ISSN No:-2456-2165

Formula Assessment of Oral Bacteria Inhibition Through the Biological Response of Plant Extracts

Basri A. Gani,[1] Subhaini Jakfar,[2] Muhammad Nazar[2]
[1]Department of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia
[2]Department of Dental Material, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia
[3]Department of Chemical Education, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia

Abstract:- Numerous natural ingredients are antimicrobial and antifungal. These characteristics must be compatible with the pathogens to be eradicated. Environmental factors and oral biology imbalances influence the development of the commensal oral cavity. Numerous plant extracts may contribute to maintaining a healthy commensal balance. Measuring the bio-tolerant properties of plant extracts is essential for determining the antibacterial and fungal properties of the extracts and maintaining the pathogen balance by preventing bacteria from becoming virulent. The bio-tolerance of plant extracts is determined using the biomass index, Temperature and pH changes, biostability, absorption and residual capacity, and pathogen opsonization. This study aims to establish the optimal concentration for future research trials; additionally, it will serve as a reference for measuring the development of oral commensals during the pathogenesis of dental and oral mucosa infections. The study was evaluated as a bio-tolerance concept to plant extracts as regulators of the oral commensal balance.

Keywords:- *Formula inhibition, Oral commensals, Plant extracts*

I. INTRODUCTION

We are researching the development of several natural ingredients such as *Moringa oleifera*, *Punica granatum* (*P. granatum*), and *Ziziphus mauritiana* for application in dental research(Soraya et al., 2020), especially related to growth control and virulence activity of oral bacteria. Oral bacteria and fungi, mandibular and maxillary bone remodeling(BURHAN et al., 2018), antioxidants, anti-oral malodor (halitosis), and several natural sources are used to prevent endocarditis infections triggered by oral commensals. Before being used as a test material, many of these natural ingredients in extracts were tested for bio-tolerance. This test confirms the effectiveness of natural ingredients when interacting with hosts and oral infectious agents. The appropriate concentration and growth control properties and balanced virulence of pathogens are references for application to the oral cavity to prevent infection of the oral cavity and toxicity to host cells such as candidiasis, halitosis, dental caries, periodontitis, and root canal infections.

Several infectious agents that cause oral infection are normal flora that must be present as a requirement for regulating the biological balance of the oral cavity. The function of natural substances applied to infection keeps the oral growth of the pathogen from developing and has a minimal population limit. The assessment of the bio-tolerant concept is adopted from the pharmacodynamic and pharmacokinetic properties of test materials such as drugs when administered to the host to prevent infection of certain diseases(Levison and Levison, 2009). Pharmacodynamic and pharmacokinetic properties are the main requirements for the duration of action of drugs in the body related to immunotolerant and immuno-compatibility properties(Gani, 2015). Many tests related to bio-tolerance are biomass index, changes in Temperature and pH, biostability, absorption and residual capacity, and the ability to opsonize against pathogens. We used some oral commensals, including *Streptococcus mutans*, *Enterococcus faecalis*, and *Porpiromonas gingivalis*, to test the bio-tolerant properties of plant extracts. These results can control the balance of oral commensal growth and prevent biofilm formation, adhesion, and invasion in oral mucosal and epithelial cells. They are called virulence factors.(Bachtiar et al., 2021)

II. MATERIAL AND METHODS

In this study, plant extracts were used as test material, and oral pathogens were used as subjects for in-vitro simulations. Several approaches were used to investigate the test material's ability to act as an anti-oral pathogen. The first stage involved producing ethanol extracts from plants using the Soraya (2020) working principle (Soraya et al., 2020). Furthermore, bacterial culture (oral pathogens) was performed using Gani's work technique (Gani et al., 2012). Mc. Farland 0.5 was used to prepare bacteria (calibrated colony count) (0.5x108). In addition, the interaction between the oral pathogen and the test material (plant extracts) was investigated. The two interacted using an incubation time approach of 24 hours, 48 hours, and 72 hours. The interaction between the two was assessed by calculating the biomass index based on weight (Soraya and Gani, 2021), temperature, and pH changes. Biostability was determined by balancing the test material's response to bacterial growth degradation, and absorption or plant extract residue was determined by the ratio of residue diameter using disc media and oral opsonization. Pathogens were evaluated using qualitative data collected by observing the opsonization process of bacterial cells by active components or chemical

compounds found in plant extracts. These outcomes were assessed using an in-vitro approach based on a formula. Our laboratory created this assessment formula to make it easier to obtain anti-oral pathogen indicators produced by plant extracts.

