International Journal of Dental Research and Allied Sciences
2022, Volume 2, Issue 2, Page No: 1-6

Available online at: www.tsdp.net

ISSN: 3062-3502

Original Article

A Comparative Assessment of the Impact of *Citrullus lanatus* Rind and Pulp Extracts on *Streptococcus mutans*

Aravinthrajkumar Govindaraj[1]*, Saravanadinesh Sudalaimani Paulpandian[2], Rajeshkumar Shanmugam[3]

[1]Department of Orthodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai, India.
[2]Department of Orthodontics and Dentofacial Orthopedics, Sri Ramakrishna Dental College and Hospital, Coimbatore, Tamil Nadu- 641006.
[3]Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical And Technical Sciences, Chennai, India.

***E-mail** ✉ draravinthrajkumar.sav@gmail.com

Received: 14 May 2022; **Revised:** 22 August 2022; **Accepted:** 27 August 2022

ABSTRACT

This study aims to explore and compare the in vitro effects of *Citrullus lanatus* rind and pulp extracts on *Streptococcus mutans*. The extracts were obtained from fresh watermelon and tested at 4 different concentrations: 25 microliter, 50 microliter, 100 microliter, and 150 microliter. Four wells on each of the four MHA plates inoculated with *S. mutans* were filled with the respective extracts, with two plates containing the varying concentrations of each extract. The plates were then incubated at 37 °C for 24 hours, and the zones of inhibition were measured. The mean inhibition zones observed around the wells containing the pulp extract at 25, 50, 100, and 150 microliters were 27, 28, 31, and 36 mm, respectively, while the corresponding zones for the rind extract were 26, 28, 31, and 35 millimeters. Both extracts demonstrated a significant increase in antibacterial activity as the concentration rose, although no major difference was noted between the pulp and rind extracts. Based on the findings, it can be concluded that *C. lanatus* extracts exhibit notable antibacterial properties against *S. mutans*. Plant-based alternatives are increasingly being explored for their affordability, reduced side effects, and accessibility. Consequently, research is focusing on herbal and plant-derived options like *C. lanatus* as viable methods to enhance oral hygiene through their antimicrobial effects.

Keywords: White spot lesions, *Streptococcus mutans*, Antibacterial activity, Watermelon

How to Cite This Article: Govindaraj A, Paulpandian SS, Shanmugam R. A Comparative Assessment of the Impact of *Citrullus lanatus* Rind and Pulp Extracts on *Streptococcus mutans*. Int J Dent Res Allied Sci. 2022;2(2):1-6. https://doi.org/10.51847/ffD4G8LgXK

Introduction

The oral cavity harbors millions of microorganisms, with *Streptococcus mutans* and *Lactobacillus* spp. being among the most prevalent [1]. These microorganisms contribute to the development or progression of various oral diseases and conditions. Plaque and calculus, which are universally common in adults, consist primarily of *Lactobacillus* spp., *S. mutans*, and mineral particles from saliva [2].

Dental caries, a widespread infectious condition, impacts approximately 60%–90% of both adults and children [3]. Its role in the global oral health burden is significantly greater than periodontal diseases and other common oral health issues, making it a pandemic. The high occurrence of untreated carious lesions leads to pain, discomfort, and impaired function [4]. The condition arises from a complex, multifactorial interaction between the tooth, bacteria, and time, resulting in the dissolution of hydroxyapatite crystals and demineralization of the tooth structure [5, 6].

In recent years, fixed orthodontic treatment has become increasingly popular, with a growing number of patients choosing this option. The length of treatment can vary, typically lasting from 1-2 years, depending on the type of malocclusion. This extended treatment period can lead to various complications. A significant clinical issue associated with fixed orthodontics is enamel demineralization around the brackets. The accumulation of plaque and calculus around orthodontic components such as brackets, elastomeric modules, wires, and bands is frequently observed. Additionally, it has been observed that patients undergoing fixed orthodontic treatment often experience alterations in the bacterial composition within their oral cavity [2].

