QUALITY IMPROVEMENT IN DENTAL AND MEDICAL KNOWLEDGE, RESEARCH, SKILLS AND ETHICS FACING GLOBAL CHALLENGES

Edited by
Armelia Sari Widyarman, Muhammad Ihsan Rizal,
Moehammad Orliando Roeslan & Carolina Damayanti Marpaung

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Quality Improvement in Dental and Medical Knowledge, Research, Skills and Ethics Facing Global Challenges

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Armelia Sari Widyarman, Muhammad Ihsan Rizal, Moehammad Orliando Roeslan and Carolina Damayanti Marpaung
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Preface

Faculty of Dentistry Universitas Trisakti (Usakti) presents FORIL XIII 2022 Scientific Forum Usakti conjunction with International Conference on Technology of Dental and Medical Sciences (ICTDMS) on December 8th–10th 2022. The theme of the conference is "Quality Improvement in Dental and Medical Knowledge, Research, Skills and Ethics Facing Global Challenges".

The triennial conference has served as a meeting place for technical and clinical studies on health, ethical, and social issues in field medical and dentistry. It is organized around 12 major themes, including behavioral, epidemiologic, and health services, conservative dentistry, dental materials, dento-maxillofacial radiology, medical sciences and technology, oral and maxillofacial surgery, oral biology, oral medicine and pathology, orthodontics, pediatrics dentistry, periodontology, and prosthodontics.

The most recent findings in fundamental and clinical sciences related to medical and dental research will be presented in the conference that will be published as part of the conference proceeding. This proceeding will be useful for keeping dental and medical professionals up to date on the latest scientific developments.

Dr. Aryadi Subrata
Chairman FORIL XIII conjunction with ICTDMS

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FAKULTAS KEDOKTERAN GIGI
UNIVERSITAS TRISAKTI

FORIL XIII 2022

FKG USAKTI SCIENTIFIC FORUM CONJUNCTION WITH INTERNATIONAL CONFERENCE IN DENTAL, MEDICAL SCIENCES AND TECHNOLOGY

"Quality Improvement in Dental Knowledge, Research, Skills and Ethics Facing Global Challenges"

8 - 10 December 2022 - JIEXPO Convention Centre and Theatre

FOREWORD

It is a great pleasure to welcome all of you, dentists, students, sponsors, and exhibitors, to this year's FORIL XIII (Forum Ilmiah) 2022. I am delighted to announce that FORIL XIII 2022 will be held at JI EXPO Convention Centre and Theatre on December 8th to 10th, 2022.

In the era of globalization, dentists have been expected to continuously pursue and update more knowledge, refine their skills, and learn advanced technology to be able to compete with dentists from all around the world and provide the best treatment for their patients. As a way to do that, our Faculty of Dentistry, Universitas Trisakti will hold FORIL, a scientific seminar with the theme "Quality Improvement in Dental Knowledge, Research, Skills, and Ethics Facing Global Challenges".

Faculty of Dentistry, Universitas Trisakti has held FORIL for many years with forefront topics on dental research and clinical applications brought by our established and professional experts from our faculty. Our organizing committe has prepared this event attentively with preeminent scientific programs, enthralling social events, and attractive dental exhibition. This event could also be the perfect place for your blissfull reunion with your colleagues. It would be an honor and privilege to have each and every one of you to participate and join us in our Faculty's acclaimed program.

drg. Aryadi, Sp. KG (K)
Chairperson, FORIL XIII 2022 Organizing Committee

Greetings from Jakarta,

It gives me tremendous pleasure to welcome all colleagues, students, sponsors and exhibitors to our Scientific Forum the “XIIIth FORUM ILMIAH” (FORIL 2022) to be held from 8 to 10 December 2022 at Jakarta International Expo Convention Centre and Theatre, Kemayoran, North Jakarta. I feel extremely proud that the XIIIth FORIL 2022 is going beyond as a part of Continuing Dental Professional Development Program.

The theme of the XIIIth FORIL 2022 is "Quality Improvement in Dental Knowledge, Research, Skills and Ethics Facing Global Challenges". This theme is to anticipate the challenges of globalization era in the field of dental health care, so that the quality of dental health professionals including dentists in Indonesia can be improved through the updating researches, clinical practices, sciences professionalism, skills and technology without leaving the ethical aspect.

The XIIIth meetings are expected to offer scientific programs, exhibition, and dentist reunion. These sessions will enrich your knowledge on the latest developments in oral and dental disciplines.

On behalf of Faculty of Dentistry Universitas Trisakti, I would like to invite everyone to be a part of this important event. I look forward to welcoming you to the XIIIth FORIL 2022.

Best wishes,
Prof Dr. Drg. Tri Erri Astoeti, MKes.
Dean of Faculty of Dentistry, Universitas Trisakti

FORIL XIII

Faculty of Dentistry Universitas Trisakti (USAKTI) presents International Conference in Dental, Medical Sciences and Technology (ICDMST) on December 8-10, 2022. With the main theme of "Quality Improvement in Dental and Medical Knowledge, Research, Skills and Ethics Facing Global Challenges", this triennial conference has served as a meeting place for researchers, practitioners, and academics to share their technical and clinical studies on health, ethical, and social issues in field medical and dentistry. The conference welcomes participants to present most recent findings in fundamental and clinical sciences related to medical and dental research under 12 major topics, including behavioral, epidemiologic, and health services, conservative dentistry, dental materials, dento-maxillofacial radiology, medical sciences and technology, oral and maxillofacial surgery, oral biology, oral medicine and pathology, orthodontics, pediatrics dentistry, periodontology, and prosthodontics. Selected papers will be published in a conference proceedings which will be useful for keeping dental and medical professionals up to date on the latest scientific developments.

