RU Revista Uningá

Revista Uningá, v. 59, eUJ4201, 2022
doi.org/10.46311/2318-0579.59.eUJ4201

ISSN 2318-0579

Health Sciences and Biological I, II, III

Psidium guajava, Phalaenopsis sp., *Syzygium aromaticum* and *Cinnamomum verum* as natural inhibitors of growth of *Streptococcus mutans*

Psidium guajava, Phalaenopsis sp., *Syzygium aromaticum* e *Cinnamomum verum* como inibidores naturais do crescimento de *Streptococcus mutans*

Letícia Maria Soares Azevedo[1,2*], Camila Mara dos Reis[2], Marisa Cristina da Fonseca Casteluber[2]

[1]Federal University of Minas Gerais – Department of Microbiology, Belo Horizonte, MG, Brazil.
[2]Minas Gerais State University – Ibirité Unit, Ibirité, MG, Brazil.
*leticiamariaazevedo12@gmail.com

ABSTRACT

Currently, an increase on research on plant extracts seeking therapeutic action in the control of diseases of the oral cavity are observed, especially dental caries. Several plant extracts are already incorporated into toothpastes and mouthwashes. The objective of this study was to evaluate *in vitro* the antibacterial action of *Psidium guajava* (guava), *Phalaenopsis* sp. (orchid), *Syzygium aromaticum* (clove) and *Cinnamomum verum* (Ceylon cinnamon) extracts. The tests were carried out with the oral cavity bacterium *Streptococcus mutans*. The antibacterial activity of plant extracts was evaluated by the disk diffusion method in agar and by minimal inhibitory concentration (MIC). The tests showed the effectiveness of the extracts through inhibition zones of bacterial growth (from 5 to 20 mm in diameter) and MIC of 100 mg. mL^{-1} *Phalaenopsis* sp. extract and 50 mg. mL^{-1} *P. guajava* extract. Complementary studies will be needed to evaluate other pharmacological actions in order to use these plant extracts in different dental specialties and mainly in the treatment and prophylaxis of dental caries.

Keywords: Antibacterial activity. Plant extract. *Streptococcus mutans*.

RESUMO

Atualmente, aumentaram as pesquisas com extratos de plantas buscando a ação terapêutica no controle de doenças da cavidade bucal, especialmente a cárie dental. Diversos extratos vegetais já são incorporados nos dentifrícios e enxaguantes bucais. O objetivo deste estudo foi avaliar *in vitro* a ação antibacteriana dos extratos de *Psidium guajava* (goiabeira), *Phalaenopsis* sp. (orquídea), *Syzygium aromaticum* (cravo-da-Índia) e *Cinnamomum verum* (canela-do-Ceilão). Os testes foram realizados com a bactéria da cavidade bucal *Streptococcus mutans*. A atividade antibacteriana dos extratos vegetais foi avaliada por método de disco difusão em ágar e por concentração inibitória mínima (CIM). Os testes mostraram a eficácia dos extratos por meio de zonas de inibição do crescimento bacteriano (de 5 a 20 mm de diâmetro) e CIM de 100 mg. mL^{-1} extrato da *Phalaenopsis* sp. e de 50 mg. mL^{-1} extrato da *P. guajava*. Estudos complementares serão necessários para avaliar outras ações farmacológicas na finalidade de usar estes extratos vegetais nas diferentes especialidades odontológicas e principalmente no tratamento e profilaxia da cárie dental.

Palavras-chave: Atividade antibacteriana. Extrato vegetal. *Streptococcus mutans*.

Received: November, 12th, 2021. Accepted: June, 07th, 2022.

INTRODUCTION

Dental caries is a self-limiting infectious disease that progress slowly until it completely destroys the tooth structure (Fejerskov & Kidd, 2005). According to Lima (2007), caries is an abnormal process of local physiological imbalance, which requires the interaction of factors such as time, susceptibility, diet and microorganisms to occur, and the main microorganism causing the disorder is bacterium *Streptococcus mutans.*

This bacterium fixes to the tooth enamel forming colonies that produce acids capable of corroding the layers of tooth enamel and causing perforations. These colonies cause a physicochemical imbalance between the tooth substrate and the biofilm present on the tooth surface, causing the destruction of tooth enamel layers (Carvalho et al., 2018).