Microorganisms have been extensively studied for their involvement in the formation of plaque and calculus, as well as in the onset of dental caries and white spot lesions in both primary and permanent teeth [7]. Research has shown that in addition to *S. mutans*, other bacteria such as *Lactobacillus*, *Propionibacterium*, *Veillonella*, *Bifidobacterium*, and non-*S. mutans* streptococci with low pH, *Actinomyces*, and *Atopobium* also contribute to the development and progression of caries. Furthermore, it has been noted that non-*S. mutans* streptococci and *Actinomyces* are involved in the early stages of caries formation [8]. Karpiński *et al*. [9] indicated that *Streptococcus* and *Lactobacillus* species play a key role in the development of caries. According to a systematic review by Tanzer *et al*. [10], *S. mutans* was found to have a major role in the initiation of caries on smooth surfaces, pits, fissures, and root surfaces.

Although chlorhexidine is renowned for its strong antibacterial and antiplaque properties, its prolonged and widespread use is restricted due to several well-documented adverse effects. The most frequent local side effect observed, particularly when used as a mouthwash, is extrinsic staining of both artificial and natural teeth, with the intensity of discoloration being directly linked to its concentration [11, 12].

In recent times, traditional medicine has gained increased recognition for its effectiveness. Plant-based products have become particularly relevant in therapy because they typically present fewer side effects, are more accessible, and are generally more affordable. *Citrullus lanatus*, commonly known as watermelon and belonging to the Cucurbitaceae family, is a widely recognized indigenous fruit. Its bioactive components—such as triterpenes, cucurbitacin, sterols, and alkaloids, along with various vitamins and minerals—have spurred research into its therapeutic benefits [13]. Various studies have explored the antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-ulcerogenic, and laxative properties of the seeds, rind, and pulp of *C. lanatus*, yielding promising results. The rind of a watermelon demonstrates superior antioxidant and antibacterial properties compared to the pulp. In addition to their potent antioxidant and antimicrobial capabilities, the rind and seeds are also rich in phenolic compounds [14]. Despite these benefits, there is limited research examining the therapeutic potential of various parts of *C. lanatus*, particularly its antibacterial effects. Furthermore, determining the optimal concentration for effective performance remains an area that requires further study. As a result, the present research aimed to evaluate and compare the antibacterial properties of the pulp and rind extracts of *C. lanatus* against *S. mutans* bacteria.

Materials and Methods

The in vitro investigation was performed at the Department of Pharmacology and the Dental Research Cell at Saveetha University, Chennai, India, following the approval from the university's scientific research council and clearance from the Institutional Human Ethical Committee (IHEC/SDC/ORTHO-1705/21/360). For the preparation of pulp and rind extracts, the study used the Kashish variety of *C. lanatus*, which is commonly grown in Tamil Nadu.

Preparation of pulp extract

The pulp of fresh watermelon was carefully sliced with a sterile knife, the seeds removed, and then mashed with a mortar and pestle to extract 100 ml of fresh juice. To create the pulp extract, the juice was heated using a Borosil HME 250 heating mantle until it was concentrated, reducing the 100 ml of juice down to 5 ml of extract. Four distinct concentrations of the pulp extract were then prepared: 25, 50, 100, and 150 microliters, as shown in **Figure 1**.

Figure 1. 100 milliliters of juice extracted from the pulp of *C. lanatus.*

Preparation of rind extract

The freshly harvested watermelon rind was thoroughly cleaned, chopped into small pieces, and mashed into a paste. This paste was then mixed with one hundred milliliters of distilled water. The mixture was heated using a Borosil HME 250 heating mantle, which facilitated the evaporation of the water, reducing the volume from 100 milliliters to 5 milliliters, yielding the rind extract. Additionally, four concentrations of the rind extract were prepared: 25, 50, 100, and 150 microliters **(Figure 2)**.

