speed of 0.5 mm/min until fracture. The maximum force applied when the specimen fractures was recorded to calculate the compressive strength values in MPa.

Statistical analysis:

The data collected were entered in the microsoft excel sheet following which statistical analyses were carried out using SPSS version 24.0 (IBM corporation). The mean MIC values were calculated using descriptive analysis. One-way analysis of variance (ANOVA) was used for comparison between the groups .The significance level was set at $P \leq 0.05$.

Results :

Table1: **Mean MIC values of S.mutans: :**

S.mutans				

Conc	1h	2h	3h	4h
01:01	0.301	0.517	0.501	0.398
01:02	0.314	0.391	0.508	0.301
02:01	0.236	0.256	0.234	0.226
control	0.58	0.556	0.535	0.663

Antimicrobial activity against S.Mutans:

The antimicrobial activity against s.mutans,mean values of dry sage leaves modified GIC are tabulated in table 1, at 2:1 concentration proved to have better results when compared to 1:1 and 1:2 and all three different concentrations showed better efficacy when compared with the control group(Figure 1).

Table 2:**Mean MIC values of Lactobacillus: :**

Lactobacillus				
Conc	**1h**	**2h**	**3h**	**4h**
01:01	0.714	0.637	0.653	0.671
01:02	0.344	0.47	0.422	0.452
02:01	0.462	0.508	0.503	0.462
control	0.258	0.333	0.383	0.345

Antimicrobial activity against Lactobacillus:

The antimicrobial activity against lactobacillus, mean values of dry sage leaves modified GIC are tabulated in table 2, control group (conventional GIC) with the least mean value of 0.258 one hourly had a better antimicrobial efficacy when compared to other other groups (Figure 2)

Compressive strength:

The compressive strength was determined by the Universal testing machine when the specimen fracture was recorded to calculate the compressive strength,where is proved that both conventional and the modified groups gave the insignificant results i.e,110 Mpa,138 Mpa,110 Mpa of group I,II and III respectively.

DISCUSSION:

In this research, the antimicrobial efficacy and the compressive strength of safe leaf modified GIC was investigated. Herbal extract with a history of safety and effectiveness instead of chemical agents to produce a modified GIC was taken in this study. The results showed that the addition of S.officinalis extract with GIC produced a dental material with direct inhibitory properties against the selected microorganisms.Glass ionomers are a class of biomaterials in widespread use in modern dentistry.(17) GICs are capable of releasing fluoride, which contributes to some reduction in the number of residual bacteria in cavities as well as remineralization of the softened dentin.(18,19) However, even after the removal of infected dentin and adequate sealing, viable bacteria have been found in the remaining affected dentine after different periods of evaluation.(20) Literature is a testimony to the fact that therapeutic benefits have been gained when antimicrobial substances like chlorhexidine and antibiotics are used in association with GIC; however, a compromise of the strength characteristics has always emerged unconcealed.(21,22)The antibacterial activity of the sage leaf modified GIC was seen against Streptococcus mutans.S. mutans bacteria as they are the most cariogenic pathogens as they survive and grow in low-pH environments.It is seen that the bacteria can survive up to 2 years even under the restored GIC.(19) This means that any intentional or unintentional residual caries under the restorations may progress and affect the vitality of the pulp and leads to patient discomfort.(20) This also questions the accuracy of ART, in which multiple carious teeth are only excavated with a hand instrument and filled with a regular GIC, especially in low socioeconomic areas of society.(21)

Different investigators used various antimicrobial agents at various concentrations with GIC in an attempt to find a new solution.(21) Broad spectrum antimicrobials such as chlorhexidine, cetrimide, and antibiotics were used in those studies. The agents were added into the powder or liquid component of the GIC, utilizing various incorporation techniques.In the study of Becci etal., the effect of adding chlorhexidine on the bond strength of GIC to healthy dentin and dentin affected by decay was investigated. The concentrations of 0.5% and 1% of chlorhexidine diacetate, increased antibacterial activity of cement and has similar bond strength in comparison with pure glass ionomer, but at a concentration of 2%, the bond strength was decreased.(22) In the study of Hatunoğlu et al., the effect of adding an ethanolic extract of propolis on antibacterial properties and bond strength of GIC used to attach orthodontic bands was investigated. The addition of materials with the natural base has no adverse effect on the properties of glass ionomer.(23)

