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Inhibition activity of water hyacinth (*Eichhornia crassipes*) leaf extract against *Prevotella intermedia*

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Abstract

Background: Water hyacinth is a plant that grows in freshwater, commonly found in Indonesia. Water hyacinth leaf extract contains antimicrobial bioactive components such as tannins, flavonoids, alkaloids, and terpenoids. *Prevotella intermedia* is one of the bacteria that cause periodontal disease. *Prevotella intermedia* increases in the subgingival plaques of patients with periodontal disease exacerbated by hormonal changes. Therefore, water hyacinth leaf extract may affect the growth of *Prevotella intermedia*.

Objective: To determine the inhibition activity of water hyacinth leaf extract on the growth of *Prevotella intermedia*.

Method: This research was conducted in vitro using water hyacinth leaf extract. Which was generated using the serial dilution method to obtain Minimum Inhibitory Concentrations (MIC) 90% and Minimum Bactericidal Concentrations (MBC) of 99,9%. The concentrations of water hyacinth leaf extract were obtained at 100%, 50%, 25%, 12.5 %, 6.25%, 3.125%, 1.57%, and 0.78%. The inhibition results of water hyacinth leaf extract were seen by calculating the growth of *Prevotella intermedia* colonies on Mueller Hinton Agar (MHA). The Kruskal-Wallis test was performed to analyze the data statistically.

Results: There were significant differences in the inhibition of *Prevotella intermedia* growth in each treatment group ($p<0.05$), with the inhibitory activity of water hyacinth leaf extract beginning at a concentration of 3.125%.

Conclusion: Water hyacinth (*Eichhornia crassipes*) leaf extract inhibits the growth of *Prevotella intermedia* with a MIC90 of 3.125% and an MBC99,9 of 6.25%.

Keywords: Water hyacinth; *Prevotella intermedia*; Inhibition activity; Antibacterial

1. Introduction

Periodontal disease, which affects 20%-50% of the global population, is the most common disease in the human oral cavity after caries[1]. Periodontal disease is an inflammatory disease consisting of gingivitis and periodontitis and is caused by the interaction of microorganisms in dental plaque and the host's immune response[2]. In addition to dental plaque, which plays a significant role in the etiology of periodontal disease, the immune response also has an essential role in the incidence of periodontal disease. The immune response is regulated by hormonal fluctuations, which

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frequently have a role in the etiology of periodontal disease. Hormone-related periodontal disease is categorized into gingivitis associated with puberty, the menstrual cycle, and pregnancy[3].

Prevotella intermedia is a gram-negative, anaerobic, spore-forming, non-motile bacteria. These bacteria are part of the normal flora that colonize the oral cavity and can become pathogenic if their numbers increase in the gingival sulcus. These bacteria were discovered to be rising in subgingival plaque in areas with periodontal disease associated with hormonal changes[4,5]. These bacteria can invade periodontal tissues and disrupt the host's defense function. *Prevotella intermedia* promotes the degradation of periodontal connective tissue and bone matrix by increasing MMP expression[6–8]. Furthermore, *Prevotella intermedia* can modulate innate immunity by secreting a protein called Interpain-A (INPA), which can degrade complement[9]. To reduce the infection process, we need an agent that can inhibit the growth of *Prevotella intermedia*.

Water hyacinth (*Eichhornia crassipes*) is a plant that has medicinal properties. Water hyacinth is a freshwater plant widely distributed in the tropics and subtropics area[10]. Phytochemical studies of water hyacinth leaf extract showed that water hyacinth leaf contains phenols, flavonoids, alkaloids, terpenoids, tannins, anthraquinones, quinones, and other metabolites that have antimicrobial, antioxidant, antitumor, and accelerate wound healing properties[11-13].

Based on the explanation above, the water hyacinth leaf extract may influence the growth of *Prevotella intermedia*. It can be a candidate for herbal medicine to inhibit the growth of *Prevotella intermedia*. However, this still needs to be proven. Therefore, in this study we aim to evaluate the inhibition activity of water hyacinth leaf extract on the growth of *Prevotella intermedia.*

2. Material and methods

This research used the agar serial dilution method to count bacterial colonies and obtained an ethical clearance certificate from Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission with certificate number 401/HRECC.FODM/VII/2021.

