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Antibacterial Activity of Red Pomegranate Ethanolic Albedo Extract Towards Prevotella Intermedia Isolated from Periodontitis Patients (In Vitro Study)

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Abstract— *Studies show that red pomegranate extracts stop the growth of gram-positive facultative anaerobes and stop biofilm from forming on dental surfaces. This study sought to evaluate the antibacterial efficacy of ethanolic albedo extract from red pomegranate against Prevotella intermedia. This study evaluated antimicrobial sensitivity, minimum inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC) utilizing agar well diffusion and two-fold serial dilution techniques. Gas chromatography-mass spectrometry was used to do a phytochemical examination. The results showed that Prevotella intermedia is sensitive to albedo extract, with a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC) of 0.39 mg/ml. The results suggest that ethanolic albedo extract could be a suitable substitute for traditional drugs in the adjunctive periodontal diseasas treatment*.

Keywords— *Plaque, Red pomegranate, Antibacterial herbs, Periodontal microbiology*.

I. INTRODUCTION

It is possible to describe Periodontal disease as an infection that is both complex and multi-factorial which is characterized by the damage it causes to the tissues supporting the teeth. In its beginnings it starts as a "reversible gingival inflammation", and if left untreated, can progress to a permanent destruction affecting these supporting tissues. For the past years, significant research has identified only a small subset of microorganisms in the sub-gingival sulcus that takes a great role in the instigation and progress of the periodontal disease[1]. *Prevotella intermedia* is an anaerobic, gram-negative, black-pigmented secondary colonizer that belongs to the orange complex. It has been associated with various forms of periodontal disease, including gingivitis that is associated with puberty, periodontitis, and severe "necrotizing ulcerative gingivitis"[2]. Regular scaling and planning of the root, together with antibiotics and antiseptic mouth rinses, are commonly used treatments for periodontal disease. However, frequent use of such mouthwashes and medications can lead to adversative side effects, like antibiotic resistance. A concept that dates back centuries is the therapeutic properties of plants, which have gained renewed interest in recent years due to their biological effectiveness and safer profile. Additionally, traditional plants were historically used to treat oral infections. Due to their broad anti-inflammatory properties, antioxidant, plant-derived medicines, and antibacterial offer a steady, nontoxic, and bioactive alternative to synthetic medications [3]. An example of such medicinal herb is red pomegranate (*Punica granatum L.*), frequently named as the 'winter jewel,' is the dominant member of two species making the Punicaceae. Various parts of red pomegranate have been utilized for their antihypertensive, antioxidant, antimicrobial and anti-inflammatory properties [4]. Early epidemiological research suggest that red pomegranate exhibits antibacterial properties against primary dental plaque colonizers, such as *Strep. Mutans, Strep. salivarius*, *Strep. anginosus* and *Strep. mitis* [5]. Polyphenolics and tannins found in red pomegranate extracts, particularly gallagic acid and Punicalagin are linked to antimicrobial activity [6]. All of these broad-spectrum effects imply that red pomegranate products could be used instead of chemical antiseptics and as extra treatments for periodontal diseases. Although various research has documented the antibacterial properties of red pomegranate against secondary and primary colonizers, its antibacterial efficacy against anaerobic periodontal infections, particularly the ethanolic seed extract, remains inadequately investigated. This study aims to assess the antimicrobial properties of red pomegranate ethanolic albedo extract toward *Prevotella intermedia*.

II. MATERIALS AND METHODS

2.1. Extraction of Albedo by Soxhlet (Hot Method)

The hot Soxhlet extraction method was used to make the ethanolic extract of red pomegranate Albedo [7]. The dried Albedo of red pomegranate were crushed and later blended, and 50 g of the material placed in the thimble of Soxhlet and left to dissolved in 500 mill of 80% ethanol, creating a 0.1 g/ml mixture. The Soxhlet was turned on for 24 hours with heat set on 45°C until the solvent in thimble chamber became colorless. The extract was subsequently filtered with Whatman No. 1 filter paper (Cat No 1001 150, England). Finally, extract was put into a spray drier to make a powder, which was then put into a sterile container and kept in the fridge for use in other studies.

