EGYPTIAN DENTAL JOURNAL

VOL. 68, 1819:1826, APRIL, 2022

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

CONSERVATIVE DENTISTRY AND ENDODONTICS

Submit Date : 2021-12-22 • Accept Date : 2022-01-22 • Available online: 2022-03-30 • DOI : 10.21608/edj.2022.111910.1924

ANTIBACTERIAL ACTIVITY AND COMPRESSIVE STRENGTH OF ROSMARINUS OFFICINALIS L. MODIFIED GLASS IONOMER CEMENT

Mayada Said Sultan* *and* Yasser Abdelaziz Abed**

ABSTRACT

Objective: To evaluate the antibacterial activity against Streptococcus mutans and compressive strength of Rosmarinus officinalis L. modified glass ionomer cement with two different concentrations.

Methods: Rosmarinus officinalis L. leaves were macerated to prepare Rosmarinus officinalis L. extract then the rosemary powdered extract was weighted and added to Fuji IX GIC to prepare 2% and 4% rosemary modified glass ionomer. A total of sixty samples were prepared and divided into 3 groups Group1; glass ionomer (GI) as control group; Group 2; 2% rosemary modified glass ionomer (2% R + GI) and Group 3; 4% rosemary modified glass ionomer (4% R + GI). The antibacterial activity against Streptococcus mutans for each group were assessed by disc diffusion methods after 48 hours. Compressive strength test was done with a universal testing machine at the rate of 1mm/min. Data were tabulated and statistically analyzed using One-way ANOVA followed by post-hoc test.

Results: Group 3 (4% R + GI) showed the statistically significant highest antibacterial activity against Streptococcus mutans and compressive strength mean values followed by group 2 (2% R + GI). However, the glass ionomer control group reported the lowest mean values.

Conclusion: Modification of glass ionomer cement with different concentrations of Rosmarinus officinalis L. enhances the antibacterial activity against Streptococcus mutans and compressive strength with superior performance for the higher concentration.

KEY WORDS: Rosmarinus officinalis L., Glass ionomer, Streptococcus mutans, Antibacterial activity, Compressive strength.

* Associate Professor, Operative Dentistry Department, Faculty of Dentistry, Fayoum University.

** Associate Professor, Operative Dentistry Department, Faculty of Dentistry, 6 October University.

INTRODUCTION

Dental caries is one of the most prevalent oral diseases worldwide, that still a challenge imposes a major public health problem due to treatment cost[1]. The incidence of dental caries is depending on Streptococcus mutans which is the main factor that trigger dental caries [2]. The microbial nature of tooth decay necessitates chemical control besides the traditional mechanical control methods. Minimal intervention dentistry focuses on the innovation and development of bioactive restorative materials that provide therapeutic effects [3].

Glass ionomer cement (GIC) is a biomaterial that characterized by its antibacterial properties and ion leachability [4]. It has a degree of antibacterial character, but the literature has proofed that it is not sufficient to diminish the cariogenic bacteria [3, 5]. Attempts have been employed to improve the antibacterial effect of glass ionomer cement by adding synthetic antibacterial agents as antibiotics [6] and chlorhexidine [7,8]. Although chlorhexidine was able to improve the antibacterial activity of glass ionomer but it has an adverse effect on microtensile and shear bond strengths [5]. Perhaps this is the reason why the combination of these antimicrobials with GICs was not yet employed in production [6].

Dental restorations are subjected to combination of forces during clinical service, these forces resulting in development of different types of stresses such as tensile, compressive, and shear stresses [9]. For this reason, the use of glass ionomer cement in high stress bearing areas is limited due to its low compressive strength[6]. Compressive strength testing is considered one of the most common employed test for evaluating the strength properties of restoratives materials [9,10].

Nowadays, alternative natural sources that has antimicrobial effect gained great attention to be incorporated into dental restorative materials. These materials provide efficient, safe, and economical alternatives for treatment and prevention of dental caries [11]. Previous trials revealed improvement in antibacterial activity of glass ionomer upon using some natural extracts such as Miswak, Proplis [12] and Aloe Vera [13].