According to Nunes and Perosa (2017), the history of caries in the Brazilian population, as well as in the world population, is related to several factors, including sociodemographic, psychosocial and environmental factors. The disease can affect the whole population, but there is a predominance of individuals in social vulnerability, because the access to dental treatments, hygiene conditions and food is more precarious. Children are the most affected mainly due to poor hygiene and preference for foods that stimulate the development of caries-causing microorganisms (Álvarez & Fernandez, 2018).

The Ministry of Health (2018) says that caries is a problem that still affects a large part of the Brazilian population, with 53.4% of children up to five years old having decayed teeth, while in adolescence, about 56.5% of the population is in the same situation. This percentage increases with the age of the individual because of the cumulative nature of the factors that favor the development of caries, such as poor hygiene throughout life.

The use of natural products for the treatment of pathologies has intensified. Among these products, plant extracts and essential oils can be highlighted, which offer pharmacologically active compounds capable of fighting and inhibiting the development of many pathogens. Both plant extracts and essential oils have been extensively studied and used clinically and therapeutically, mainly to increase immune resistance and reduce the side effects caused by synthetic antibiotics (Carvalho, 2016; Azevedo, 2019).

The search for alternative treatments using medicinal plants has intensified in Dentistry in recent decades, seeking ways to minimize serious dental problems, such as caries (Pinheiro, Brito, Almeida, Cavalcanti & Padilha, 2012). Many studies show the effectiveness of natural extracts and tinctures against the development of *S. mutans*, as demonstrated by Freires et al. (2010).

Plant extracts and essential oils may be able to suppress or stop the growth of *S. mutans*, and this proposal is an alternative to help prevent infections caused by this bacterium. The antibacterial efficacy of these extracts can provide subsidies for the replacement of antibacterial chemical components in the composition of toothpastes and other oral hygiene products. Therefore, the aim of this research was to evaluate the antibacterial potential *P. guajava*, *Phalaenopsis* sp., *S. aromaticum* and *C. verum* extracts against *S. mutans in vitro.*

MATERIAL AND METHODS

The research was developed in the Applied Microbiology Laboratory of the State University of Minas Gerais – Ibirité Unit (LAMAP). All steps were carried out in a Laminar Flow Chapel, avoiding contact and contamination from the external environment.

The bacterium *Streptococcus mutans* (ATCC 31989) was donated by the Laboratory of Oral and Anaerobic Microbiology of the Department of Microbiology of the Institute of Biological Sciences of the Federal University of Minas Gerais, and then forwarded to the Laboratory of Applied Microbiology of the University of the State of Minas Gerais – Ibirité. The cultivation of *Streptococcus mutans* was performed on Mueller Hinton agar, kept in a bacteriological oven at 37°C for 48 hours

in microaerophilicity and submitted to the Gram Stain test according to the Ministry of Health manual (2001), to confirm the microorganism (Freire et al., 2014; Carvalho, 2016).

The extracts were prepared using: *Psidium guajava* (guava tree); flowers of *Phalaenopsis* sp. (orchid) with all the structures; the dried flower bud *Syzygium aromaticum* (clove of India); and the bark of *Cinnamomum verum* (cinnamon). The plant components of *P. guajava* and *Phalaenopsis* sp. were obtained in the form *in natura* in the vegetable garden and orchard of the Botany Laboratory of the State University of Minas Gerais – Ibirité. The plant components of *S. aromaticum* and *C. verum* were obtained commercially in a natural products store in the Central Market of Belo Horizonte.

The plant extracts were obtained according to the protocol for obtaining extracts proposed by the Brazilian Pharmacopoeia (Agência Nacional de Vigilância Sanitária [Anvisa], 2018). After cleaning, the plant parts used, such as leaves, flowers and bark, were fractionated into small fragments and macerated in ethanol (70% v/v), in which for each 1g of plant material, 5mL of grain alcohol were added. The macerate was stored for seven days, in an amber bottle, at room temperature and in the absence of light, after which it was filtered through a paper filter and stored for later use.

A suspension of the *S. mutans* bacterium was obtained to carry out the tests that evaluated the antibacterial activity of the tested extracts; according to the following experimental protocol. First, a portion of this microorganism, taken from a culture grown in a Petri dish, was inoculated into 50 mL of Mueller Hinton broth. The suspension was shaken and then incubated for 12 hours at 37°C in microaerophilia. After inoculum growth, the suspension was centrifuged at 1500 rpm for 10 minutes. The supernatant was discarded and the pellet resuspended in 15 ml of Mueller Hinton broth.

