Ferreira DCA, Polizeli SAF, da Silva LAB, Küchler EC, de Rossi A. Antibacterial Efficacy of Epigallocatechin-3-gallate against *Streptococcus mutans*: A Systematic Review. Univ Odontol. 2017 Ene-Jun; 36(76). https://doi.org/10.11144/Javeriana.uo36-76.aees

SECCIÓN: Dossier Revisiones Sistemáticas de la Literatura en Odontología
TITULILLO: Epigallocatechin-3-gallate and S. mutans

Antibacterial Efficacy of Epigallocatechin-3-gallate against *Streptococcus mutans*: A Systematic Review

Eficacia antibacteriana de la epigalocatequina-3-galato contra el Streptococcus mutans: Revisión Sistemática

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HOW TO CITE THIS ARTICLE

Ferreira DCA, Polizeli SAF, da Silva LAB, Küchler EC, de Rossi A. Antibacterial Efficacy of Epigallocatechin-3-gallate against *Streptococcus mutans*: A Systematic Review. Univ Odontol. 2017 Ene-Jun; 36(76). https://doi.org/10.11144/Javeriana.uo36-76.aees

Submitted for publication: 25/04/2016

Accepted for publication: 01/05/2017

Available at: http://www.javeriana.edu.co/universitasodontologica

ABSTRACT

Background*:* Green tea, obtained from the *Camellia sinensis*, is one of the most popular drinks worldwide and has recently been in the focus of scientific research due to its beneficial effects on general health. Several studies suggest that, among the polyphenols found on green tea, epigallocatechin-3-gallate (EGCG) is the most bioactive compound and is responsible for its antibacterial activity. ***Purpose:*** To conduct a qualitative systematic review of literature evaluating the antibacterial efficacy of EGCG against *Streptococcus mutans* (*S. mutans*). ***Methods:*** Relevant published studies included in the Pubmed (1966- June 2015), Scopus (1960- June 2015), Web of Science (1900- June 2015), and Google Scholar databases were identified. Publications of *in vitro* studies, which studied EGCG antibacterial efficacy against *S. mutans*, were extracted and pooled in a table. The evaluation included inhibition zone measures, reduction of the number of microorganisms, and biofilm formation. ***Results:*** Twelve studies were selected to compose this systematic review. Eleven of them showed that EGCG has antibacterial efficacy against *S. mutans*. ***Conclusions:*** *In vitro* evidence available confirms the antibacterial activity of EGCG against *S. mutans*.

KEYWORDS

Antimicrobial Agents; *Camellia sinensis*; Dental Caries, Epigallocatechin-3-gallate

THEMATIC FIELDS

Cariology; Catechins; Microbiology

RESUMEN

Antecedentes*:* El té verde, obtenido de la *Camellia sinensis*, es una de las bebidas más populares en el mundo y ha estado recientemente en el foco de atención de la investigación científica por sus efectos benéficos en la salud general. Varios estudios sugieren que, entre los polifenoles encontrados en el té verde, la epigalocatequina-3-galato (EGCG) es el compuesto más bioactivo y es el responsable de su actividad antimicrobiana. ***Objetivo:*** Realizar una revisión sistemática cualitativa de la literatura donde se evalúe la actividad antibacteriana de la EGCG contra el

Streptococcus mutans (*S. mutans*). ***Métodos:*** Se identificaron estudios relevantes incluidos en las bases de datos bibliográficas Pubmed (1966- junio 2015), Scopus (1960- junio 2015), Web of Science (1900- junio 2015) y Google Académico. Los datos de estudios in vitro que investigaron la eficacia antibacterial de la EGCG contra el *S. mutans* se seleccionaron y organizaron en una tabla. La evaluación de los estudios incluyó los criterios: medidas de las zonas de inhibición, reducción del número de microorganismos y formación de biopelícula. ***Resultados:*** Se seleccionaron 12 estudios para la revisión sistemática. Once de ellos comprobaron la eficacia antibacteriana de la EGCG contra el *S. mutans*. ***Conclusiones:*** La evidencia in vitro disponible confirma que la EGCG tiene un efecto antibacteriano contra el *S. mutans*.