2.1. Extraction

The ethanol extract of water hyacinth leaves used in this study was processed at UPT Materia Medica, Batu, East Java, Indonesia. Water hyacinth leaves are washed under running water until clean. Then cut into several pieces and dry in an oven at 50 ℃. The results of drying are fed into the pollination machine. Water hyacinth leaf powder (1 kg) was then macerated with 8 L of 70% ethanol for five days by stirring on an orbital shaker. After five days, the 70% ethanol filtrate was separated from the residue using a funnel Buncher lined with filter paper. The filtrate was evaporated using a rotary evaporator at 60 °C with a pressure of 175 Mb until the solvent evaporated and the pure extract was obtained.

2.2. Quantitative Phytochemical Test

2.2.1. Total Alkaloid

A total of 2.5 g water hyacinth extract was diluted in 50 ml of 10% acetic acid in ethanol. The solution was shaken for 4 hours with a magnetic stirrer, then filtered, evaporated, and dripped with ammonium hydroxide until an alkaloid precipitate formed. Next, the filter paper to be used is prepared and weighed. The alkaloid precipitate was then filtered and washed with a 1% ammonium hydroxide solution. The filter paper containing the precipitate was then dried in an oven at 60 °C for 30 minutes. After cooling, the filter paper that contained the precipitate was weighed to produce a consistent weight. The alkaloid content was obtained from the difference between the weight of the filter paper with precipitate and the clean filter paper.

2.2.2. Total Tannin

A total of 0.1 g of water hyacinth leaf extract was weighed, then dissolved in 10 ml of methanol. After that, 1 ml of the sample solution was pipetted, and 7.5 ml of methanol and 0.5 ml of Folin Denis reagent were added. Leave it for 3 minutes. After that, 1 ml of saturated Na2CO3 solution was added. The absorbance was measured using a spectrophotometer at a wavelength of 740 nm. The results obtained were calculated using a standard curve made using tannic acid.

2.2.3. Total Flavonoid

A total of 1 mL of water hyacinth leaf extract was added to a test tube, followed by 2 mL of 2% aluminum chloride, and vortexed. The mixed solution was then incubated for 30 minutes, and the absorbance value was measured using a

spectrophotometer with a wavelength of 415 nm. The Quercetin linear regression equation curve was used to determine the sample's total flavonoid content, expressed in mg QE/g extract.

2.2.4. Total Terpenoid

A total of 10 grams of extract samples were soaked in ethanol for 24 hours. The solution was filtered, and the filtrate was extracted with Petroleum ether. Petroleum ether extracts were treated as total terpenoids.

2.3. Inhibition Activity Test

This study employs in vitro laboratory experimental research with a Post-Test only control group design. The test uses *Prevotella intermedia* ATCC 25611 as bacterial samples and is performed at the Research Center of the Faculty of Dentistry at Airlangga University in Surabaya. The study was conducted in 4 repetitions.

The serial dilution method was used in this study to obtain the concentration of water hyacinth leaf extract from 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. First, nine test tubes and one bacterial suspension test tube were prepared. The test tube with label no. 1 contains 10 mL of water hyacinth leaf extract, while the test tubes with labels no. 2 until no. 9 contain 5 mL of Brain Heart Infusion Broth (BHIB). 5 mL of water hyacinth leaf extract from test tube no. 1 is transferred to test tube no. 2 containing BHIB media and then stirred until homogeneous. Then, 5 ml of the solution from test tube no. 2 is transferred to test tube no. 3 and stirred again. This process is repeated until tube no. 8 is reached. Take 5 ml of solution from tube no. 8 and discard it so that each test tube has the same volume.

A 0.05 ml of *Prevotella intermedia* bacterial suspension equalized with 0.5 Mc Farland turbidity (1.5x108 CFU/ml) was added to each test tube containing a mixture of BHIB and water hyacinth leaf extract. Following that, each test tube was incubated for 1x24 hours at 37 °C. The spread method was used to cultivate bacteria on Mueller Hinton Agar (MHA) media from each tube. The process is repeated four times to obtain a valid result. In addition, colonies of *Prevotella intermedia* grown on MHA media were counted and expressed in CFU/ml units.

2.4. Statistical Analysis

The statistical analysis was done using SPSS with the Shapiro-Wilk normality test, Levene's homogeneity test, and the Kruskal-Wallis comparison test. Furthermore, a post hoc test using the Mann-Whitney test to determine the differences between pairs of different groups.