The bacterial concentration was adjusted to obtain $5x10^{-5}$ UFC mL^{-1} for plating, as described by Veloz, Alvear e Salazar (2019). The bacterium *S. mutans* was inoculated with Mueller Hinton agar by the scattering technique, to perform the disk-diffusion test on agar. Filter paper discs with a diameter of 6 mm, previously soaked in the extracts to be tested, were dispersed on the plate. The discs separately contained *Psidium guajava* (10 µg per disc); *Phalaenopsis* sp. (10 µg per disc); *Syzygium aromaticum* (10 µg per disc); and *Cinnamomum verum* (10 µg per disc) extracts; amoxicillin in the concentration of 10 µg per disc according Sánchez, Varona, Ortega e Ciódaro (2015) (positive control) and alcohol 70% (v/v) (negative control) (10 µg per disc). Alcohol was used as a negative control to demonstrate that the effectiveness of the extracts was due to their antibacterial properties and not to the alcohol present in the extracts.

The plates were incubated for 12 hours in a bacteriological oven at 37º C under anaerobic conditions. Seven tests were performed in triplicate. The bacterial growth inhibition zones were measured after the incubation time with the aid of a caliper and the data obtained were analyzed by the program Graph Pad Prism 5.0 by statistical test TwoWayANOVA.

Minimum inhibitory concentration (MIC) tests were performed according to the methodology described by Carvalho (2016) with modifications, using the micro-dilution method in 96-well micro-plates, with all extracts tested in duplicate. Sterile liquid Mueller Hinton medium was used for dilution by adding 100 µL in each well. The desired concentrations of the extracts were 200 mg. mL^{-1}, 100 mg. mL^{-1}, 50 mg. mL^{-1}, 25 mg. mL^{-1}, 12,5 mg. mL^{-1}, 6,25 mg. mL^{-1}, 3,125 mg. mL^{-1} and 1,5625 mg. mL^{-1} and to get them, 100 µL of the extract was deposited in the first well, homogenized, being removed 100 µL and deposited in the next well. The process was repeated until the concentration of 1,5625 mg. mL^{-1} in the last well, thus obtaining eight different concentrations for the tested extracts. Dilution of 70% alcohol (v/v) (negative control) and amoxicillin (positive control) at the initial concentration of 100 mg. mL^{-1}.

The micro-plate was incubated in microaerophilia (candle flame method) in a bacteriological oven at 37°C for 12 hours (Melo et al., 2006). After incubation, the plate was read on the TP micro-plate reader – Reader NM Thermo Plate at a wavelength of 630 nm. Os resultados foram registrados. The Excel program (2013) was used for data analysis.

Statistical analysis was performed using the Graphpad Prism 5.0 program, Two-wayANOVA tool, performing canalysis of variance and means compared by Tukey's test ($P \leq 0,05$).

RESULTS AND DISCUSSION

The *Psidium guajava*, *Phalaenopsis* sp., *Syzygium aromaticum* and *Cinnamomum verum* extracts were able to inhibit the growth of *Streptococcus mutans*, with MIC of 50 mg. mL^{-1}, 100 mg. mL^{-1}, 50 mg. mL^{-1} and 50 mg. mL^{-1} respectively (Figure 1).