PALABRAS CLAVE

agentes antimicrobianos; *Camellia sinensis*; caries dental; epigalocatequina-3-galato

ÁREAS TEMÁTICAS

cariología; catequinas; microbiología

INTRODUCTION

The green tea, originating from the *Camellia sinensis* plant, is one of the most popular drinks and has received special attention by presenting known effects on general health (1,2). Among the components of this tea, Epigallocatechin-3-gallate (EGCG) is the substance found in greater amounts, constitutes about 59% of total substances and the most bioactive component (3).

In addition to these properties, can be highlight the antimicrobial action, several studies have reported the inhibitory effects of EGCG on both Gram-positive and Gram-negative bacteria, such as *Enterococcus spp.*, *Staphylococcus aureus*, *Streptococcus spp.*, *Salmonella spp.*, and *E. coli* (1,2,4-6). In Dentistry, this substance was proposed in toothpaste (7), because it presents a broad spectrum of action against *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*). The action of catechin occurs preventing bacterial adhesion to the tooth surface by inhibiting glucosyltransferase enzyme and bacterial amylase (1, 2, 8).

The primary role of *S. mutans* in the pathogenesis of dental caries resides with it is ability to assemble an insoluble polymeric matrix (9). This microorganism is considered the main and predominant etiologic agent of dental caries and contributes significantly to the virulence of the biofilm, especially in the presence of sucrose (10). There are many studies regarding the use of EGCG in medicine, however few and recent studies have been conducted on the use of EGCG in Dentistry, since this substance is found in a very popular drink that contacts the oral mucosa, it may have antimicrobial efficacy and play an anticariogenic role. The research question behind this systematic review was: Does EGCG have antibacterial efficacy against *S. mutans*? The hypothesis of this study was: EGCG performs an antibacterial efficacy against the microorganisms *S. mutans*. This systematic review evaluated the antibacterial efficacy of the EGCG against *S. mutans*.

METHODS

Originals *in vitro* studies that investigated the EGCG as an antibacterial agent were included, as well as those who evaluated the antibacterial activity of this substance against *S. mutans*, which have been published in the English language.

In vivo, ex vivo, in situ, animals, clinical studies, letters to the editor, case reports, case series, review and studies with explicit convenience sample (institutionalized/hospitalized individuals, vulnerable population, sample selection performed according to the researcher interest) were excluded, as well as patents, conferences, studies evaluating other extracts of green tea, other microorganisms and other studies that have been published in non-English language.

Electronic searches were conducted to identify the published literature in the Pubmed Medline (1966- Feb 2016), SciVerse Scopus (1960- Feb 2016), Web of Science (1900- Feb 2016) and Google Scholar databases. The Google Scholar was used as a program aimed at the international literature.

The search strategy was based on the following Medical Subject Heading terms (MeSH) and free terms: (“epigallocatechin-3-gallate” [MeSH] OR “EGCG” [MeSH] OR “*Camellia sinensis*” [MeSH] OR “green tea” [all]) AND (“*Streptococcus mutans*” [MeSH] OR “*S. mutans*” [all] OR “*Mutans streptococci*” [all]). Data was extracted and pooled in a table, to facilitate the visualization of information.