Another study done by Shahriar Shahriari et al stated that there was a direct inhibitory activity of *S. officinalis*-containing GIC against *S. mutans* and *L. casei* in a dose-response manner(24). S. officinalis modified GIC possesses a direct antibacterial effect against S. mutans and L. casei, according to the limitations of this investigation. This in turn offers numerous benefits for usage as a base, liner, or restorative material in operating on caries teeth and managing their problems. The significance of herbal agents in creating new dental materials with astounding qualities is also emphasized.The use of the S. officinalis modified GIC may prevent and control the inflammatory condition of the pulp. It is recommended to conduct experiments to investigate such abilities.(25)The reasons for selecting the S. officinalis in this research are the long history of successes and no history of any negative side effect documented for, in traditional and contemporary medical literature.[26] It is also reported that S. officinalis extract has superior efficiency compared to the antibiotics and has also less chance of drug resistance when used in high concentrations.[27] In addition, researchers are recently investigating possible applications of S. officinalis essential oil extract in oral care products. The studies reported inhibitory effect of the extract on many oral and fungi responsible for carious and periodontal infections.[26,28,29,30] They also reported positive effect on reducing the number of plaque bacterial colonies, impairing plaque formation,[31,32] and fungi attachment to prosthetic surfaces. Since the effect of S. officinalis extract modified GIC were effective against oral pathogens, other investigations are needed to evaluate the mechanical, physical, and biocompatibility properties of the material before undergoing routine clinical applications.

CONCLUSION:

Within the limitations of this study, it is concluded that a S. officinalis modified GIC has shown a good antibacterial property against S. mutans and no changes in terms of compressive strength. This, in turn, provides numerous advantages to use as a base, liner, or restorative material in operative procedures and management of the complications of caries teeth. It also emphasizes the significance of the herbal agents in developing new dental materials with good medicinal properties.

Acknowledgement : Nil

Conflict of interest :Nil

REFERENCE:

1. Sookto T, Srithavaj T, Thaweboon S, Thaweboon B, Shrestha B. In vitro effects of Salvia officinalis L. Essential oil on Candida albicans. Asian Pac J Trop Biomed. 2013;3:376–80.

2. Craig RG, Powers JM. Restorative dental materials. 11th ed. St. louis: Mosby-Elsevier Science Ltd; 2002.

3. Kermanshah H, Kamangar SH, Arami S, Mirsalehian A, Kamalineghad M, Karimi M, et al. In vitro evaluation of antibacterial activity of hydroalcoholic extract of Salvia officinalis and Pimpinella anisum against cariogenic bacteria. J Dent Med. 2009;22:149–54. [Google Scholar]

4. Borhan-Mojabi K, Azimi S. Antimicrobial natural products in oral health. In: Mendez-Vilas A, editor. Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. Vol. 2. Spain: Formatex Research Center; 2013. pp. 932–9.

5. Türkün LS, Türkün M, Ertuğrul F, Ateş M, Brugger S. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent. 2008;20:29–44. [PubMed] [Google Scholar]

6. Holmgren CJ, Roux D, Doméjean S. Minimal intervention dentistry: Part 5. Atraumatic restorative treatment (ART) – A minimum intervention and minimally invasive approach for the management of dental caries. Br Dent J. 2013;214:11–8. [PubMed] [Google Scholar]

7. Hook ER, Owen OJ, Bellis CA, Holder JA, O'Sullivan DJ, Barbour ME, et al. Development of a novel antimicrobial-releasing glass ionomer cement functionalized with chlorhexidine hexametaphosphate nanoparticles. J Nanobiotechnology. 2014;12:3. [PMC free article] [PubMed] [Google Scholar]
8. Nomot R, Komoriyama M, McCabe J, Hirano S. Effect of mixing method on the porosity of encapsulated glass ionomer cement. Dent Mater. 2004;20:972–8. https://doi.org/10.1016/j. Dental.2004.03.001.

9. Sidhu SK. Glass ionomer cement restorative materials: A sticky subject? Aust Dent J. 2011;56(Suppl 1):23–30. [PubMed] [Google Scholar]

10. Herrera M, Castillo A, Baca P, Carrión P. Antibacterial activity of glass-ionomer restorative cements exposed to cavity-producing microorganisms. Oper Dent. 1999;24:286–91. [PubMed] [Google Scholar]

11. Türkün LS, Türkün M, Ertuğrul F, Ateş M, Brugger S. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent. 2008;20:29–45. [PubMed] [Google Scholar]

12. McCabe JF, Yan Z, Al Naimi OT, Mahmoud G, Rolland SL. Smart materials in dentistry. Aust Dent J. 2011;56(Suppl 1):3–10. [PubMed] [Google Scholar]