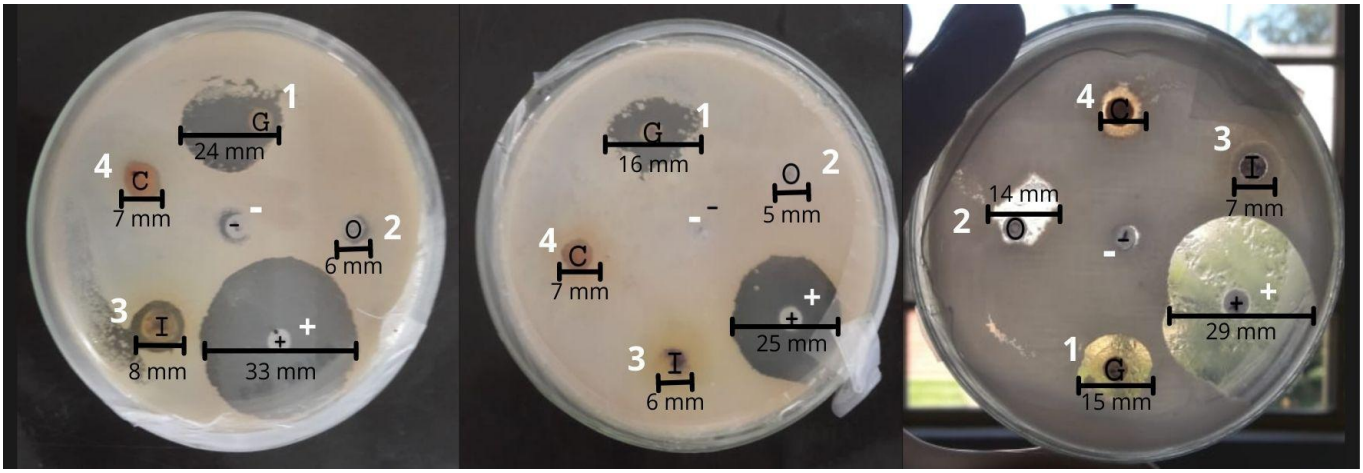

Figure 1. Assay of Antibacterial Activity by disc diffusion method on Agar. Zones of inhibition for *Psidium guajava* (G - 1) (24 mm, 16 mm, 15 mm); *Phalaenopsis* sp. (O – 2) (6 mm, 5 mm, 14 mm); *Syzygium aromaticum* (I – 3) (8 mm, 6 mm, 7 mm), *Cinnamommum verum* (C – 4) (7 mm, 7 mm, 10 mm), Amoxicillin (positive control - +) (33 mm, 25 mm, 29 mm) and alcohol 70% (negative control) (no inhibition zone).
Source: The authors.

Seven disk-diffusion tests were performed, in triplicate, in which the inhibition zones, after 12 hours of incubation in a bacteriological oven, were measured with the aid of a caliper. The average of the inhibition zones in each extract tested is shown in Table 1.

Table 1
Distribution of the averages of the Diameters of the inhibition Zones of the growth the *S. mutans* (mm).

	P. guajava	***Phalaenopsis* sp.**	***S. aromaticum***	***C. verum***	**Amoxicillin**	**Alcohol 70%**
Test 1	16 mm	9 mm	6 mm	8 mm	33 mm	0 mm
Test 2	19 mm	6 mm	8 mm	8 mm	27 mm	0 mm
Test 3	14 mm	7 mm	7 mm	8 mm	30 mm	0 mm
Test 4	20 mm	6 mm	7 mm	8 mm	35 mm	0 mm
Test 5	17 mm	6 mm	10 mm	8 mm	34 mm	0 mm
Test 6	12 mm	6 mm	6 mm	7 mm	27 mm	0 mm
Test 7	16 mm	6 mm	7 mm	8 mm	28 mm	0 mm

Source: The authors.
Notes: The Table expresses the means of inhibition zones obtained in the seven (7) tests performed for the *P.guajava*, *Phalaenopsis* sp., *S. aromaticum*, *C. verum* extracts, positive and negative control.

After seven (7) tests in triplicate the inhibition zones of bacterial growth were measured and the means described in mm were obtained. The *P. guajava* extract obtained an average of inhibition

zones between 12 mm and 20 mm. The *Phalaenopsis* sp. extract obtained an average of inhibition zones between 6 mm and 9 mm. The *S. aromaticum* extract obtained an average of inhibition zones between 6 mm and 10 mm. The *C. verum* extract obtained an average of inhibition zones between 7 mm and 8 mm.

The data obtained demonstrate that the *P. guajava* extract with inhibition zones from 12 to 20 mm in diameter, was the one that showed the greatest ability to inhibit the growth of *S. mutans*, with relevant statistical differences ($p<0.001$) when compared to *Phalaenopsis* sp. extract (6 mm diameter inhibition zones), *S. aromaticum* ($p<0.01$) (8 mm diameter inhibition zones) and *C. verum* ($p<0.01$) (8 mm inhibition zones), showing to be more effective in inhibiting the studied bacterium. Statistical analysis demonstrates the effectiveness among the tested extracts (Figure 2).