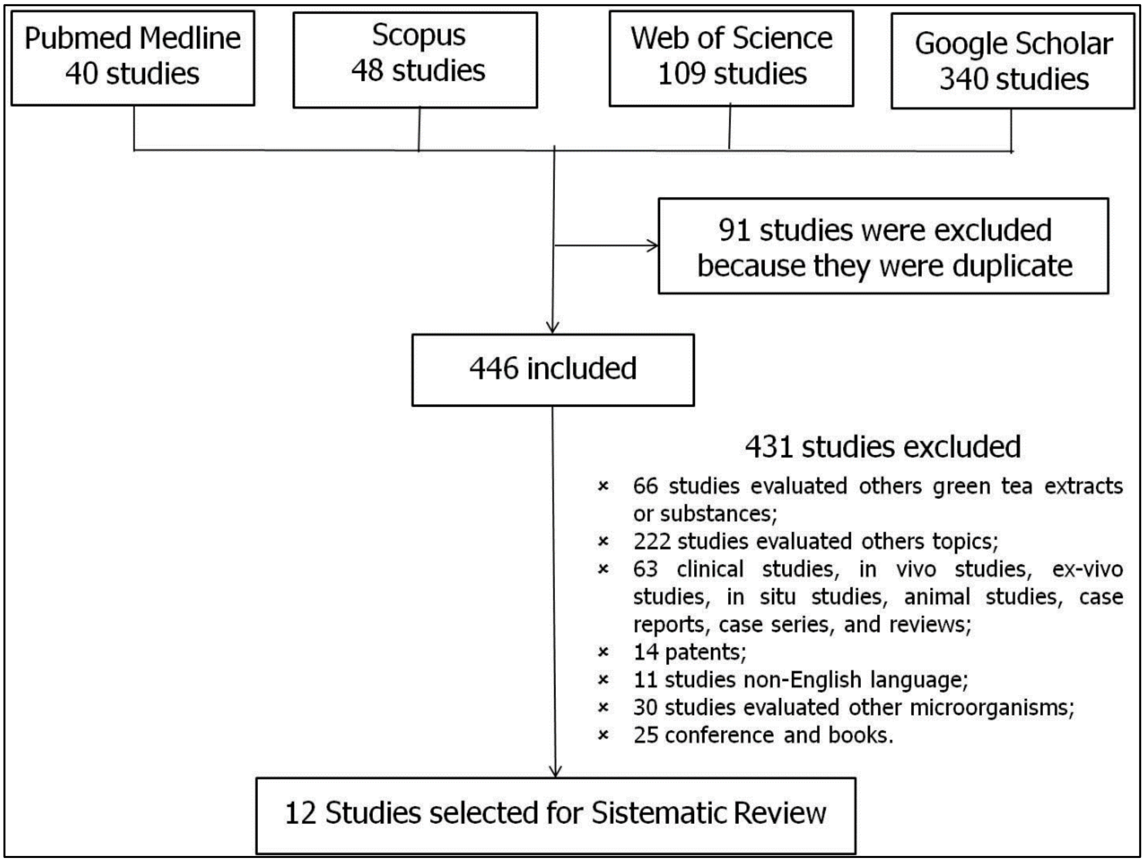
All papers were managed using the software EndNote X7 (Thomson Reuters, New York, NY, US). Duplicate papers were excluded. Titles and abstracts were screened independently by two reviewers based on the criteria mentioned above, (DCAF and SAFP). When there was some sort of disagreement, a third examiner was requested (ECK). After initial screening of titles and abstracts, full articles were evaluated by the same two reviewers. Structured data collection worksheets were employed for the assessment of each publication.

RESULTS

The Figure 1 presents a flow diagram, describing the process of selection of the studies about antimicrobial activity of EGCG against *S. mutans*. In Pubmed Medline database 40 studies were found, in Scopus database 48 studies were found, in Web of Science 109 studies were found, and finally 340 studies were found in the Google Scholar database.

Studies were compared across all database and the studies in duplicate were excluded (91 studies). The remaining studies were analyzed and those, which did not meet the inclusion criteria, were excluded (434 studies). At the end, 12 studies were included in the full-text analysis and all were selected for inclusion in the systematic review. Table 1 (at the bottom of the manuscript) shows the characteristics of the selected studies. The results demonstrated that EGCG present a considerable efficacy against *S. mutans*, by using different methods to analyze the antibacterial activity against this microorganism. Of the 12 studies evaluated, 11 demonstrated that EGCG showed efficacy against the lineage of microorganisms in question. However, 2 of them presented low efficacy, verifying interference in the formation of the biofilm, without causing the total inhibition. Only 1 study showed that EGCG is not an effective agent against *S. mutans*.

FIGURE 1.
FLOW DIAGRAM SHOWING THE PROCESS OF SELECTION OF THE STUDIES



DISCUSSION

Dental caries is the most common oral disease and is an irreversible infectious disease of multifactorial origin, which leads to the destruction of dental hard tissue (10). The biofilm plays an important role in the cause of tooth decay, the cariogenic microorganisms such as *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus oralis* and *Lactobacillus acidophilus* play a vital role in the etiology of the dental caries (11). *Streptococcus mutans* is the major causative agent of dental caries and plays an important role in cariogenic biofilm formation (12, 13). It has been demonstrated that *S. mutans* are the primary etiological agent of caries (12).

Medicinal plants have been used for therapeutic purposes for thousands of years and although their use has been popularly propagated between generations or described in pharmacopoeias, it has also aroused the interest of the current scientific community. Teas generally are rich in biologically active compounds such as flavonoids, catechins, polyphenols, alkaloids, vitamins and minerals that may contribute to the prevention and treatment of various diseases (14).