III. RESULTS AND DISCUSSION

➢ *Measurement of Biomass Index*

The principle of this test is to measure the total solid dissolved (biomass) formed as a result of the interaction between the test material (plant extracts) and pathogens using an incubation time basis of 24 hours, 48 hours, and 72 hours. The value of the biomass index was measured using analytical scales based on the sample weight before and after incubation. The difference in value is a reference to justify whether the test material (plant extracts) can eliminate oral pathogens or maintain growth balance, based on the higher the index biomass, the stronger the bacterial activity, meaning that the material is less sensitive to pathogens or vice versa, the lower the biomass index, the better it is to maintain the balance of bacteria. This activity is based on the formula: Biomass Value = A– B (A: value before incubation) (B: Value after incubation). Then the resulting index biomass value = C / D x 100%, (C: Biomass value); (D: Total Value of samples). Best (<20%); Good (21-50); 51-75 (Moderate); 76-100% (low effect).

➢ *Changes in Temperature and pH*

The principle of this test is to measure changes in the pH of the solution due to the influence of the oral activity of the pathogen to maintain a stable environment that is beneficial to the pathogen by lysing several active plant components to escape death. Examination of the Temperature and pH of the solution can be an indicator of pathogenic activity. Gani (2012) showed in his research that each oral pathogen has mutual control over changes in salivary pH(Gani et al., 2012). This test examined changes in the solution's pH (pathogens with plant extracts) using a formula is quantity pH change = A / B (A: pH of the treatment group); (B: Pathogen pH in medium or control). Meanwhile, to assess the adaptation of pH of plant extracts using a formula, pH response = 100% - A (A: percentage of every sample of the number of pH changes). Best response (80-100%); Good responses (65-79%); moderate (40-64%); Low responses (<40%).

➢ *Biostability*

The principle of this test is to assess the stability of the test material (plant extract) against the influence of pathogens. This value determines the lowest preferred concentration, which still shows strong stability against the influence of bacterial activity based on changes in time of 24 hours, 48 hours, and 72 hours. The biostability value was taken from the solution (plant and bacterial extracts) and then centrifuged at 3000 rpm for 15 minutes. The supernatant was measured by spectrophotometry at a wavelength of 590 nm. Biostability Formula Index = A / B (A: OD Sample); (B: OD Control). The scale of the biostability index was OD 1> (strong); OD 0.91-0.99 (moderate); <OD 0.9 (weak).

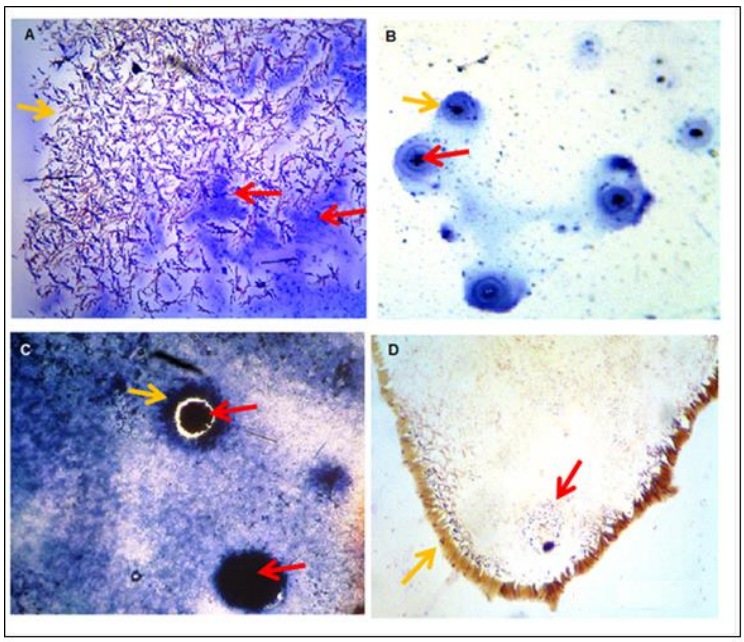

Fig 1:- Opsonization of *E. faecalis* cells by *Punica granatum*. (A) the atmosphere of active component interaction of *P. granatum* (yellow arrow) and *E. faecalis* cells trapped in the active component of *P. granatum* (red arrow) at 400x magnification. (B) Profile of E. faecalis cells opsonized by the active ingredient of *P. grantum* (red arrow) and opsonization, the active component (yellow arrow) of 1000x magnification. (C) Extreme opsonization of E. faecalis cells experienced toxicity and lysis (red arrows) and opsonization (yellow arrows) 1000x magnification. (D) The stage of opsonizing *E. faecalis* cells by the active component of *P. granatum*. The images were taken of different *P. granatum* concentrations tested in this study