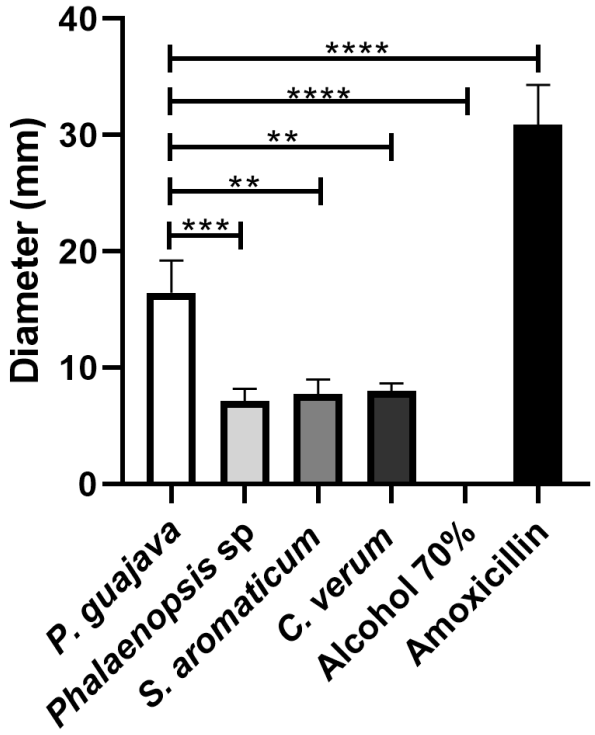

Figure 2. Efficacy of extracts tested as inhibitors of *Streptococcus mutans*. The graph shows the statistical difference in the mean inhibition zones between the tested extracts and the positive and negative controls.
Source: The authors.

The *P. guajava* extract showed a statistical difference between all the tested extracts and the controls. *P. guajava* and *Phalaenopsis* sp.: statistical difference of $p<0.001$. *P. guajava* and *S. aromaticum*: statistical difference of $p<0.01$. *P. guajava* and *C. verum*: statistical difference of $p<0.01$. *P. guajava* and Amoxicillin: statistical difference of $p<0.0001$. *P. guajava* and alcohol 70%: statistical difference of $p<0.0001$. The other extracts have not showed no statistical difference between them or between the negative control.

When compared to the positive control, the inhibition presented by *P. guajava* extract was not as effective as amoxicillin ($p<0.0001$). In the study by Alves, Queiroz, Pereira and Pereira (2009), the *P. guajava* extract was able to inhibit bacteria that colonize the mouth with MIC of 1:4, in addition to some genera of *Candida*, with MIC of 1:32. Brighenti et al. (2008) showed that other species of the genus *Psidium* have antimicrobial potential against *S. mutans*. The aqueous extract of the leaf of *P. guajava* also has effects on cariogenic bacteria, being able to prevent the appearance of caries in

rats, according to the results of the work of Menezes, Delbem, Brighenti, Okamoto and Gaetti-Jardim (2010). In the present study, the MIC for *P. guajava* found was 50 mg. mL^{-1}. Even so, the fact that *P. guajava* extract was able to inhibit the bacterium under study, it represents an alternative in times when microorganisms have been increasingly resistant to widely used synthetic antibiotics.

As for the *Phalaenopsis* sp. extract, Bertolino (2015) showed inhibition using extracts from orchid species other than *Phalaenopsis*. Orchids of the genus *Dendrobium* showed better inhibition of *Enterococcus faecalis* and *Escherichia coli* at a concentration of 50 mg/mL, and the extract of orchids of the genus *Cymbiduim* was able to inhibit *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* also at a concentration of 50 mg/mL. Research on antibacterial activity against *S. mutans* with this plant species has not been reported. However, in the research in question, the MIC of the extract of this plant was 100 mg. mL^{-1}.

The *S. aromaticum* essential oil was used by Nogueira, Diaz, Tagami and Lorscheide (2007) to inhibit the growth of *Streptococcus mutans* and *Lactobacillus casei*, in the agar diffusion test with *S. mutans*, inhibition zones of 9 mm were observed. In the present study, inhibition zones of approximately 8 mm and MIC of 50 mg. mL^{-1} were observed for the extract of the same plant species. However, in this study, the alcoholic extract was used, which is easily accessible and cost-effective.