It is important to emphasize that this systematic review has shown that although the methods used in the included studies are different, the results clearly support the important role of EGCG as an antibacterial agent against *S. mutans*. The EGCG agent inhibits the formation of biofilm and prevents infections (15), it is effective in inhibiting the formation of fermentable carbohydrates involved in the caries formation, which explains it is antimicrobial role (16). Per the findings of this review, 11 studies showed favorable results for EGCG as an effective antibacterial agent against *S. mutans* in *in vitro* studies, acting both inhibiting the growth of the microorganism, as in the biofilm formation and in decreased acids production. Only one study found a negative result regarding the use of EGCG in the decontamination of infected dentin. This lack of effectiveness was justified in the study by the authors for perhaps be some interaction between EGCG with specific sites in collagen molecular structure (17).

Several antimicrobial agents have been suggested, but the agent considered the gold standard is chlorhexidine gluconate (CHX). CHX widely used antimicrobial in Dentistry (18), due to it is strong antibacterial activity and ability to reduce the accumulation of oral biofilms (19). Its efficacy can be attributed to bactericidal and substantivity effects (20), broad spectrum against microorganism, both aerobic and anaerobic, and selectively suppress the growth of caries-associated *S. mutans* (21). CHX presents antimicrobial efficacy *in vitro* and *in vivo* (mouthwash) against salivary microbiota cariogenic and has a role in preventing the development of gingivitis (22). Some studies compared EGCG with CHX (17, 23-26). The results of the comparison between CHX and EGCG showed that both substances were effective in inhibiting microbial the growth and biofilm formation. Other studies (26-31) used as negative control the absence of EGCG and in these situations; it was observed that there was a marked growth of microorganisms compared to the experimental group. One study compared the antimicrobial activity of EGCG whit black, oolong and Pu-erh tea against growth of *S. mutans* (32) and another study had no control group (33).

Different methods have been used to evaluate the efficacy of EGCG against microorganisms, such as inhibition zone measurement, counting colonies forming units, counting of microorganisms and biofilm formation. In addition, different forms of manipulation of the EGCG, in some studies the solution was used (29-31), others incorporated into dental materials (24, 27, 28), and even as specific formula (26). Despite all these variety in the method and material handling, the results suggested that it is a stable substance and with antimicrobial properties and able to inactivate the *S. mutans*, and thus inhibit the formation of biofilm. This systematic review demonstrates a clear efficacy of EGCG against *S. mutans* in *in vitro* studies. However, clinical studies are needed to determine this substance as a potential agent for the prevention of dental caries.

ACKNOWLEDGEMENTS

No conflicts of interest to declare.

REFERENCES

1. Cai Y, Kurita-Ochiai T, Hashizume T, Yamamoto M. Green tea epigalocatechin-3-gallate attenuates *Porphyrmonas gingivalis* – induced atherosclerosis. Pathog Dis. 2013 Feb; 67(1):76-83.
2. Subramanian P, Eswara U, Maheshwar Reddy KR. Effect of different types of tea on *Streptococcus mutans*: an *in vitro* study. Indian J Dent Res. 2012; 23:43-8.
3. McKay DL, Blumberg JB. The role of tea in human health: an update. J Am Coll Nutr. 2002; 21:1-13.
4. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against *Enterococcus faecalis* biofilm formed on tooth substrate: an *in vitro* study. J Endod. 2010; 36:83-6.
5. Reygaert WC. The antimicrobial possibilities of green tea. Front Microbiol. 2014 Aug 20; 5:434.
6. Steinmann J, Buer J, Pietschmann T, Steinmann E. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Br J Pharmacol. 2013 Mar; 168(5):1059-73.
7. Hrishi T, Kundapur P, Naha A, Thomas B, Kamath S, Bhat G. Effect of adjunctive use of green tea dentifrice in periodontitis patients – A Randomized Controlled Pilot Study. Int J Dent Hyg. 2015; 17.