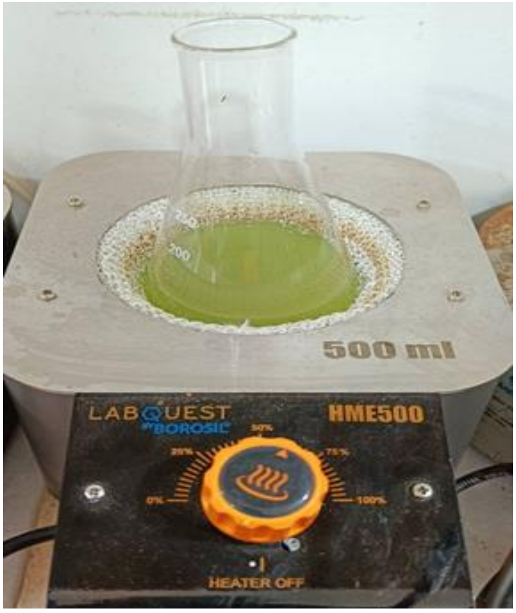

Figure 2. The rind extract was heated using a Borosil HME 500 ml heating mantle.

Evaluation of antimicrobial activity

Muller Hinton agar (MHA) was prepared and sterilized at 120 lbs for 45 minutes. Once sterilized, the media was poured into four sterile plates and left to solidify. *S. mutans*, obtained from cultures isolated and identified from oral swabs of orthodontic patients, was used for the research. The test organisms were swabbed onto the surface of the four MHA agar plates. Using a gel puncher, four wells were made in each plate, and the 4 different concentrations of pulp extract (25, 50, 100, and 150 microliters) were added to the wells of two of the plates. A similar method was followed for the rind extract, with two additional plates being prepared in the same way. The plates were incubated at 37 °C for 24 hours. After incubation, the inhibition zones for both pulp and rind extracts were measured using a scale. The collected data was recorded, tabulated, and analyzed for both intragroup and intergroup comparisons **(Figures 3 and 4)**.

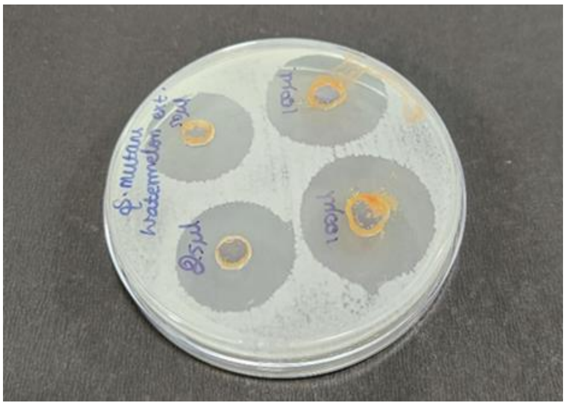

Figure 3. *S. mutans* swabbed MHA plates with wells loaded with different doses of pulp extract of *C. lanatus* (25, 50, 100, and 150 microliters).

Figure 4. *S. mutans* swabbed MHA plates with wells loaded with different doses of pulp extract of *C. lanatus* (25, 50, 100, and 150 microliters).

Results and Discussion

After 24 hours of incubation, the MHA plates displayed clear zones of inhibition in both groups at various concentrations. The average zone of inhibition around the wells with 25, 50, 100, and 150 microliters of rind extract was 26, 28, 31, and 35 mm, respectively. A comparison within the group showed that antimicrobial activity increased with higher concentrations, with the 150 microliter concentration showing the greatest inhibitory effect compared to the 25 microliter concentration, which had the least **(Table 1, Figure 5)**.

Similarly, the mean inhibition zone for wells with 25, 50, 100, and 150 of pulp extract was 27, 28, 31, and 36 mm, respectively. The intra-group comparison revealed a rise in antimicrobial activity with increasing concentration, with the 150 microliter pulp extract concentration exhibiting the highest inhibitory effect, while the 25 microliter concentration demonstrated the lowest **(Table 1, Figure 5)**.

Table 1. The zone of inhibition measured in different concentrations of the extract

	25 µL	50 µL	100 µL	150 µL
Rind extract	26 mm	28 mm	31 mm	35 mm
Pulp extract	27 mm	28 mm	31 mm	36 mm

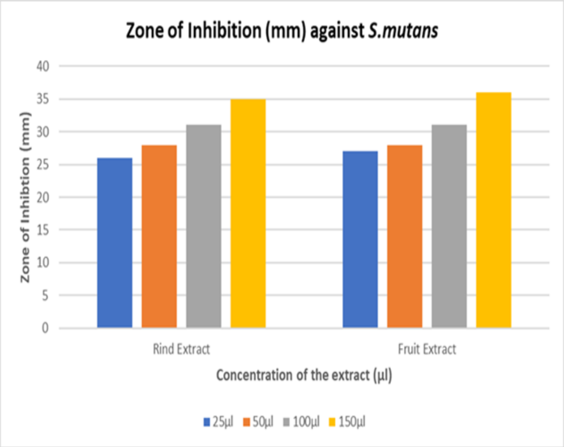

Figure 5. The zone of inhibition measured in different concentrations of the rind and pulp extracts.