Rosmarinus officinalis L. is a medicinal plant belongs to the Lamiaceae family which is commonly known as rosemary [14]. It is a perennial bush, originated from the Mediterranean region however, it could be found all over the world [15]. It is an aromatic plant, characterized by its sessile, curved edges, dark green leaves, and very characteristic smell [16]. Rosmarinus officinalis L. can promote several pharmacological effects include antioxidant, antifungal, antimicrobial, and anti-inflammatory actions [17].

Rosmarinus officinalis L. has been used in dentistry due to its antibacterial action against oral bacteria [16]. Rosemary extract was able to reduce bacterial biofilms viability [18]. It has been applied in an experimental toothpaste, and it was effective in inhibiting the growth of bacteria [16] and controlling the bacterial plaque [19]. No studies have been conducted to investigate the effect of incorporation of Rosmarinus officinalis L. extract into GICs. Therefore, this in vitro study was designed to evaluate the antibacterial activity against Streptococcus mutans and compressive strength of Rosmarinus officinalis L. modified glass ionomer with two different concentrations.

MATERIALS AND METHODS

Preparation of Rosmarinus Officinalis L. Extract

Rosmarinus officinalis L. leaves were collected from the experimental plant station, faculty of pharmacy, Cairo university. Leaves were washed under running water, chopped into small pieces, left to air-dried for 6 days in a dark, well-ventilated room, and then ground using electrical blender (Moulinex Genuine Blender, France). A total of 100 g of the powdered leaves were macerated in 200ml ethanol (100%) for 72 hours. The filtrates were collected, evaporated till dryness under vacuum device (Buchi labortechnik AG 9230 Flawil, Switzerland) and subjected to freeze

– drying to yield a dark brown amorphous powdered extract (16g). The extract was prepared in Faculty of Pharmacy, 6 October university.

Preparation of Rosmarinus Officinalis L. Modified Glass Ionomer

A total of 100 mg of the powdered extract were weighed using 4 digit balance (Libror AEG 220, Denver Instrument Company, USA) and dissolved in 5g (4ml) liquid of Fuji IX pack (Gold Label – high strength posterior restorations, GC Corporation, Tokyo, Japan) to obtain 2% concentration. A concentration of 4 % was prepared by dissolving 200mg of the powdered extract in 5g Fuji IX liquid of another pack.

Specimens Grouping

A total of sixty samples were used in the study and divided into 3 groups (n=20, 10 specimens for antibacterial activity test and 10 specimens for compressive strength test). Group1; glass ionomer (GI) as control group; Group 2; 2% rosemary modified glass ionomer (2% R + GI) and Group 3; 4% rosemary modified glass ionomer (4% R + GI).

Antibacterial Activity Test

Thirty glass ionomer specimens (10 specimens for each group) were prepared using a sterilized custom split Teflon mold (5mm in diameter x 2mm in height) for antibacterial assessment. For specimens' preparation, sterilized glass slide covered with a Mylar strip (Stripmat, Polydentia, CH-6805 Mezzovico, Switzerland) was used. The glass ionomer powder and liquid were proportioned according to the manufacturer' instructions and mixed until a homogenous mix was obtained. The materials were packed into the mold in one increment, covered with another Mylar strip and a glass slide, then pressed for 10 seconds to remove the excess material and achieve a uniform smooth surface. Glass ionomer mix was left into the mold till complete setting. The specimens were stored for 24 h in a dry condition till testing.

ATCC 25175 Type strain Streptococcus mutans (16S rRNA gene, Serotype c. carious dentin) were obtained from MIRCEN (Microbiological Resources Centre, Cairo, Egypt) and used in this in vitro study. Bacteria were cultured at 37°C overnight in Trypticase Soy agar (BD 236950, Difco ™, USA) and used as inoculums. The McFarland 0.5 turbidity standard (Densimat, BioMerieux, France) was used to determine the turbidity of the suspension. On that absorbance, the concentration of bacteria is standardized to about 1x CFU/ml and used as a working microbial solution.

A total of 100µl of the previously prepared working microbial solutions was spread evenly over blood agar (blood agar sheep base, CM0854, Oxiod, UK) plate (TSA, Difco, USA). The plates were incubated at 37 °C a bacteriological incubator. The antibacterial effect for all groups were appraised by measuring the diameter of bacterial growth inhibition zones after 48 hours. The diameter of the bacterial growth inhibition zones was measured in millimeters using an electronic digital caliper (STECO, Germany) at three different points to obtain three measurements for each specimen.