3. Results

The result of the quantitative phytochemical content of water hyacinth leaf extract showed alkaloid as the most dominant component of the extract, followed by tannin, flavonoid, and terpenoid in chronological order (**Figure 1**).

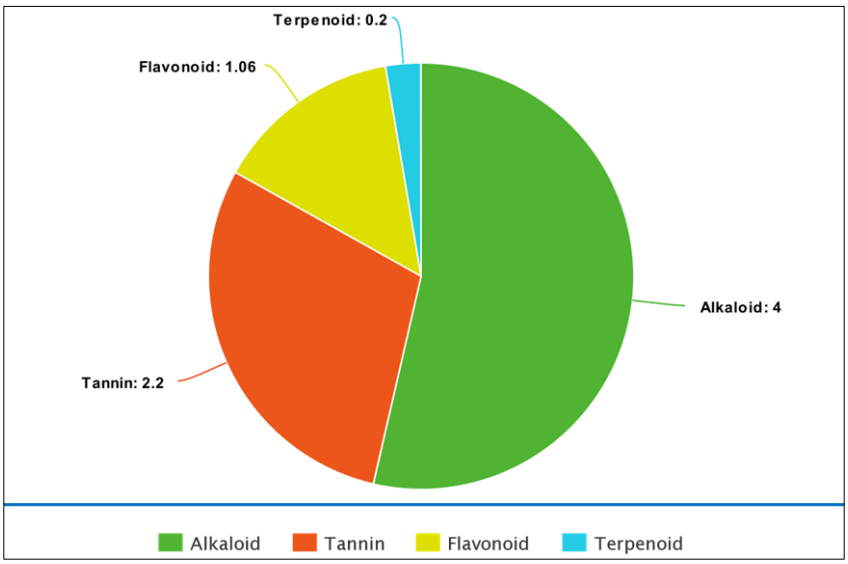

Figure 1 Phytochemical Test Results of Water Hyacinth Leaf Extract (%)

The inhibition activity of water hyacinth leaf extract in every research group toward *Prevotella intermedia* can be seen on the number of bacterial colonies on MHA media. It showed that the concentration of the extract demonstrated the ability to reduce the number of bacterial colonies, which was more significant as the concentration increased from 0,78%, 1,56%, and lastly 3,125%, while research groups with the concentration above 3,125% show no growth of *Prevotella intermedia* after 2x24 hours (Table 1).

Table 1 Bacterial Colonies on MHA Media, Shapiro-Wilk normality test, Levene's homogeneity test, and the Kruskal-Wallis comparison test

	Mean+SD (CFU/ml)	**Shapiro-Wilk (Sig.)**	**Levene's test (Sig.)**	**Kruskal Wallis (Asymp. Sig.)**
Negative control	0±0	-	-	-
Positive control	176±4.3	0.970	0.000	0.000
0,78%	58±3.0	0.952		
1,56%	31±2.1	0.995		
3,125%	14±2.0	0.850		
6,25%	0±0	-	-	-
12,5%	0±0	-	-	-
25%	0±0	-	-	-
50%	0±0	-	-	-
100%	0±0	-	-	-

The Shapiro-Wilk tests showed that the data were normally distributed ($p > 0.05$). Using Levene's test, the homogeneity test demonstrated that the data variance was not homogeneous ($p < 0.05$). Thus, the difference test was continued using the Kruskal-Wallis test, which indicated a significant difference between the treatment groups in the growth of *Prevotella intermedia* ($p< 0.05$) (Table 1). Meanwhile, the result of the post hoc Mann Whitney test showed significant differences that occurred at concentrations of 3.125%, 1.56%, and 0.78% with concentrations of 100% to 6.25% and the control group ($p < 0.05$) (Table 2).