As for the extract of *C. verum*, studies have demonstrated the inhibitory potential of the species. Carvalho (2016) demonstrated the inhibitory action of *C. verum* against *S. mutans* with inhibition zones of approximately 8 mm in diameter in the antimicrobial activity assay. In the present study, inhibition zones of the same diameter were observed in the tests performed. The MIC of the reported study was lower than in the current research, being 0,156 mg. mL^{-1} for the first case and 50 mg. mL^{-1} no for the second case. It is worth mentioning that the study conducted by Carvalho (2016) used the essential oil of *C. verum*, which has a higher concentration of antibacterial compounds when compared to the crude extract of the plant.

All extracts tested as potential inhibitors of *S. mutans* growth showed inhibition zones. Furthermore, the MIC values of the *P. guajava*, *Phalaenopsis* sp., *S. aromaticum* and *C. verum* extracts tested were satisfactory, considering that the crude extract has less antibacterial components compared to the essential oil of these vegetables. However, the extract with the best results for both methodologies used (disk-diffusion test in agar and test of the minimum inhibitory concentration) was the extract of *P. guajava*, since it presented larger zones of inhibition.

CONCLUSION

The results showed that the *P. guajava*, *Phalaenopsis* sp., *S. aromaticum* and *C. verum* extracts have potential antibacterial action against strains of *S. mutans*, observed through inhibition zones of bacterial growth. However, none of the plant extracts tested was statistically similar or superior to the inhibition potential of the tested antibiotic, Amoxicillin. The data obtained in this study allow us to propose that new tests using the essential oil of *P. guajava* can be studied against *S.mutans*, since, in *in vitro* tests, this was the extract that showed the best antibacterial activity. Thus, this extract becomes the best option for future studies that can make it viable as a potential component of oral hygiene products, which can be used both as a treatment and as a prophylaxis of dental caries formation.

REFERENCES

Álavarez, J. J. R., & Fernandez, D. Y. B. (2018). Conocimientos de higiene oral de acudientes y su relación con caries en menores de 5 años. *Revista Facultad Nacional de Salud Pública*, *36*(2), pp. 1-17. doi: 10.17533/udea.rfnsp.v36n2a03

Alves, P. M., Queiroz, L. M. G., Pereira, J. V., & Pereira, M. S. V. (2009). Atividade antimicrobiana, antiaderente e antifúngica *in vitro* de plantas medicinais brasileiras sobre microrganismos do

biofilme dental e cepas do gênero *Candida. Revista da Sociedade Brasileira de Medicina Tropical*, *42*(2), pp. 222-224. doi.org/10.1590/S0037-86822009000200028

Anvisa. Agência Nacional de Vigilância Sanitária. (2018). *Formulário de Fitoterápicos Farmacopeia Brasileira.* 1. ed., Primeiro Suplemento. Recuperado de https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/formulario-fitoterapico

Azevedo, L. M. S. (2019). *Psidium guajava, Phalaenopsis sp., Syzygium aromaticum e Cinnamomum verum como inibidores naturais do crescimento de Streptococcus mutans.* (Monografia de Graduação em Ciências Biológicas). Universidade do Estado de Minas Gerais, Ibirité, Brasil.

Bertolino, B. F. C. (2015). *Avaliação da atividade antimicrobiana dos extratos de Dendrobium sp e Cymbidium sp*. (Monografia de Graduação em Farmácia). Faculdade de Pindamonhangaba, Pindamonhangaba, Brasil.

Brasil. Ministério da Saúde. Secretaria de Políticas de Saúde. Programa Nacional de DST e Aids. (2001). *Técnica de Coloração de Gram*. Recuperado de https://bvsms.saude.gov.br/bvs/publicacoes/115_03gram.pdf

Brasil. Ministério da Saúde. (2018). *A saúde bucal no Sistema Único de Saúde.* Brasília: Ministério da Saúde. Recuperado de https://aps.saude.gov.br/biblioteca/visualizar/MTI2Nw==

Brighenti, F. L., Luppens, S. B. I., Delbem, A. C. B., Deng, D. M., Hoogenkamp, M., Gaetti-Jardim E. Jr., ... Cate, J. M. (2008). Effect of *Psidium cattleianum* leaf extract on *Streptococcus mutans* viability, protein expression and acid production. *Caries Research, 42*(2), pp. 148-154. doi: 10.1159/000121439

Carvalho, I. O. (2016). *Atividade antibacteriana de óleos essenciais e cremes dentais fitoterápicos em bactérias cariogênicas*. (Dissertação de Mestrado em Bioquímica Aplicada). Universidade Federal de Viçosa, Viçosa, Brasil.