Compressive Strength Evaluation

Thirty cylindrical glass ionomer specimens (n = 10) were prepared for compressive strength test using a split plastic mold (4mm in diameter x 6 mm in height). Specimens were prepared in the same manner as mentioned before and stored for 24h at 37ºC in distilled water to ensure complete setting. Specimens were subjected to compressive loading using a universal testing machine (Instron model 3345 England). Compressive force was applied via compression plate at across head speed of 1 mm/min up to specimen failure. The maximum load at fracture was recorded and the compressive strength was calculated using computer software BlueHill.

Statistical Analysis

Numerical data were tested for normality and variance homogeneity using Shapiro-Wilk and Levene's tests respectively. Data showed parametric distribution and variance homogeneity, so they were presented as mean and standard deviation values and were analyzed using one-way ANOVA followed by Tukey's post hoc test. Correlation between antibacterial activity and compressive strength was analyzed using Pearson correlation coefficient. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows*.

RESULTS

One-way ANOVA results showed that there was a significant difference between the tested groups ($p<0.001$). Descriptive statistics of antibacterial activity after 48 hours were represented as mean and standard deviation (SD) values in table (1). Post hoc pairwise comparisons between the tested groups revealed that group 3; (4% R + GI) showed the statistically significantly highest mean inhibition zone diameter followed by group 2; (2% R + GI). Group1; (GI control) showed the lowest mean inhibition zone diameter. Figure (1) showed inhibition zone diameter after 48 hours against Streptococcus mutans around the tested groups.

Descriptive statistics of compressive strength were represented as mean and standard deviation (SD) values in table (2). Post hoc pairwise comparisons between the tested groups revealed that group 3; (4% R + GI) showed the highest mean compressive strength value followed by group 2; (2% R + GI) while group1; (GI control) showed the lowest value.

* R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Results of correlation analysis presented in figure (2) showed that there was a strong positive correlation between antibacterial activity against Streptococcus mutans and compressive strength which was statistically significant ($r=0.980$, $p<0.001$).

TABLE (1) Mean, standard deviation values and intergroup comparisons for antibacterial activity

Parameter	Mean±SD			p-value
	GI (Control)	2% R + GI	4% R + GI	
Antibacterial activity (Bacterial inhibition zone in mm)	11.84 ±0.27^{C}	13.65 ±0.10^{B}	14.75 ±0.25^{A}	**<0.001***

**** Significant (p<0.05)***

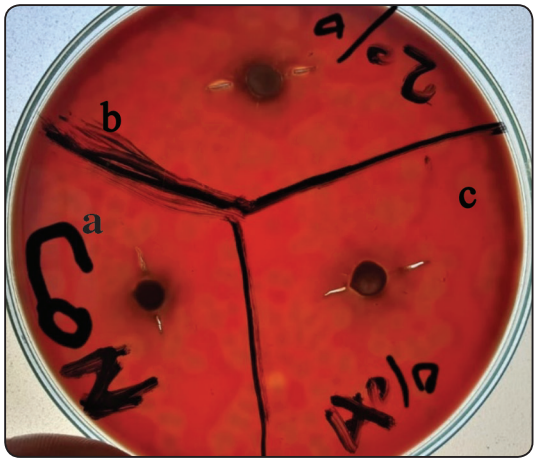

Fig. (1) The bacterial growth inhibition zone diameter after 48 hours against Streptococcus mutans for the different groups; a) GI (control), b) 2% R+ GI, c) 4% R+ GI.

TABLE (2) Mean, standard deviation values and intergroup comparisons for compressive strength

Parameter	Mean±SD			p-value
	GI (Control)	2% R + GI	4% R + GI	
Compressive strength (MPa)	135.72 ±2.42^{C}	197.78 ±1.87^{B}	255.88 ±3.94^{A}	<0.001*