2.2. GC-MAS Analysis of Albedo

Agilent Technologies 7820A GC System (USA) was used to do gas chromatography-mass spectrometry (GC-MS) analysis to figure out what was in the extract. One microliter of extract sample solution was put into the GC-MS apparatus. The mass spectrometer used a conventional capillary column with a film thickness of 30 mm × 0.25 mm × 0.25 µm. The

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column was first kept at 104°C for 6 minutes after that, then slowly raised to 241°C at a rate of 12°C/min. It stayed at that temperature for around 12 minutes. The carrier gas was helium, which flowed at a steady rate of 1 ml/min. The mass spectrum analyzer (MS) worked in electron impact ionization mode at 1,555 V. The ion's generator setting was kept at 246°C, while the MS quadrant temperature was kept at 156°C. Mass spectral data was collected by The GC-MSD Agilent ChemStation Software. We compared the identified parts to the MS values in the NIST collection to make sure they were correct.

2.3. Sampling of Subgingival Plaque

Individuals whom had received periodontal therapy, utilized antimicrobial agents within the preceding 3 months, smokers, or had systemic disorders (such as diabetes) and pregnant, were eliminated from the present study. 10 men in good health who suffered from stage 3 Periodontitis, that is defined by clinical attachment loss of no less than 5 mm, having a probing depth of no less than 6 mm and the loss of radiographic bone that extends to the middle third portion of the root, were chosen to gather sub-gingival plaque samples for the clinical isolation of *Prevotella intermedia*. Before taking a subgingival plaque sample, a sterile curette was used to scale the region above and below the gum line. This was done to make sure that no plaque or calculus was left behind, which could have contaminated the blood on the day of the sample. After a week, the patients were asked to come back for a sample of plaque from below the gum line. The specimens were collected from pockets resembling the oral aspects of the upper left molar teeth, which were confirmed to exceed a depth of 7 mm. Cotton roll used to separate chosen pocket on the buccal side of the lower left first molar [8]. Then, a clean strip paper was carefully put into the periodontal pocket and left for sixty seconds till tissue resistance became apparent [8]. After that, the paper tip was carefully taken off and marked on a Columbia agar plate that had around 6% human blood (Oxoid, UK), 2 µg/ml vitamin K1 (Himedia), and 6 µg/ml hemin in it. For 5 to 7 days at 36 °C, inoculated plates were grown in an anaerobic jar (OxoidTM AnaeroJar) with an anaerobic gas pack (thermo-scientific).

2.4. Identification, Isolation, and Confirmation Prevotella Intermedia

The preliminary identification of Prevotella intermedia relied on traditional culture methods, encompassing colony morphology, black pigment production, Gram stain properties, and the necessity for anaerobic growth. The tentative identity was later confirmed using molecular techniques. Genomic DNA was isolated from chosen colonies using a boil-prep technique and underwent conventional polymerase chain reaction (PCR)[9]. The amplification focused on a 575-base pair segment of the 16S rRNA gene utilizing species-specific primers (Forward: 5′-ATT GTT GGG GAG TAA ACG GGC-3′; Reverse: 5′-TGA ACA TGT CTG TAT CCT GGC T-3′). The PCR results were analyzed using agarose gel electrophoresis, and the resultant amplicons were purified. The species identity was conclusively confirmed using automated sequencing of the purified PCR products (macrogen, South Korea).

2.5. Bacterial Suspension Preparation

Using sterile loops taken from agar plates, three milliliters of Mueller Hinton Broth, with 6 µg/ml of hemin and 2 µg/ml of menadione added, were added to pure isolated colonies of *P. intermedia*. Using the 0.5 McFarland turbidity standard at an optical density of 650 nm, the bacterial suspension was changed to 1.5×10^8 CFU/ml [10].

2.6. Agar Diffusion Test

The diffusion technique based on agar wells was used to find out how sensitive *P. intermedia* was to ethanolic albedo extract [11]. An L-shaped loop was used to transfer 50 µl of *P. intermedia* from a bacterial culture to Mueller Hinton Agar (MHA) plates, which were then allowed to dry. A sterile micropipette tip was used to make 5 mm holes in each MHA plate. Then 100 µl of albedo extract at concentrations (6.25, 12.5, 25, and 50 mg/ml) into the wells. 100 µl of sterilized distilled water was used as a positive control and 0.12% chlorhexidine (CHX; periokin) as a negative control. For 48 hours, the plates were kept at 36°C without oxygen. Using a millimeter ruler, the inhibition zones were measured and recorded as the average width around each well that had the extract solution test. If there are inhibitory zones larger than 8 mm, it means that the extract was effective against the bacteria [12].