Table 2 Post hoc Mann Whitney Test

Significant	**100%**	**50%**	**25%**	**12.5%**	**6.25%**	**3.125%**	**1.56%**	**0.78%**	**Control (+)**	**Control (-)**
100%	-	-	-	-	-	*0.014	*0.014	*0.014	*0.014	-
50%	-	-	-	-	-	*0.014	*0.014	*0.014	*0.014	-
25%	-	-	-	-	-	*0.014	*0.014	*0.014	*0.014	-
12.5%	-	-	-	-	-	*0.014	*0.014	*0.014	*0.014	-
6.25%	-	-	-	-	-	*0.014	*0.014	*0.014	*0.014	-
3.125%	*0.014	*0.014	*0.014	*0.014	*0.014	-	*0.021	*0.021	*0.021	*0.014
1.56%	*0.014	*0.014	*0.014	*0.014	*0.014	*0.021	-	*0.021	*0.021	*0.014
0.78%	*0.014	*0.014	*0.014	*0.014	*0.014	*0.021	*0.021	-	*0.021	*0.014
Control (+)	*0.014	*0.014	*0.014	*0.014	*0.014	*0.021	*0.021	*0.021	-	*0.014
Control (-)	-	-	-	-	-	*0.014	*0.014	*0.014	*0.014	-

*Significant differences

4. Discussion

Based on this research, Water hyacinth (*Eichhornia crassipes*) leaf extract can inhibit the growth of *Prevotella intermedia* bacteria, with a MIC_{90} of 3.125% and an $MBC_{99,9}$ of 6.25%. The results of this study are in line with Krismariono *et al.* (2022) that state, water hyacinth leaf extract was potential effective in inhibiting bacterial plaque growth in patients with gingivitis[14], as well as Afidati *et al.* (2019) that state, water hyacinth leaf extract has inhibition activity against the gram-negative bacteria *Aggregatibacter actinomycetemcomitans*[15].

Herbal plants such as *Camellia sinensis, Heteropyxis natalensis,* and *Curcuma longa* have been shown in previous studies to inhibit the growth of *Prevotella intermedia*[16]. *Prevotella intermedia* can obtain nutrients through the invasion of buccal epithelial cells. Increased levels of menadione or vitamin K in the gingival crevicular fluid can stimulate the growth of *Prevotella intermedia*, resulting in increased plaque formation[17]. Thus, inhibiting these bacteria is expected to reduce the severity of periodontal disease.

Water hyacinth plants have been shown to have antibacterial activity against both gram-negative and gram-positive bacteria[13]. According to previous research on the periodontal pathogenic bacteria, *Aggregatibacter actinomycetemcomitans*, water hyacinth leaf extract can partially inhibit bacteria at a minimum concentration of 3.125%, and the total inhibitory effect is achieved at a concentration of 6.25%[15].

The present studies demonstrated that the higher the concentration, the fewer bacterial colonies are present in MHA media. According to theory, a concentration of 3.125% is the minimum inhibitory concentration (MIC) of water hyacinth leaf extract against *Prevotella intermedia* growth because it results in a reduction in bacterial growth of up to 92% (> 90%). While the Minimum Bactericidal Concentration (MBC) in this study was 6.25%, it is the lowest water hyacinth leaf extract concentration that can kill 99.9% of *Prevotella intermedia* colonies seen by the absence of bacterial colony growth on agar media[18].

The growth of *Prevotella intermedia* in media is influenced by environmental factors such as temperature, pH, osmotic pressure, oxygen, and other chemical components contained in the media[19]. In this study, the water hyacinth leaf extract containing bioactive compounds most influenced bacterial growth.

Prevotella intermedia is a gram-negative bacteria with three primary layers: an outer membrane, peptidoglycan in the cell wall, and an inner membrane or plasma membrane. Bacterial outer membranes function as permeable barriers to protect cells from harmful environmental conditions. The plasma membrane comprises proteins involved in energy metabolism and synthesizing molecules necessary for bacterial growth. Peptidoglycan is found between the outer membrane and the plasma membrane. Because of its rigidity, peptidoglycan plays a role in determining cell shape. Furthermore, peptidoglycan prevents turgor pressure, which causes bacterial cell lysis. Thus, the death of *Prevotella intermedia* by water hyacinth leaf extract is most likely due to damage to plasma membranes, cell metabolism disruptions, and cell wall formation[20].

Based on quantitative phytochemical tests, water hyacinth leaf extract contains bioactive compounds such as alkaloids, tannins, flavonoids, and terpenoids. Alkaloids have the highest percentage of levels at 4%. Following that are tannins, flavonoids, and terpenoids, which account for 2.2%, 1.06%, and 0.2%, respectively. All of the components tested contribute to inhibiting the growth of *Prevotella intermedia*.