** Significant (p<0.05)*

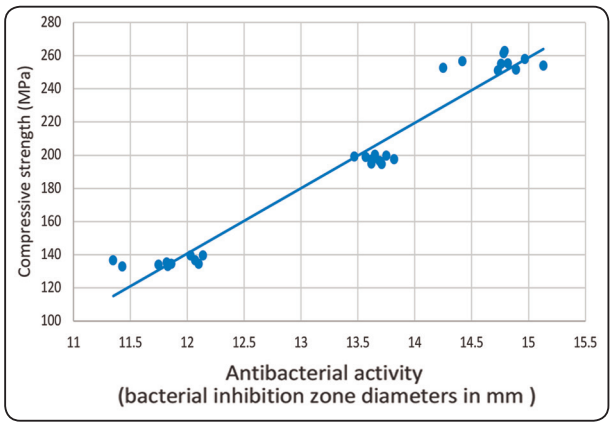

Fig. (2) Scatter plot for the correlation between antibacterial activity against Streptococcus mutans (bacterial inhibition zone diameters in mm) and compressive strength

DISCUSSION

The present study was designed to evaluate the antibacterial activity against Streptococcus mutans and compressive strength of Rosmarinus officinalis L. modified glass ionomer with two different concentrations.

Natural products are valuable sources of potentially effective antimicrobials that have least cost and low levels of toxicity. Recently, plant extracts and their compounds are efficient natural alternatives in prevention of dental caries. One of these materials is Rosmarinus officinalis L., which is a valuable raw material for therapeutic products [20, 21]. Rosemary has been widely used in cooking as food spices to modify and enhance flavors [20]. Also, it used in traditional medicine being a highly appreciated medicinal plant to prevent pain of muscles and joints, cure colds, and rheumatism. Nowadays, rosemary is one of the most popular sources of natural bioactive compounds, as it possesses various pharmacological activities such as antibacterial, antidiabetic, anti-inflammatory, antitumor, and antioxidant [14]. It shows no toxicity, as it is considered 'generally recognized as safe' (GRAS) by the US Food and Drug Administration (FDA) [22].

In the present study, Rosmarinus officinalis L. ethanolic extract was prepared by maceration method in which the powdered materials are left in ethanol solvent for at least three days at room temperature under agitation then, the solution was filtered. This preparation method allows the phytocompounds to be released in the solvent by breaking the cell wall of plant cells [15].

In this in vitro study, all tested groups showed statistically significant effect on mean inhibition zone diameter after 48 hours. Group 3; (4% R + GI) showed the highest mean inhibition zone diameter after 48 hours followed by group 2; (2% R + GI) while the control group showed lowest mean value. This antimicrobial activity is attributed to the active component of Rosmarinus officinalis L. extract as it contains several phytocompounds include caffeic acid, carnosic acid, chlorogenic acid, monomeric acid, oleanolic acid, rosmarinic acid, ursolic acid, camphor, carnosol, eucalyptol, rosmadial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol [15]. These compounds pass through the cell wall and cytoplasmic membrane, disrupting the different layers of fatty acids, phospholipids, and polysaccharides in bacterial cell wall, thereby leading to cell permeability. This led to leakage of ions and other cell contents resulted in bacterial cell death [23].

The results showed that group 3; (4% R + GI) showed the highest mean inhibition zone diameter after 48 hours in comparison to group 2; (2% R + GI). In other words, the higher percentage of Rosmarinus officinalis L. extract in glass ionomer, the greater the antibacterial effect. This might be attributed to the increase in the percentage of terpenoids,

flavonoids, and polyphenols (most commonly diosmin, luteolin, and alpha-pinene) in rosemary extract. Such phytocompounds have been demonstrated antimicrobial activity due to inhibition of nucleic acid synthesis and alteration of cytoplasmic membrane [24]. Results of this study was in accordance with **Valones et al., 2016** [16] who evaluated the antimicrobial activity of a dentifrice containing an alcoholic rosemary extract on Streptococcus mutans. They demonstrated that a dentifrice containing rosemary extract has the capability of inhibiting the growth of Streptococcus mutans. This result was in agreement with another clinical study [19] reported that experimental toothpaste containing Rosmarinus officinalis was effective in controlling bacterial plaque in comparison to conventional fluoridated toothpaste. Also, **Oliveira et al., 2017** [18] analyzed the antimicrobial effect of rosemary extract on Streptococcus mutans biofilm viability and reported significant reduction in biofilm viability.