2.7. *MIC and MBC Determination of the Extracts of Moringa Oleifera L. Seeds*

A standard macrobroth two-fold serial dilution method validated the MIC of the red pomegranate ethanolic albedo extract. Ten test tubes were filled with 900 µL of Mueller Hinton Broth (MHB). Put 900 µL of extract with a concentrations of 12.5 mg/mL in the first tube. A progressive two fold dilution using 900 ul from tube 1 to tube 8 produced extract concentrations of 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09, and 0.04 mg/mL. Tube 9 was the negative growth control, while tube 10 was the positive control containing 900 µL of 0.12% chlorhexidine (CHX) in MHB. By adding 100 ul of Prevotella intermedia solution to each dilution, we were able to get total volume of 1,000 ul each tube. An Oxoid™ AnaeroJar with Thermo-Scientific gas packs was used to incubate the samples anaerobically at 37°C for 48 hours. A spectrophotometer (V-1100 DIGITAL SPECTROPHOTOMETER, Germany) was used to detect turbidity and optical density at 625 nm after incubation. This was done to see how much bacteria had grown. The minimum extract concentration that completely inhibited detectable growth was the MIC. Antimicrobial tests were done three times with two samples each time to be sure the results were the same.

To find MBC, a 50 µL sample from tubes that didn't show any growth after the MIC test was sub cultured onto Mueller Hinton Agar (MHA) plates. These plates were kept in an anaerobic environment at 36°C for 48 hours. The MBC was the lowest amount of extract that didn't promote growth or less

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than three colonies on the cultural plate, which meant that it killed 99.0–99.5% of the bacteria[13].

2.8. Statistical Analysis

The data analysis employed SPSS version 26.0 for Windows software. The antimicrobial sensitivity test findings were first looked at using the Kruskal-Wallis test at a 5% significance level. We used the Mann-Whitney U test to see if there was a difference between the groups at a significance level of 0.5%.

III. RESULTS

The Soxhlet extraction yielded 8 grams of albedo crude extract. In Figure 1, you can see that the ethanolic albedo GC-MS analysis made 7, 9, and 10 peaks, in that order. Furfural (34.44%), 3,4,5-trihydroxybenzoic acid (23.9%), and gallic acid (21.7%) were the main parts of the extracts that went up the most. Table 1 shows all of the parts of the extract. With comparing the spectrum data of the components to NIST references, peaks were sorted into groups based on the types and amounts of phytochemical constituents they showed

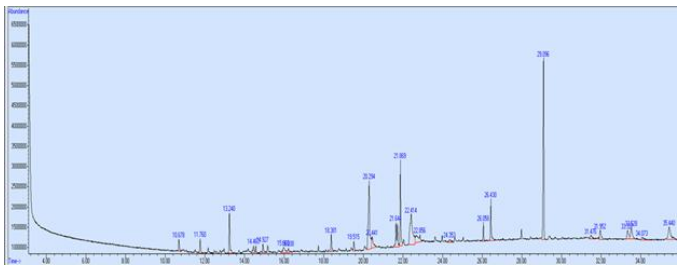

Figure 1. Gc-Mas Analysis of Ethanolic Albedo Crude Extract

TABLE 1. Gc-Mas Component of Ethanolic Albedo Crude Extract

Peck	Retention time	Area %	Name
1	10.623	4.54	Cyanoacetyl-3,5-dimethylpyrazole
2	11.678	5.60	2-Cyclohexen-1-one 2-Imino-4-methylpentanenitrile
3	12.624	0.89	3-hydroxyanthocyanidins
4	14.933	0.91	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
5	16.628	2.07	5-Hydroxymethylfurfural
6	18.324	1.43	Hexadecanoic acid, methyl ester
7	20.422	21.7	gallic acid
8	22.837	34.44	Furfural
9	25.424	1.11	13-Octadecenal, (Z)-cis-11-Hexadecenal
10	29.091	23.9	3,4,5-trihydroxybenzoic acid

3.2. Identification of P. Intermedia

70 Percent (N=7) of the investigated sample contained anaerobic bacteria exhibiting black pigmentation. the colonies were small, round, and grew in a convex shape. after 48 hours, they turned opaque. Figure 2 shows how black colonies grow on lysed blood after 7 to 10 days. figure 3 shows that sequencing of 16s rrna showed that all the pure colonies and the p. intermedia atcc 25611 strain had the same genetic makeup.