13. Hook ER, Owen OJ, Bellis CA, Holder JA, O'Sullivan DJ, Barbour ME, et al. Development of a novel antimicrobial-releasing glass ionomer cement functionalized with chlorhexidine hexametaphosphate nanoparticles. J Nanobiotechnology. 2014;12:3. [PMC free article] [PubMed] [Google Scholar]

14. Weerheijm KL, Kreulen CM, de Soet JJ, Groen HJ, van Amerongen WE. Bacterial counts in carious dentine under restorations: 2-year in vivo effects. Caries Res. 1999;33:130–4. [PubMed] [Google Scholar]

15. Weng Y, Guo X, Gregory R, Xie D. A novel antibacterial dental glass-ionomer cement. Eur J Oral Sci. 2010;118:531–4. [PubMed] [Google Scholar]

16. Sidhu S, Nicholson J. A review of glass-ionomer cements for clinical dentistry. J Funct Biomater. 2016;7:16. https://doi.org/10. 3390/jfb7030016.

17. Sidhu SK. Glass ionomer cement restorative materials: A sticky subject? Aust Dent J. 2011;56(Suppl 1):23–30. [PubMed] [Google Scholar]

18. Herrera M, Castillo A, Baca P, Carrión P. Antibacterial activity of glass-ionomer restorative cements exposed to cavity-producing microorganisms. Oper Dent. 1999;24:286–91. [PubMed] [Google Scholar]

19. Türkün LS, Türkün M, Ertuğrul F, Ateş M, Brugger S. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent. 2008;20:29–45. [PubMed] [Google Scholar]
20. Yesilyurt C, Er K, Tasdemir T, Buruk K, Celik D. Antibacterial activity and physical properties of glass-ionomer containing antibiotics. Oper Dent. 2009;34:18–23. [PubMed] [Google Scholar]

21. Herrera M, Castillo A, Bravo M, Liébana J, Carrión P. Antibacterial activity of resin adhesives, glass ionomer and resin-modified glass ionomer cements and a compomer in contact with dentin caries samples. Oper Dent. 2000;25:265–9.

22. Becci A, Marti L, Zuanon A, Brighenti F, Spolidorio D, Giro E. Inuence of the addition of chlorhexidine diacetate on bond strength of a high-viscosity glass ionomer cement to sound and articial caries-affected dentin. J Rev Odontol UNESP 2014;43:1-9.

23. Hatunoğlu E, Oztürk F, Bilenler T, Aksakallı S, Simşek N. Antibacterial and mechanical properties of propolis added to glass ionomer cement. Angle Orthod 2014;84:368-73.

24. Shahriari, Shahriar, et al. "Evaluation of preventive antibacterial properties of a glass-ionomer cement containing purified powder of Salvia officinalis: An in vitro study." *International Journal of Preventive Medicine* 10 (2019).
25. Salimpour F, Ebrahimiyan M, Sharifnia F, Tajadod G. Numerical taxonomy of eight Salvia L. species using anatomical properties. Ann Biol Res. 2012;3:795–805.\

26.Hamidpour M, Hamidpour R, Hamidpour S, Shahlari M. Chemistry, pharmacology, and medicinal property of sage (Salvia) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer. *J Tradit Complement Med.* 2014;4:82–8.

27.Rami K, Li Z. Antimicrobial activity of essential oil of *Salvia officinali* L. collected in Syria. *Afr J Biotech.* 2011;10:8397–402.

28.Sookto T, Srithavaj T, Thaweboon S, Thaweboon B, Shrestha B. *In vitro* effects of *Salvia officinalis* L. Essential oil on *Candida albicans*. *Asian Pac J Trop Biomed.* 2013;3:376–80

29.Kermanshah H, Kamangar SH, Arami S, Mirsalehian A, Kamalineghad M, Karimi M, et al. *In vitro* evaluation of antibacterial activity of hydroalcoholic extract of *Salvia officinalis* and *Pimpinella anisum* against cariogenic bacteria. *J Dent Med.* 2009;22:149–54

30.Beheshti-Rouy M, Azarsina M, Rezaie-Soufi L, Alikhani MY, Roshanaie G, Komaki S, et al. The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: A randomized clinical trial. *Iran J Microbiol.* 2015;7:173–7.

31.Smullen J, Finney M, Storey DM, Foster HA. Prevention of artificial dental plaque formation *in vitro* by plant extracts. *J Appl Microbiol.* 2012;113:964–73.