In the present study, Fuji IX GIC was used as control as it is considered the gold standard in high strength posterior restorations. Concerning the compressive strength of Rosmarinus officinalis L. modified glass ionomer, group 3; (4% R + GI) showed the significantly highest mean value, followed by group 2; (2% R + GI), then control group showed the lowest compressive strength value.

This increase in the compressive strength may be attributed to the chemical composition of Rosmarinus officinalis L. extract as it contains caffeic acid and carnosic acid. These carboxylic acids when added to glass ionomer liquids, they showed increase in the degree of cross-linking together with polysalt bridge formation and subsequently the increase in mechanical properties of the set cement [25].

Moreover, Rosmarinus officinalis L. extract consists of phytocompounds that have OH and COOH functional groups in their chemical formulas such as rosmadial, chlorogenic acid, oleanolic acid, rosmarinic acid, ursolic acid, carnosol, caffeic acid, carnosic acid, and rosmanol [14, 26]. Functional groups OH and COOH are also present in the chemical structure of glass ionomer, which participates in the acid-base reaction between polyacrylic acid and glass particles [26]. The similarity of chemical formula for these compounds with functional groups present in GIC can be explained the increase in compressive strength.

The results of this study showed that group 3; (4% R + GI) has the statistically significant highest compressive strength values in comparison to group 2; (2% R + GI). This direct correlation between the increased concentration of Rosmarinus officinalis L extract in glass ionomer and the increase in compressive strength may be attributed to the increase in the percentage of active ingredients in rosemary modified glass ionomer. It seems that the addition of Rosmarinus officinalis L. extract at both 2% and 4% concentrations improve the compressive strength of glass ionomer cement. As there are no available studies in the literature about the antibacterial activity against Streptococcus mutans and compressive strength of Rosmarinus officinalis L. modified glass ionomer, the result of this study cannot be directly compared.

CONCLUSION

Based on the results of this study with concern to its limitations, modification of glass ionomer cement with different concentrations of Rosmarinus officinalis L. enhances the antibacterial activity against Streptococcus mutans and compressive strength with superior performance for the higher concentration.

RECOMMENDATIONS

1. Further investigations to evaluate the color properties of Rosmarinus officinalis L. modified glass ionomer.
2. Further investigations to evaluate the bond strength of Rosmarinus officinalis L. modified glass ionomer to dentin.