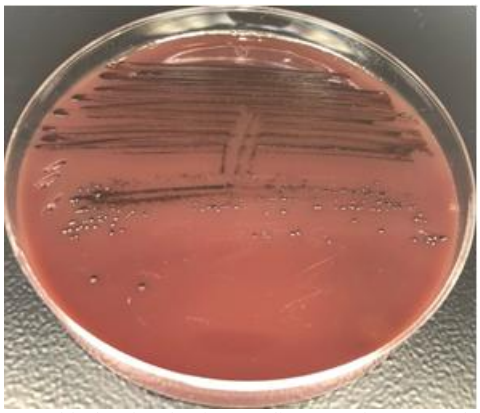

Figure 2. Pure black colored P. intermedia Seven days post-subculturing

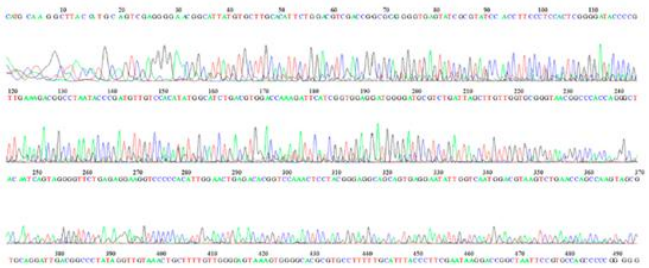

Figure 3. A graph showing the genome sequencing of P. intermedia at 16s RNA

3.3 Antibacterial Evaluation of the Ethanolic Albedo Extract

The agar well diffusion test showed that the drug had dose-dependent antibacterial activity at all tested doses. The average inhibition zone at a dose of 6.25 mg/ml was 5.2±0.2 mm, whereas the highest inhibition zone at 50 mg/ml was 13±0.6 mm. When tested against red pomegranate Albedo, CHX 0.12% had the biggest inhibitory zone, which was 19.5±0.2 mm, as shown in table 2. We used successive macro dilutions and optical density (OD) measurements to find the MIC values of ethanolic albedo extract against P. intermedia. The lowest concentration that showed antibacterial action against P. intermedia was 0.39 mg/ml (OD=0.027). The values for the MBC, which are shown in Table 3, were found to be 0.39 mg/ml

TABLE 2. The Response of *P. Intermedia* to Various Concentrations of Ethanolic Albedo Extracts

Extract	inhibition zone (mm)	Standard deviation	*P*-value
CHX (0.12%)	21.5000	0.21* ^	
Albedo 50 mg	13.566	0.62* ^	
Albedo 25mg	10.164	0.69* ^	
Albedo 12.5 mg	8.343	0.25* ^	0.000
Albedo 6.25mg	5.261	0.22* ^	
CHX: Chlorhexidine; *Compared to CHX by Kruskal, significant at P < 0.05. ^ Examining similarities and differences			

TABLE 3. MIC, MBC, and OD values of ethanolic albedo extract and 0.12% chlorhexidine in broth twards *P. intermedia*.

Extract	MBC (mg/ml)	MIC (mg/ml)	OD (SD)	Versus	*P*-value
Albedo	0.39	0.39	0.027(0.00229)	CHX	0.001*
CHX	0.06	0.06	0.013 (0.00033)	Broth	0.000*

(CHX) Chlorhexidine 0.06%; (MIC)Minimum inhibitory concentration; (OD)Optical density; (MBC)minimum bactericidal concentrations;
*Comparison using Mann-Whitney test, significance level at < 0.05

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IV. Discussion

Plant-based medicine has been around for a long time and is still commonly used as a regular way to treat people in many poor countries. Research also shows that these medicinal plants work well for treating dental problems, notably periodontal diseases [14]. Chemical antiseptics, like as CHX, have various benefits as supplemental therapy for periodontal diseases; nevertheless, they also exhibit specific drawbacks. For example, changes in taste perception and discoloration of teeth. These traits make it harder to figure out if chemical antiseptics would still be the best option if there were no other good options. Herbal medicine has a wide spectrum of antibacterial, antioxidant, and anti-inflammatory effects, which suggests that it could be a stable, safe, and bioactive replacement for regular drugs. *P. intermedia* is regarded as crucial for the advancement of periodontal disorders. Many studies have focused on the antibacterial properties of natural herbs in relation to *P. intermedia*. While multiple in vivo and in vitro studies have established the efficacy of red pomegranate extracts against primary and secondary invaders, there remains insufficient evidence regarding their antibacterial effectiveness against *P. intermedia*, indicating a need for further research. This study seeks to assess the antibacterial efficacy of the ethanolic Albedo extract against *P. Intermedia*.