8. Sakanaka S, Kim M, Taniguchi M, Yamamoto T. Antibacterial substance in Japanese Green tea extract against *Streptococcus mutans*, a cariogenic bacterium. Agr Biol Chem. 1989; 53:2307-2311.
9. Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol. 2015; 5:10.
10. Kt S, Kmk M, NB, Jimson SRS. Dental caries vaccine - a possible option? J Clin Diagn Res. 2013;7:1250-3.
11. Janardhanan S, Mahendra J, Girija AS, Mahendra L, Priyadharsini V. Antimicrobial Effects of Garcinia Mangostana on Cariogenic Microorganisms. J Clin Diagn Res. 2017 Jan;11(1):ZC19-ZC22.
12. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. 1986; 50: 353-80.
13. Hamada S, Slade HD. Biology, Immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev. 1980; 44(2): 331–384.
14. Trevisanato SI, Kim YI. Tea and health. Nutr Rev 2000; 58:1-10.
15. Asahi Y, Noiri Y, Miura J, Maezono H, Yamaguchi M, Yamamoto R, Azakami H, Hayashi M, Ebisu S. Effects of the tea catechin Epigallocatechin Gallate on *Porphyromonas gingivalis* biofilms. J Appl Microbiol. 2014; 116:1164-71.
16. Narotzki B, Reznick AZ, Aizenbud D, Levy Y. Green tea: a promising natural product in oral health. Arch Oral Biol. 2012; 57:429-35.
17. Assis JS, Lima RA, Lima JPM, Rodrigues LKA, Santiago SL. Effect of epigallocatechin-3-gallate application for remaining carious dentin disinfection. J Conserv Dent. 2015; 18: 51-55.

18. Twetman, S. Antimicrobials in future caries control? A review with special reference to chlorhexidine treatment. Caries Res. 2004; 38(3):223-9.

19. Drago L, Bortolin M, Taschieri S, De Vecchi E, Agrappi S, Del Fabbro M, Francetti L, Mattina R. Erythritol/chlorhexidine combination reduces microbial biofilm and prevents its formation on titanium surfaces *in vitro*. J Oral Pathol Med. 2016 Dec 9.

20. García-Caballero L, Quintas V, Prada-López I, Seoane J, Donos N, Tomás I. Chlorhexidine substantivity on salivary flora and plaque-like biofilm: an *in situ* model. PLoS One. 2013; 8:e83522.

21. Marsh PD. Microbiological aspects of the chemical control of plaque and gingivitis. J Dent Res. 1992; 71:1431-8.

22. Osso D, Kanani N. Antiseptic mouthrinses: an update on comparative effectiveness, risks and recommendations. J Dent Hyg. 2013; 87:10-8.

23. Anita P, Sivasamy S, Madan Kumar PD, Balan IN, Ethiraj S. *In vitro* antibacterial activity of *Camellia sinensis* extract against cariogenic microorganisms. J Basic Clin Pharm. 2014; 6:35-9.

24. Hu J, Du X, Huang C, Fu D, Ouyang X, Wang Y. Antibacterial and physical properties of EGCG-containing glass ionomer cements. J Dent. 2013; 41:927-34.

25. Mankovskaia A, Lévesque CM, Prakki A. Catechin-incorporated dental copolymers inhibit growth of *Streptococcus mutans*. J Appl Oral Sci. 2013; 21:203-7.

26. Tamura M, Saito H, Kikuchi K, Ishigami T, Toyama Y, Takami M, Ochiai K. Antimicrobial activity of Gel-entrapped catechins toward oral microorganisms. Biol Pharm Bull. 2011; 34:638-43.

27. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent. 2012; 40: 485-92.
28. Saito K, Hayakawa T, Kuwahara NS, Kasai K. Antibacterial Activity and Bond Strength to Enamel of Catechin-Incorporated 4-META/MMA-TBB Resin as an Orthodontic Adhesive Resin. Journal of Hard Tissue Biology. 2011; 20:203-210.
29. Shumi W, Hossain MA, Park DJ, Park S. Inhibitory Effects of Green Tea Polyphenol Epigallocatechin Gallate (EGCG) on Exopolysaccharide Production by *Streptococcus mutans* under Microfluidic Conditions. BioChip J. 2014; 8: 179-186.
30. Xu X, Zhou XD, Wu CD. Tea catechin epigallocatechin gallate inhibits *Streptococcus mutans* biofilm formation by suppressing *gtf* genes. Arch Oral Biol. 2012; 57:678-83.
31. Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. Antimicrob Agents Chemother. 2011; 55:1229-36.
32. Yoshino K, Nakamura Y, Ikeya H, Sei T, Inoue A, Sano M, Tomita I. Antimicrobial activity of tea extracts on cariogenic bacterium *(Streptococcus mutans)*. J. Food Hyg. Soc. Japan. 1996; 37:104-8.
33. Sakanaka S, Kim M, Taniguchi M, Yamamoto T. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. Agric. Biol. Chem. 1989; 53:2307-11.