When comparing the two groups, it is noteworthy that no significant difference was observed between the inhibition zones of the rind and pulp extracts of *C. lanatus*. Based on the findings from this research, it can be concluded that both rind and pulp extracts of *C. lanatus* demonstrated effective antibacterial properties at varying concentrations, showing distinct zones of inhibition on MHA plates.

The findings of this study indicate that both rind and pulp extracts of *C. lanatus* exhibit substantial antibacterial activity against *S. mutans* at the different tested concentrations. The common side effects of antibacterial mouth rinses, including staining and altered taste sensation, highlight the growing need for alternative solutions in oral hygiene maintenance with minimal or no adverse effects. This study, therefore, sought to explore natural and herbal alternatives as potential reinforcements for improving oral hygiene, driven by the need to investigate the effectiveness of such products.

Researchers globally have redirected their attention toward natural alternatives, recognizing their benefits which were previously known but lacked precise scientific validation. As a result, various natural products have been explored. Cildir *et al*. [15] found that consuming yogurt with probiotic bacteria daily reduced the presence of *S. mutans* in the saliva of individuals undergoing fixed orthodontic treatment. They also highlighted that consuming fruit yogurt with *Bifidobacterium animalis* daily could lower *S. mutans* levels in saliva. According to Khameneh *et al*. [16], phytochemicals are a significant source of bioactive compounds with powerful antibacterial properties.

Limsong *et al*. [17] examined the inhibitory effect of *Andrographis paniculata*, *Cassia alata*, Chinese black tea, and *Harrisonia perforata* on *S. mutans* adhesion, with similar results found for propolis extract, which also exhibited a notable impact on the bacteria [18]. Philip *et al*. [19] proposed that cranberry extract not only effectively hindered the virulence of *S. mutans* without harming bacterial viability but could also play an ecological role as a non-bactericidal agent, potentially altering the pathogenic characteristics of cariogenic layers on teeth.

Various components of *C. lanatus* have been studied for their antimicrobial properties. Research shows that the seeds of *C. lanatus* have medicinal applications, including antibacterial, antifungal, anti-inflammatory, and antiulcer effects, similar to the pulp and rind of the fruit [20]. Mohammed *et al*. [21] investigated the antibacterial activity of ethanolic extracts from the seeds and rind of *C. lanatus*, targeting both gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*, as well as gram-negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Their study revealed significant inhibition zones against these microorganisms [21]. In a similar vein, the present study assessed the antibacterial potential of *C. lanatus* pulp and rind extracts against *S. mutans*, employing the agar well diffusion method, which showed favorable outcomes. Previous research has indicated that pulp extracts, derived by centrifuging fresh juice of *C. lanatus*, effectively combat common oral bacteria, including *Lactobacillus* [1]. This finding aligns with the current study, though it focused on *S. mutans* instead.

Numerous studies have also explored the impact of plant-based, green-synthesized nanoparticles on a variety of general and oral bacteria, demonstrating significant antimicrobial effects. For example, research has shown that zinc oxide nanoparticles derived from *Punica granatum* and *Elettaria cardamomum* exhibit strong anti-inflammatory and antimicrobial properties [22]. Another investigation by Kunte *et al*. [23] found that pomegranate peel extract has a notable antibacterial effect against *S. mutans*. Recent research by Nomura *et al*. [24] revealed that extracts from *C. unshiu* fruits, particularly its albedo, can inhibit *S. mutans*, suggesting its potential for use in caries prevention due to its high polyphenol content, neutral pH, and inhibitory characteristics. Plant-derived exosome-like nanoparticles (PENs) are emerging as promising therapeutic options for disease treatment and drug delivery [25]. These studies align with the present research, reinforcing the antimicrobial potential of natural, plant-derived substances.