REFERENCES

1. Maheswari SU, Raja J, Kumar A, Seelan RG. Caries management by risk assessment: A review on current strategies for caries prevention and management. J Pharm Bioallied Sci. 2015;7(2): S320-S324.
2. Dziedzic A, Wojtyczka RD, Kubina R. Inhibition of oral Streptococci growth induced by the complementary action of berberine chloride and antibacterial compounds. Molecules. 2015; 20:13705-13724.
3. Fucio SBP, Paula AB, Sardi JCO, Duque C, Correr-Sobrinho L, Puppin-Rontani RM. Streptococcus mutans biofilm influences on the antimicrobial properties of glass ionomer cements. Braz Dent J. 2016; 27(6): 681-687.
4. Kumar RS, Ravikumara N, Kavithaa S, Mahalaxmia S, Jayasreeb R, Sampath Kumarb TS, Haneesha M. Nanochitosan modified glass ionomer cement with enhanced mechanical properties and fluoride release. Int J Biol Macromol. 2017; 104: 1860–1865.
5. Tüzüner T, Dimkov A, Nicholson JW. The effect of antimicrobial additives on the properties of dental glass-ionomer cements: A review. Acta Biomater Odontol Scand. 2019; 5(1): 9–21.
6. Mitta S, Soni H, Sharma DK, Mittal K, Pathania V, Sharma S. Comparative evaluation of the antibacterial and physical properties of conventional glass ionomer cement containing chlorhexidine and antibiotics. J Int Soc Prev Community Dent. 2015; 5: 268-275.
7. Yadik JV, Jampanapalli SR, Konda S, Inguva HC, Chimata VK. Comparative evaluation of the antimicrobial properties of glass ionomer cements with and without chlorhexidine gluconate. Int J Clin Pediatr Dent. 2016; 9(2):99-103.
8. Sreekumar S, Hegde VK. Comparative evaluation of antibacterial effect of three commercially available herbal products against Streptococcus mutans: An in vitro study. J Indian Assoc Public Health Dent. 2018; 16 (1): 75-77.
9. Kiran KV, Tatikonda A, Jhajharia K, Raina S, Lau H, Katare D, Kaur RK. In vitro evaluation of compressive strength of microhybrid and nanocomposite. Oral Health Dent Manag 2014; 13(4):1171- 1173.
10. Souza JCM, Silva JB, Aladim A, Carvalho O, Nascimento RM, Silva FS, Martinelli AE, Henriques B. Effect of zirconia and alumina fillers on the microstructure and mechanical strength of dental glass ionomer cements. Open Dent J. 2016; 10:58-68.
11. Wolde T, Kuma H, Trueha K, Yabeker A. Anti-bacterial activity of garlic extract against human pathogenic bacteria. J Pharmacovigil. 2018; 6(1)1000253: 1-5.
12. Elgamily H, Ghallab O, El-Sayed H, Nasr M. Antibacterial potency and fluoride release of a glass ionomer restorative material containing different concentrations of natural and chemical products: An in-vitro comparative study. J Clin Exp Dent. 2018; 10(4): e312-e320.
13. Sultan MS. Antibacterial effect of Aloe Vera and glass ionomer modified by Aloe Vera on Streptococcus mutans. Egypt Dent J. 2019; 65 (3): 1407-1416.
14. Andrade JM, Faustino C´, Garcia C, Ladeiras D, Reis CP, Rijo P. Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity. Future Sci OA. 2018; 4(4): 283- 299.
15. De Oliveira JR, Camargo SEA, Dias de Oliveira L. Rosmarinus officinalis L. (rosemary) as therapeutic and prophylactic agent. J Biomed Sci. 2019; 26(5):1-22.
16. Valones MAA, Higino JS, Eleutério Souza PR, Crovella S, Caldas Júnior AF, Tavares Carvalho AA. Dentifrice containing extract of Rosmarinus officinalis Linn.: An antimicrobial evaluation. Braz Dent J. 2016; 27(5): 497-501.
17. Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F. Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. Ind Crops Prod. 2015; 71:197–204.
18. Oliveira JR, Jesus D, Dias de Oliveira L. Rosmarinus officinalis L. (Rosemary) extract decreases the biofilms viability of oral health interest. Braz Dent Sci. 2017; 20(1): 64- 69.
19. Valones MAA, Silva ICG, Gueiros LAM, Leão JC, Caldas AF, Carvalho AAT. Clinical assessment of rosemary based toothpaste (Rosmarinus officinalis Linn.): A randomized controlled double-blind study. Braz Dent J. 2019; 30 (2): 146-151.
20. Azad MF, Schwiertz A, Jentsch FR. Adjunctive use of essential oils following scaling and root planning – a randomized clinical trial. BMC Complement Altern Med. 2016; 16(171): 1-8.
21. Mahyari S, Mahyari B, Emami SA, Malaekeh-Nikouei B, Jahanbakhsh SP, Sahebkar A, et al. Evaluation of the efficacy of a polyherbal mouthwash containing Zingiber officinale, Rosmarinus officinalis and Calendula officinalis extracts in patients with gingivitis: A randomized double-blind placebo-controlled trial. Complement Ther Clin Pract. 2016; 22:93-98.
22. Bilgin, G, Yanıkoğlu, F, Tagtekin D. Remineralization potential of herbal mixtures: An in situ study. Indian J Res. 2016; 5(2):264-268.

23. Stojiljkovic J, Trajchev M, Nakov D, Petrovska M. Antibacterial activities of rosemary essential oils and their components against pathogenic bacteria. Adv Cytol Pathol. 2018; 3(4):93–96.
24. Liu Y, McKeever CL, Malik NSA. Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. Front Microbiol. 2017; 8 (113): 1-8.
25. Prentice L, Tyas M, Burrow M. The effect of oxalic acid incorporation on the setting time and strength of a glass-ionomer cement. Acta Biomater. 2006; 2:109–12.
26. Forouzanmehr M, Barekatain M, Shahtalebi MA. An in vitro evaluation of the compressive strength and shear bond strength of resin-modified glass-ionomer cement containing purified powder of Salvia officinalis. Dent Res J. 2020; 17 (5): 319- 325.