Carvalho, T. P., Maske, T. T., Signori, C., Brauner, K. V., Oliveira, E. F., & Cenci, M. S. (2018). Desenvolvimento de lesões de cárie em dentina em um modelo de biofilme simplificado *in vitro*: um estudo piloto. *Revista de Odontologia da UNESP, 47*(1), pp. 40-44. doi.org/10.1590/1807-2577.06017

Fejerskov, O., & Kidd, E. (2005). *Cárie dentária: a doença e seu tratamento clínico.* 1. ed. São Paulo: Santos.

Freire, I. C. M., Pérez, A. L. A. L., Cardoso, A. M. R., Mariz, B. A. L. A., Almeida, L. F. D., Cavalcanti, Y. W., & Padilha, W. W. N. (2014). Atividade antibacteriana de óleos essenciais sobre *Streptococcus mutans* e *Staphylococcus aureus. Revista Brasileira de Plantas Medicinais, 16(*2), suppl. 1. doi.org/10.1590/1983-084X/12_053

Freires, I. A., Alves, L. A., Jovito, V. C., Almeida, L. F. D., Castro, R. D., & Padilha, W. W. N. (2010). Atividades antibacteriana e antiaderente *in vitro* de tinturas de *Schinus terebinthinfolius* (Aroeira) e *Solidago microglossa* (Arnica) frente a bactérias formadoras do biofilme dentário. *Odontologia Clínico-Científica, 9*(2), pp. 139-143.

Lima, J. E. O. (2007). Cárie dentária: um novo conceito. *Revista Dental Press Ortodontia e Ortopedia Facial*, 12(6), pp. 119-130. doi.org/10.1590/S1415-54192007000600012

Melo, A. F. M., Santos, E. J. V., Souza, L. F. C., Carvalho, A. A. T., Pereira, M. S. V., & Higino, J. S. (2006). *In vitro* antimicrobial activity of an extract of *Anacardium occidentale L.* against *Streptococcus* species. *Revista Brasileira de Farmacognosia., 16* (2). doi.org/10.1590/S0102-695X2006000200012

Menezes, C. T. E., Delbem, A. C. B., Brighenti, F. L., Okamoto, A. C., & Gaetti-Jardim, E. (2010). Protective efficacy of *Psidium cattleianum* and *Myracrodruon urundeuva* aqueous extracts against caries development in rats. *Pharmaceutical Biology, 48*(3), pp. 300-305. doi: 10.3109/13880200903122202

Nogueira, M. A., Diaz, M. G., Tagami, P. M., & Lorscheide, J. (2007). Atividade microbiana de óleos essenciais e extratos de própolis sobre bactérias cariogênicas. *Revista de Ciências Farmacêuticas Básica e Aplicada, 28*(1), pp. 93-97.

Nunes, V. H., & Perosa, G. B. (2017). Cárie dentária em crianças de 5 anos: fatores sociodemográficos, lócus de controle e atitudes parentais. *Ciência Saúde Coletiva, 22*(1), pp. 191-201. doi.org/10.1590/1413-81232017221.13582015

Pinheiro, M. A., Brito, D. B. A., Almeida, L. F. D., Cavalcanti, Y. W., & Padilha, W. W. N. (2012). Efeito antimicrobiano de tinturas de produtos naturais sobre bactérias da cárie dentária. *Revista Brasileira em Promoção da Saúde, 25*(2), pp. 197-201. doi: 10.5020/18061230

Sánchez, S. B., Varona, F. S. S., Ortega, H. D., & Ciódaro, A. R. (2015). Resistencia a la amoxicilina de cepas de *Streptococcus mutans* aisladas de indivíduos con antibioticoterapia previa y sin esta. *Universitas Odontológica, 34*(72), pp. 101-106. doi.org/10.11144/Javeriana

Veloz, J. J., Alvear, M., & Salazar, L. A. (2019). Antimicrobial and antibiofilm activity against *Streptococcus mutans* of individual and mixtures of the main polyphenolic compounds found in Chilean propolis. *BioMed Research International*, 7602343, pp. 1-7. doi: 10.1155/2019/7602343