Alkaloids reduce the number of colonies by interfering with the peptidoglycan constituent components in the bacterial cell wall, resulting in incomplete cell wall formation and bacterial death. Furthermore, alkaloids inhibit nucleic acid and ATP synthesis. The nitrogen-containing alkaline group affects the antibacterial activity of the alkaloids. When the alkaloids interact with *Prevotella intermedia*, the alkaline groups react with the amino acids that form the cell wall and react with bacterial DNA. This reaction will alter the amino acid composition and cause DNA chain imbalances, damaging DNA. When DNA in the cell nucleus is damaged, the cell nucleus lyses, resulting in cell damage. Cell damage impairs bacterial cell metabolism, resulting in the death of *Prevotella intermedia*[21,22].

Water hyacinth leaf extract contains tannin that has antibacterial properties due to its ability to inhibit bacterial attachment to surfaces by inactivating bacterial cell adhesion and inhibiting protein transport[23]. Furthermore, tannins target bacterial cell wall polypeptides, resulting in incomplete cell wall formation[24]. According to Akiyama *et al*.[25], tannin toxicity as an antibacterial includes the mechanism of damaging cell membranes. As a result of the research, tannins are considered substances that can inhibit the growth of *Prevotella intermedia.*

Water hyacinth leaf extract contains flavonoids that inhibit nucleic acid synthesis and cytoplasmic membrane function, providing antibacterial activity on *Prevotella intermedia*. Ring B flavonoids inhibit nucleic acid synthesis by forming hydrogen bonds with bacterial nucleic acids, inhibiting DNA and RNA synthesis. Meanwhile, by impairing the cytoplasmic membrane's function, flavonoids can either penetrate the lipid bilayer directly or interfere with the cell's defense function or indirectly by defacing the permeability of cell membranes, resulting in cell membrane damage and the expulsion of intracellular compounds. *Prevotella intermedia* will die due to the release of intracellular compounds such as proteins and nucleotides involved in bacterial cell metabolism[26,27]. The presence of terpenoid compounds containing lipophilic compounds also affects cell membrane damage[28]. When terpenoids bind to the membrane protein *Prevotella intermedia*, they cause an increase in membrane permeability. Increased cell membrane permeability allows antibacterial substances to enter the bacterial cell and results in the lysis of the *Prevotella intermedia* bacterial cell membrane[29].

Last but not least, besides the aims and results of the study, the sample used in this study is quite small, and the concentration range of the research groups is too wide. The authors consider these few factors as the weakness of the study. Therefore, it is hoped that this can be a recommendation for further research to use a shorter concentration range and increase the sample.

5. Conclusion

Water hyacinth (*Eichhornia crassipes*) leaf extract can inhibit the growth of *Prevotella intermedia* bacteria, with a MIC_{90} of 3.125% and an $MBC_{99,9}$ of 6.25%.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

Statement of ethical approval

The ethical clearance of this study was obtained from Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission, number: 401/HRECC.FODM/VII/2021.

References

[1] Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim). 2017;1(2):72–80.

[2] Ferreira MC, Dias-Pereira AC, Branco-de-Almeida LS, Martins CC, Paiva SM. Impact of periodontal disease on quality of life: a systematic review. Vol. 52, Journal of Periodontal Research. Blackwell Munksgaard; 2017. p. 651–65.

[3] Bhardwaj A, Bhardwaj SV. Effect of Androgens, Estrogens and Progesterone on Periodontal Tissues. J Orofac Res. 2012;2(3):165–70.

[4] Newman MG, Takei HH, Klokkevold PR, Carranza FA. Clinical Periodontology. 13th ed. Philadelphia: Elsevier Saunders; 2018. 19-21,33,34,38,41,62,212,465-474 p.

[5] Elias-Boneta AR, Ramirez K, Rivas-Tumanyan S, Murillo M, Toro MJ. Prevalence of gingivitis and calculus in 12-year-old Puerto Ricans: A cross-sectional study. BMC Oral Health. 2018;18(1):1–10.

[6] Guan S-M, Shu L, Fu S-M, Liu B, Xu X-L, Wu J-Z. *Prevotella intermedia* induces matrix metalloproteinase-9 expression in human periodontal ligament cells. 2008;283(1):47–53. Available from: https://academic.oup.com/femsle/article/283/1/47/547662

[7] Guan S-M, Shu L, Fu S-M, Liu B, Xu X-L, Wu J-Z. *Prevotella intermedia* upregulates MMP-1 and MMP-8 expression in human periodontal ligament cells. FEMS Microbiol Lett. 2009 Oct 1;299(2):214–22.