ISSN No:-2456-2165

➢ *Level Absorbtion and Residual of Plant Extract*

The principle of this test is to ensure that the test material (plant extract) is absorbed by the target cells, both as an antibacterial and antioxidant function. The residual value is an indicator of metabolism that is not absorbed by cells, and this is to avoid excessive response from target cells (bacterial cells and host cells). In this test, if the absorption value is greater than the residual value, then the concentration of choice becomes a reference for further testing on hosts and bacteria. Still, if the residual is more extensive, it is necessary to screen the molecular size of the test material (plant extract) so that the residual value is smaller. The assessment of the absorption and residual power of plant extracts was carried out using a culture medium basis by forming a well before which the surface of the medium was coated with antibiotics to prevent contamination from bacteria and fungi. Then the plant extract was filtered with Whatman paper Grade S2: 8 µm was recommended to filter the plant extract (http://rothsteinchemicals.com/filter%20paper.html), then measured the absorption zone and the residual zone. The value of absorption power uses the Absorption Level formula: $\frac{\text{Ratio Diameter Residual}}{\text{Total Diameter Residual} + \text{Diameter absorbs zone}}$ Using a scale, Strong (<0.50 mm), Moderate (0.51-0.75 mm), Weak (0.76-1 mm), and Negative (1mm more).

➢ *Opsonization of Pathogens cells*

- We are examining pathogenic cell opsonization to determine the cellular response of plant extracts to pathogenic cells and assess its ability to eliminate pathogenic cells. This examination is to obtain the standard or number of opsonization cells as an indicator of cytotoxicity and lysis, which can reduce the degree of virulence of the pathogen in the pathogenicity of oral infections. In general, pathogenic cells that have been opsonized by Plant extract (antibacterial) can disrupt communication between oral pathogenic cells in the phases of biofilm formation, colonization, and adhesion on the tooth surface and oral mucosa(Kolenbrander et al., 2002). Fig.1 shows one of the plant extracts (Punica granatum) when the Enterococcus faecalis bacterial cell opsonization occurred.

IV. CONCLUSION

Several tests in our review laboratory mentioned the biotolerant assay we practiced before assessing bacterial growth, biofilm formation, and anti-adhesin of oral bacteria like *S. mutans* and *E. faecalis*. The results of our research show that many plant extracts we use can balance the oral activity of bacteria in the pathogenesis of infection in-vitro. In addition, this biotolerant concept and opsonization of bacterial cells can be used to measure drug hydrophilic and hydrophobic activity.

ACKNOWLEDGMENT

The authors would like to acknowledge Grant No. 172/UN11/SPK/PNBP/2021, February 22 2021, LPPM Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia

REFERENCES

[1]. Bachtiar BM, Gani BA, Deviana A et al. The discrepancy between clove and non-clove cigarette smoke-promoted Candida albicans biofilm formation with precoating RNA-aptamer. *F1000Research* 2021;10: 372.
[2]. Burhan E, Thahar B, Gani BA et al. Analysis of reactivity of Punica granatum polyphenols to the osteocalcin, bone morphogenetic protein-2, and collagen type-1. Asian Journal Pharmaceutical and Clinical Research. 2018;11(12):544-7.
[3]. Gani BA. Immuno-biokompatibilitas Pada Material Implan (Immuno-biocompatibility of implant materials). Cakradonya Dental Journal 2015**;7**:842-847.
[4]. Gani BA, Soraya C, Sunnati S et al. The pH changes of artificial saliva after interaction with oral micropathogen. Dental Journal (Majalah Kedokteran Gigi) 2012;45:234-238.
[5]. Kolenbrander PE, Andersen RN, Blehert DS et al. Communication among oral bacteria. Microbiology and Molecular Biology Reviews 2002;66:486-505.
[6]. Levison ME, Levison JH. Pharmacokinetics and pharmacodynamics of antibacterial agents. Infectious Disease Clinics 2009;23:791-815.
[7]. Soraya C, Alibasyah ZM, Gani, BA. Biomass index and viscosity values of Moringa oleifera influenced by Enterococcus faecalis. Journal of Syiah Kuala Dentistry Society 2021; **6**:1-5.
[8]. Soraya C, Mubarak Z and Gani BA. The growth and biofilm formation of Enterococcus faecalis in ethanol extract of Citrus aurantiifolia Indonesian species. Journal of Pharmacy & Pharmacognosy Research 2020;8:558-568.