32.George J, Hegde S, Rajesh KS, Kumar A. The efficacy of a herbal-based toothpaste in the control of plaque and gingivitis: A clinico-biochemical study. *Indian J Dent Res.* 2009;20:480–2

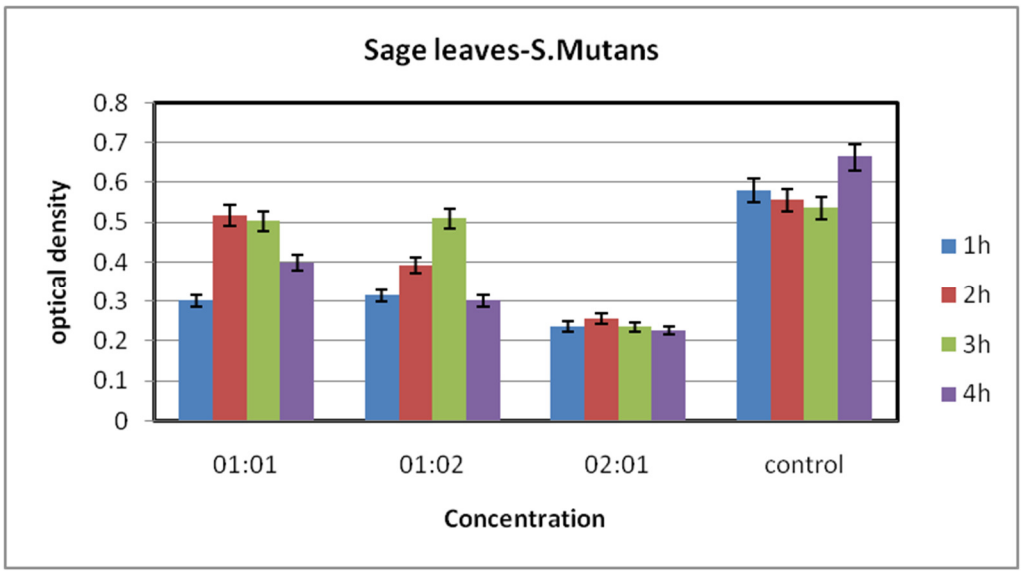

Figure 1:Antimicrobial efficacy against S.mutans

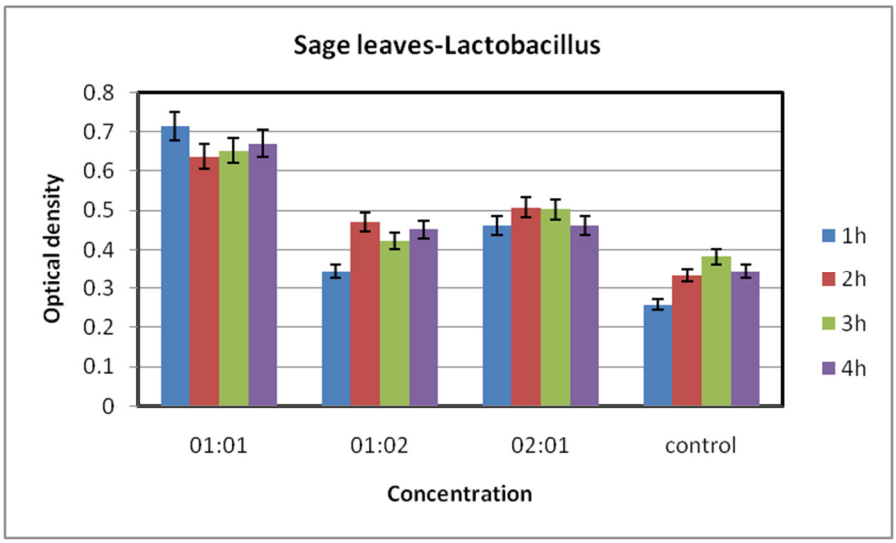

Figure 2:Antimicrobial efficacy against Lactobacillus

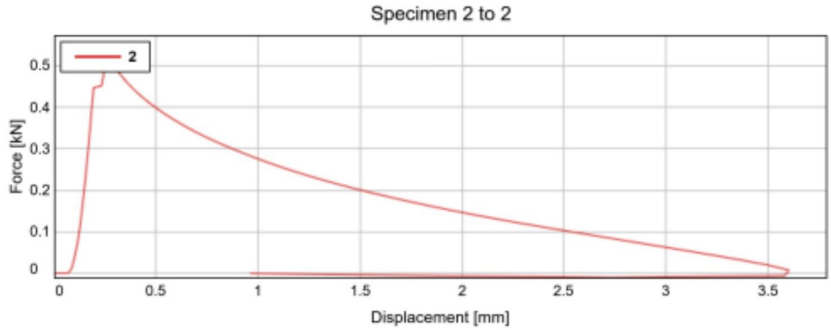

Figure 3:Linear graph compressive strength values of sage leaves modified GIC.