[8] Pelt P, Zimmermann B, Ulbrich N, Bernimoulin JP. Effects of lipopolysaccharide extracted from *Prevotella intermedia* on bone formation and on the release of osteolytic mediators by fetal mouse osteoblasts in vitro. J Anim Sci. 2002;80(1):859–66.

[9] Potempa M, Potempa J, Kantyka T, Nguyen KA, Wawrzonek K, Manandhar SP, et al. Interpain A, a cysteine proteinase from *Prevotella intermedia*, inhibits complement by degrading complement factor C3. PLoS Pathog. 2009;5(2):1–14.

[10] Ayanda OI, Ajayi T, Asuwaju FP. *Eichhornia crassipes* (Mart.) Solms: Uses, Challenges, Threats, and Prospects. Sci World J. 2020;2020:1–12.

[11] Mala A, Tulika T, Mala A. Pharmaceutical Potential of Aquatic Plant Pistia stratiotes (L.) and *Eichhornia crassipes*. J Plant Sci Spec Issue Med Plants. 2015;3(1):10–8.

[12] Isebe TI. PhytochemicalCompositionAnd Antibacterial Activity Of *Eichhornia crassipes* In Lake Victoria, Kisumu. Int J Sci Technol Res. 2016;5(09):45–52.

[13] Haggag WM, Abou El Ella SM, Abouziena HF. Phytochemical analysis, antifungal, antibacterial activities and application of *Eichhornia crassipes* against some plant pathogens. Planta Daninha. 2017;35:1–11.

[14] Krismariono A, Setiawatie EM, Rachmawati RY, Setiawan YA, Padmarini HN, Apriliyanti NA, Al-Shekhli AA, Soulissa AG, Ummah SR, Lombardo B, Widyarman AS. Antibacterial Activity of Water Hyacinth (*Eichhornia crassipes*) Leaf Extract Against Bacterial Plaque from Gingivitis Patients. Journal of International Dental and Medical Research. 2022 Jul 1;15(3):966-71.

[15] Afidati YI, Savitri IJ, Krismariono A. Inhibition activity of water hyacinth leaf extract (*Eichhornia crassipes*) against Aggregatibacter Actinomycetemcomitans. Asian J Pharm Clin Res. 2019;12(6):122–5.

[16] Dib K, Ennibi OK, Alaoui K, Cherrah Y, Filali-Maltouf A. Antibacterial activity of plant extracts against periodontal pathogens: A systematic review. J Herb Med. 2021;29(August 2019):100493. → aslinya 14 jadi 16.

[17] Ramak P, Talei GR. Chemical composition, cytotoxic effect and antimicrobial activity of Stachys koelzii Rech.f. essential oil against periodontal pathogen *Prevotella intermedia*. Microb Pathog. 2018;124:272–8.

[18] Turgeon ML. Linnê and Ringsrud's Clinical Laboratory Science- Concepts, Procedures, and Clinical Applications. 7th ed. St. Louis, Missouri: Elsevier Inc.; 2016. 514 p.

[19] Jufri RF. The Effect of Environmental Factors on Microbial Growth. J La Lifesci. 2020;1(1):12–7.

[20] Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2010;2:1–16.

[21] Yan Y, Li X, Zhang C, Lv L, Gao B, Li M. Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics. 2021;10(3):2–30.

[22] Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents. 2014;44(5):377–86.

[23] Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials-A minireview. Materials (Basel). 2020;13(14).

[24] Bossi A, Rinalducci S, Zolla L, Antonioli P, Righetti PG, Zapparoli G. Effect of tannic acid on Lactobacillus hilgardii analysed by a proteomic approach. J Appl Microbiol. 2007;102(3):787–95.

[25] Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against Staphylococcus aureus. J Antimicrob Chemother. 2001;48(4):487–91.

[26] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343–56.

[27] Ahmad A, Kaleem M, Ahmed Z, Shafiq H. Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections-A review. Food Res Int. 2015;77:221–35.

[28] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82.

[29] Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, et al. Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules. 2019;24(13):1–12.