Research has indicated that phytochemical analysis of *C. lanatus* revealed the presence of free-reducing sugars and alkaloids across all its parts, while no steroids were detected in any of the extracts. Alkaloids, known for their antibacterial properties, play an important role as medicinal agents [26]. As such, the antibacterial effect observed in the extracts of *C. lanatus* may be attributed to the presence of these alkaloids. Miranda-Cadena *et al*. [27] demonstrated that compounds like carvacrol, cinnamaldehyde, and thymol are effective fungicides against Candida planktonic cells, and phytochemicals offer promising alternatives to combat Candida biofilms. New studies have highlighted the influence of toothpaste ingredients on oral health-related quality of life, with toothpaste containing natural active components improving daily oral hygiene [28]. The deep layers of bacterial cells within biofilms are challenging for conventional antibiotics to fully eliminate, contributing to the global issue of drug resistance. Consequently, innovative solutions, such as the use of nanoparticles, should be developed and further researched to address the resistance of bacterial biofilms to treatment [29].

One of the key limitations of this study is its in vitro design, which means that clinical trials are needed to confirm the findings. Additionally, the antibacterial effectiveness of a standard agent like chlorhexidine was not included in the comparison, which could have helped assess the relative efficacy of the extracts. Future research should focus on evaluating the antimicrobial properties of various parts of *C. lanatus*, their concentrations, effects on a range of microorganisms, and different extraction methods. Furthermore, exploring the use of nanoparticles within these extracts and developing dental products such as mouthwashes, pastes, powders, and varnishes incorporating them is an area with great potential for further investigation.

Conclusion

Despite the limitations of the current in-vitro study, the results suggest that both the rind and pulp extracts of *C. lanatus* exhibit substantial antibacterial activity against *S. mutans* at concentrations of 25, 50, 100, and 150 microliters. Additional studies focusing on its effects on other oral pathogens, as well as the methods of formulation and application, are necessary. However, the antibacterial properties of this natural extract offer the potential for enhancing oral hygiene practices.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: Ethical clearance was granted by the institutional Human Ethical Committee, with the IHEC reference number IHEC/SDC/ORTHO-1705/21/360.

References

1. Govindaraj A, Dinesh SP, Muralidharan NP. Efficiency of watermelon extract against oral microflora particularly lactobacillus-an in vitro study. Drug Invent Today. 2019;11(1).
2. Govindaraj A, SP SD. Effect of novel watermelon mouthwash on oral micro flora in patients undergoing orthodontic treatment. J Evol Med Dent Sci. 2020;9(37):2674-80. doi:10.14260/jemds/2020/582
3. Balakrishnan M, Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. Aust Dent J. 2000;45(4):235-45. doi:10.1111/j.1834-7819.2000.tb00257.x
4. Petersen PE. The world oral health report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO global oral health programme. Community Dent Oral Epidemiol. 2003;31(s1):3-24. doi:10.1046/j..2003.com122.x
5. Mehta R, Nandlal B, Prashanth S. Comparative evaluation of remineralization potential of casein phosphopeptide-amorphous calcium phosphate and casein phosphopeptide-amorphous calcium phosphate fluoride on artificial enamel white spot lesion: an in vitro light fluorescence study. Indian J Dent Res. 2013;24(6):681. doi:10.4103/0970-9290.127610
6. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res. 2010;89(11):1187-97. doi:10.1177/0022034510376046
7. Govindaraj A, Dinesh SP. Effect of Chlorhexidine varnish and fluoride varnish on white spot lesions in orthodontic patients-a systematic review. Open Dent J. 2021;15(1):151-9. doi:10.2174/1874210602115010151

8. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol. 2008;46(4):1407-17. doi:10.1128/JCM.01410-07
9. Karpiński TM, Szkaradkiewicz AK. Microbiology of dental caries. J Biol Earth Sci. 2013;3(1):M21-4.
10. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ. 2001;65(10):1028-37.
11. Lang NP, Lindhe J, editors. Clinical periodontology and implant dentistry, 2 volume set. John Wiley & Sons; 2015.
12. Mathur S, Mathur T, Srivastava R, Khatri R. Chlorhexidine: the gold standard in chemical plaque control. Natl J Physiol Pharm Pharmacol. 2011;1(2):45.
13. Erhirhie EO, Ekene NE. Medicinal values on *Citrullus lanatus* (watermelon): pharmacological review. Int J Res Pharm Biomed Sci. 2013;4(4):1305-12.
14. Neglo D, Tettey CO, Essuman EK, Kortei NK, Boakye AA, Hunkpe G, et al. Comparative antioxidant and antimicrobial activities of the peels, rind, pulp and seeds of watermelon (*Citrullus lanatus*) fruit. Sci Afr. 2021;11(5):e00582. doi:10.1016/j.sciaf.2020.e00582
15. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthod. 2009;31(4):407-11. doi:10.1093/ejo/cjn108
16. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8(1):1-28.
17. Limsong J, Benjavongkulchai E, Kuvatanasuchati J. Inhibitory effect of some herbal extracts on adherence of *Streptococcus mutans*. J Ethnopharmacol. 2004;92(2-3):281-9. doi:10.1016/j.jep.2004.03.008
18. Duailibe SA, Gonçalves AG, Ahid FJ. Effect of a propolis extract on *Streptococcus mutans* counts in vivo. J Appl Oral Sci. 2007;15(5):420-3. doi:10.1590/S1678-77572007000500009
19. Philip N, Bandara HM, Leishman SJ, Walsh LJ. Inhibitory effects of fruit berry extracts on *Streptococcus mutans* biofilms. Eur J Oral Sci. 2019;127(2):122-9. doi:10.1111/eos.12602
20. Alka G, Anamika S, Ranu P. A review on watermelon (*Citrullus lanatus*) medicinal seeds. J Pharmacogn Phytochem. 2018;7(3):2222-5.
21. Mohammed MF, Kannan HB, Abdalqader M, Alwan MR, Alhoot MA, Ghazi HF. Antibacterial activities of watermelon (*Citrullus lanatus*) rind and seed extracts against selected gram-positive and gram-negative bacteria. Int J Med Toxicol Legal Med. 2020;23(3and4):95-100. doi:10.5958/0974-4614.2020.00052.2
22. Shanmugam R, Hannah R, Kumar R. In vitro anti-inflammatory activity using fruit formulation mediated zinc oxide nanoparticles. Plant Cell Biotechnol Mol Biol. 2020;30:58-66.
23. Kunte S, Kadam N, Patel A, Shah P, Lodaya R, Lakde L. Comparative evaluation of antimicrobial properties of pomegranate peel extract against *Streptococcus mutans* and Lactobacillus-an in vitro study. Int Dent Med J Adv Res. 2018;4(1):1-6. doi:10.15713/ins.idmjar.88
24. Nomura R, Ohata J, Otsugu M, Okawa R, Naka S, Matsumoto-Nakano M, et al. Inhibitory effects of flavedo, albedo, fruits, and leaves of Citrus unshiu extracts on *Streptococcus mutans*. Arch Oral Biol. 2021;124:105056. doi:10.1016/j.archoralbio.2021.105056
25. Kim J, Li S, Zhang S, Wang J. Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian J Pharm Sci. 2021;17(1):53-69.
26. Tabiri B, Agbenorhevi JK, Wireko-Manu FD, Ompouma EI. Watermelon seeds as food: nutrient composition, phytochemicals and antioxidant activity. Int J Nutr Food Sci. 2016;5(2):139-44. doi:10.11648/j.ijnfs.20160502.18
27. Miranda-Cadena K, Marcos-Arias C, Mateo E, Aguirre-Urizar JM, Quindós G, Eraso E. In vitro activities of carvacrol, cinnamaldehyde and thymol against Candida biofilms. Biomed Pharmacother. 2021;143:112218.
28. Machorowska-Pieniążek A, Morawiec T, Olek M, Mertas A, Aebisher D, Bartusik-Aebisher D, et al. Advantages of using toothpaste containing propolis and plant oils for gingivitis prevention and oral cavity hygiene in cleft lip/palate patients. Biomed Pharmacother. 2021;142:111992.
29. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019;8(1):1-0.