Table 2 shows that Albedo had dose-dependent inhibitory effects on *P. intermedia* at all dosages given. Albedo exhibited antibacterial activity in this investigation at minimum inhibitory concentrations (MICs) of 0.39 mg/ml, as shown in Table 3. Table 1 shows that the ethanolic Albedo extract of red pomegranate had big increases in Furfural (34.44%), 3,4,5-trihydroxybenzoic acid (23.9%), and gallic acid (21.7%). It has been demonstrated that each of these elements possesses antibacterial properties against P. intermedia in vitro [15]. confirming the results of the present investigation. Gallic acid damages the membranes of bacterial cells, which causes cytoplasmic leakage and cell lysis [16]. Furthermore, it was shown This study detected furfural, which was proven to disrupt DNA synthesis and impact the survival of *P. intermedia*[17-18]. In the realm of polymicrobial interactions, being within a biofilm is more advantageous than achieving a free planktonic condition [19].

The study's inability to pinpoint particular physiologically active components in red pomegranate albedo using GC-MS analysis is a significant shortcoming. The results may be due to the solvent not being able to extract the components or the components being extracted before the GC-MS analysis could find them. Future study should utilize alternate methodologies for phytochemical screening, such as high-performance liquid chromatography, to find components that exhibit resistance to evaporation or destruction at extreme temperatures [20]. More research is needed to confirm the study's results, especially about how the combination works against biofilms, which could help make better oral health products.

V. Conclusions

This in vitro investigation unequivocally revealed the antibacterial properties of red pomegranate ethanolic albedo extract against clinically isolated *P. intermedia*. It is advisable to do additional research to isolate and determine the physiologically active constituents of these oil extracts. It is also advised to perform in vivo studies to validate their effectiveness and to integrate them with mechanical periodontal therapy.