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Table 1. Characteristics of the selected studies in regarding the method used for antimicrobial efficacy evaluation of EGCG on SM.

Authors/ Year	**Hypothesis/ Objective**	**Comparative group**	**Antimicrobial activity evaluation**	**Authors' Results**	**Additional Findings**	**EGCG Efficacy**
Assis et al.	To evaluate the efficacy of EGCG on the dentin caries	2% Chlorhexidine digluconate	Count forming units of bacteria colonies	EGCG did not reduce SM levels	________	Not effective
Anita et al.	To evaluate antimicrobial activity of EGCG on SM	0.2% Chlorhexidine Digluconate	Inhibition zone measurement	EGCG has antimicrobial activity against SM	________	**Effective**
Shumi et al.	To investigate the inhibitory effect of EGCG on SM biofilm formation	Petri dish without EGCG	Bacteria count	-EGCG did not affect bacterial viability and growth. -Lower acid was produced as EGCG increased	The biomass of biofilm decreased since there was an increase in the concentration of EGCG	Low efficacy
Hu et al.	To evaluate the effect of EGCG added the GIC as antimicrobial	1% Chlorhexidine was added into the GIC	Inhibition zone measurement	EGCG group had lower microbial growth	EGCG increased the flexural strength and hardness, and did not interfere with fluoride release	Low efficacy
Mankovskaia et al.	To evaluate the growth of SM in composite resin incorporated with EGCG	Chlorhexidine	Count forming units of bacteria colonies	EGCG incorporated into composite resin showed growth inhibition of SM	________	**Effective**
Du et al.	To evaluate the antimicrobial potential of a dental adhesive incorporated with EGCG	Dental adhesive	Spatial distribution and architecture of biofilm using SEM	Incorporation of EGCG showed growth inhibition of SM	EGCG did not change the parameters settings of microtensile and degree of conversion.of the resin	**Effective**
Xu et al.	EGCG suppresses glucosyltransferase genes in SM	Wells contained medium without EGCG	- Biofilm formation inhibition (chamber slide model); -Virulence factors (RT-PCR)	EGCG inhibited the *in vitro* growth of SM	EGCG inhibited the Glucosyltransferase B, C, D genes expression.	**Effective**
Xu et al.	EGCG contributes to the suppression of virulence factors of SM	Medium without EGCG	Count forming units of bacteria colonies	EGCG inhibited the *in vitro* growth of SM and biofilm formation.	Acidurity and the virulence factors of SM at the transcriptional and enzymatic levels was suppressed	**Effective**
Saito et al.	To assess the antibacterial efficacy of incorporating catechins into resin against SM	Resin without EGCG	Count forming units of bacteria colonies	EGCG incorporated into resin showed growth inhibition of SM.	EGCG changed shear strength.	**Effective**
Tamura et al.	To evaluate the antimicrobial activities of gel-entrapped catechin on oral microorganisms *in vitro*	-Gel-entrapped catechin (GEC) -Gel-entrapped EGCG (GEEG) -Gel containing 0.03% CHX (CHX-gel)	Inhibition zone measurement	The GEC showed antibacterial effects similar to, or in some cases greater than, those of GEEG . CHX-gel present similar effects to those of GEC.	ECGC was used as a positive control group and not as experimental group.	**Effective**
Yoshino et al.	To compare the antimicrobial activities of extracts of various tealeaves on SM	Others extracts	Count forming units of bacteria colonies	EGCG could suppress the growth of SM.	________	**Effective**
Sakanaka et al.	To determine EGCG mode of inhibition of microbial growth	________	Count forming units of bacteria colonies	EGCG showed growth inhibition of SM.	________	**Effective**

Note: Bold form indicates the EGCG superiority against the control. GIC means glass ionomer cement; SEM means scanning electron microscopy; CHX means chlorhexidine; SM means *Streptococcus mutans.*