References

1. Könönen, E., Gursoy, M. and Gursoy, U.K. (2019). Periodontitis: a multifaceted disease of tooth-supporting tissues. *Journal of Clinical Medicine*, 8(8), p.1135. DOI: 10.3390/jcm8081135
2. Rasheed, A.H., Gul, S.S. and Azeez, H.A. (2022). Antibacterial and Antibiofilm Profiles of Thymus Vulgaris Essential Oil on Clinically Isolated Porphyromonas Gingivalis and Prevotella Intermedia: An in vitro Study. *Dent J*, 9(2), pp.53-63. https://doi.org/10.3390/dj9020053 .
3. Smiley, C.J., Tracy, S.L., Abt, E., Michalowicz, B.S., John, M.T., Gunsolley, J., Cobb, C.M., Rossmann, J., Harrel, S.K., Forrest, J.L., Hujoel, P.P., Noraian, K.C., Greenwell, H., Frantsve-Hawley, J., Estrich, C. and Hanson, N. (2015). Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *The Journal of the American Dental Association*, 146(7), pp.508-524.e5. DOI: 10.1016/j.adaj.2015.01.026.
4. Madhloom, A.F., Al-Taweel, F.B.H., Sha, A.M. and Abdulbaqi, H.R. (2022). Antimicrobial Effect of Moringa Oleifera L. and Red Pomegranate against Clinically Isolated Porphyromonas gingivalis: in vitro Study. *Archives of Razi Institute*, 77(4), pp.1405–1419. DOI: 10.22092/ARI.2022.357513.2051 .
5. Mathabe, M.J., Nikolova, R.V., Lall, N. and Nyazema, N.Z. (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *Journal of Ethnopharmacology*, 105(1-2), pp.286-293. DOI: 10.1016/j.jep.2006.01.029.
6. Reddy, M.K., Gupta, S.K., Jacob, M.R., Khan, S.I. and Ferreira, D. (2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. *Planta Medica*, 73(5), pp.461-467. DOI: 10.1055/s-2007-967167.
7. López-Bascón, M.A. and De Castro, M.L. (2020). Soxhlet extraction. In: C.F. Poole, ed., *Liquid-Phase Extraction*. Elsevier, pp.327-354. https://doi.org/10.1016/B978-0-12-816911-7.00011-6.
8. Madhloom, A.F., Al-Ghanim, K.M., Hussein, A.M., Yousif, A., Abdulrasool, N.M. and Jubran, A.S. (2024). Antimicrobial Activity of Moringa Oleifera L. Ethanolic Seeds Extract Against Orally Isolated Prevotella Intermedia from Periodontally Diseased Patients: An in Vitro Study. *J Bras Patol Med Lab*, 60, pp.e492. https://doi.org/10.5935/1676-2444.20240010
9. Kim, M.J., Hwang, K.H., Lee, Y.S., Park, J.Y. and Kook, J.K. (2011). Development of Prevotella intermedia-specific PCR primers based on the nucleotide sequences of a DNA probe Pig27. *Journal of Microbiological Methods*, 84(3), pp.394-397. DOI: 10.1016/j.mimet.2010.12.019
10. Wunsch, C.M. and Lewis, J.P. (2015). Porphyromonas gingivalis as a model organism for assessing interaction of anaerobic bacteria with host cells. *Journal of Visualized Experiments*, (106), p.53476. DOI: 10.3791/53476.
11. Elleuch, L., Shaaban, M., Smaoui, S., Mellouli, L., Karray-Rebai, I., Hu, L.B., et al. (2010). Bioactive secondary metabolites from a new terrestrial Streptomyces sp. TN262. *Applied Biochemistry and Biotechnology*, 162(2), pp.579-593. DOI: 10.1007/s12010-009-8869-4.
12. Saquib, S.A., Al-Qahtani, N.A., Ahmad, I., Kader, M.A., Al-Shahrani, S.S. and Asiri, E.A. (2019). Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: an in vitro microbiological study. *Antibiotics*, 8(3), p.89. https://doi.org/10.3390/antibiotics8030089.
13. Cavalieri, S.J. (2005). *Manual of Antimicrobial Susceptibility Testing*. American Society for Microbiology.

Ali Faisal Madhloom, "Antibacterial Activity of Red Pomegranate Ethanolic Albedo Extract Towards Prevotella Intermedia Isolated from Periodontitis Patients (In Vitro Study)," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 9, Issue 1, pp. 35-39, 2025.

14. Anand, B. (2017). Herbal therapy in periodontics: a review. *Journal of Research in Pharmaceutical Sciences*, 3(5), pp.1-7.
15. Zhu, W., Guo, J., Li, X., Li, Y., Song, L., Li, Y., Feng, B., Bao, X., Li, J., Gao, Y. and Xu, H. (2025). Effects of Gallic Acid on In Vitro Ruminal Fermentation, Methane Emission, Microbial Composition, and Metabolic Functions. *Animals*, 15(13), p.1959. https://doi.org/10.3390/ani15131959.
16. Choi, E.-Y., Kim, H.-R., Choi, J.-I., Kim, H.-S., Lee, S.-H. and Kim, C.-S. (2022). Nitrooleic acid inhibits macrophage activation induced by lipopolysaccharide from Prevotella intermedia. *Nutrition Research*, 106, pp.35-46. DOI: 10.1016/j.nutres.2022.06.006.
17. Choi, J.-S., Park, N.-H., Hwang, S.-Y., Sohn, J.-H. and Kwak, I. (2013). The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens. *Journal of Environmental Biology*, 34(4), pp.673-676. PMID: 24617106.
18. Kakad, A.V., Laddha, U.D., Kshirsagar, S.J. and Khairnar, S.J. (2022). Traditional herbal remedies for periodontitis. *Biosciences Biotechnology Research Asia*, 19(4), pp.1079-1091. https://doi.org/10.13005/bbra/3065.
19. Lynch, A.S. and Robertson, G.T. (2008). Bacterial and fungal biofilm infections. *Annual Review of Medicine*, 59, pp.415-428. DOI: 10.1146/annurev.med.59.110106.132000.
20. Das, K., Tiwari, R.K.S. and Shrivastava, D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2), pp.104-111. https://academicjournals.org/journal/JMPR/article-full-text-pdf/89A44D03017 .