AGENDA

May 16, 2022
First Call for Abstract

August 30 2022
Abstract Submission Deadline

September 9 2022
Announcement of Abstract Acceptance

October 14 2022
Full Paper Submission and Payment Deadline

December 8 2022
Conference Day 1

December 9 2022
Conference Day 2

December 10 2022
Conference Day 3

Jl. Kyai Tapa No.260, RT.4/RW.16, Grogol, Kec. Grogol petamburan, Kota Jakarta Barat, Daerah Khusus Ibukota Jakarta 11410

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FORIL XIII 2022

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ABSTRACT

SHORT LECTURE

RECONSTRUCTION OF LARGE POST- ENUCLEATION MANDIBULAR DEFECT WITH BUCCAL FAT PAD

ABS-090

Nyoman Ayu Anggayanti, Agus Dwi Sastrawan, Oyagi Shuka

Background: The ideal intraoral reconstruction should mimic speech, mastication, articulation, and aesthetical function of previous soft and hard tissue. Buccal Fat Pad (BFP) is a vascularized graft with potent regenerative ability. However, reports in BFP application especially in mandibular defects are somewhat limited.
Case Report: A 47-year-old female patient came to Wangaya Regional Hospital, Bali, Indonesia with chief complaint of swelling on left lower jaw. Radiograph examination showed a large cystic lesion in posterior left mandible region.
Case Management: After extraction of affected teeth #36-38, overlying bone was removed, the cyst was enucleated and sent for biopsy. Necrotomy was performed, leaving a defect of 3.5 cm x 1.5 cm. Buccal extension of BFP was herniated via blunt dissection, placed into the post-enucleation defect, covered with flap, and sutured. The defect showed progressive and stable healing at one day, one week, and one-month post-reconstruction follow up.
Discussion: BFP has been increasingly used for intraoral reconstruction especially in oroantral communication and cleft palate cases. It has been reported to give successful result even in previously failed graft site. BFP has a low infection rate, is rich in vascularity, close to recipient site, has quick epithelization rate, and only needs minimal dissection to be harvested hence minimal morbidity at donor site. The main disadvantage of BFP is possible post-surgical contraction. Conclusion: BFP graft is a practical technique that could be applied clinically to achieve an ideal intraoral reconstruction, mimicking both aesthetic and functionality of antecedent removed tissues.

Keywords: Mandibular defect; Intra oral reconstruction; Buccal fat pad

INTERCEPTIVE ORTHODONTIC TREATMENT NEED AND ITS RELATING DEMOGRAPHIC FACTORS IN DKI JAKARTA AND KEPULAUAN SERIBU

ABS-091

Y Yusra, J Kusnoto, H Wijaya, T E Astoeti, B Kusnoto

Background: Interceptive orthodontic is an orthodontic treatment procedure that aims to minimize the effect of malocclusion and decrease the need for a more complex malocclusion treatment, high cost of treatment, and eventually declining the need for corrective orthodontic treatment. DKI Jakarta and Kepulauan Seribu has 763.666 primary school aged children thus screening for the need of interceptive orthodontic treatment would be highly useful in identifying children that would benefit from getting interceptive orthodontic treatment. Aim. To investigate the need for interceptive orthodontic treatment and identifying its relating factors in 8-11 years old children in DKI Jakarta and Kepulauan Seribu. Method. This research is observational analytic research with cross sectional study design utilizing the Indeks Kebutuhan Perawatan Ortodonti Interseptif (IKPO-I). Each indicator is scored based on the subjects intra oral conditions then the data gathered was used to quantify the need for interceptive orthodontic treatment and its relating factors. Result. Based on 2020 subjects it is found that 18.96% of subjects does not need orthodontic treatment, 59.36% require interceptive orthodontic treatment, and 21.68% need corrective orthodontic treatment. There is a significant correlation between need for interceptive orthodontic treatment with parents' income (r= -0.07; p= 0.02). Conclusion. IKPO-I can be used as an interceptive orthodontic treatment screening instrument. More than half of the subjects require interceptive orthodontic treatment. Parents' income is the only demographic factor relate to the need for interceptive orthodontic treatment.

Keywords: Interceptive orthodontic, treatment need, IKPO-I, DKI Jakarta and Kepulauan Seribu

PEPSODENT FORIL XIII AWARD

WHAT IS PEPSODENT FORIL XIII AWARD?

Pepsodent Foril XIII Award is a prestigious competition organized by Foril Scientific Committee to honour the participants with outstanding research, case reports or literature reviews. We welcome everyone from different institutes and countries who wishes to participate in Pepsodent Foril XIII Award. The winner of Pepsodent Foril XIII Award will be granted prize money from our sponsor.

CATEGORIES OF COMPETITION
Participants can choose to enter into one of the following categories in the competition during the online abstract submission:

1. Dentists Category:
Participants has acquired their dental degree, is a dental practitioner, or enrolled in a post-graduate or PhD program. The participant of this category can choose to submit abstract on one of the following criteria:
a. Research
b. Case Report
c. Literature Review.

2. Student Category:
This category will be limited to only students who have completed their research as part of undergraduate dental programs.

PRIZE FOR PEPSODENT FORIL XIII AWARD WINNERS

1. Dentist Categories

a. Research
The winner will receive Rp.12.000.000,-
The first runner up will receive Rp.9.000.000,-
The second runner up will receive Rp.7.000.000,-

b. Case Report
The winner will receive Rp.10.000.000,-
The first runner up will receive Rp.8.000.000,-
The second runner up will receive Rp.6.000.000,-

c. Literature Review
The winner will receive Rp.9.000.000,-
The first runner up will receive Rp.7.000.000,-
The second runner up will receive Rp.5.000.000,-

2. Student Categories
The winner will receive Rp.8.000.000,-
The first runner up will receive Rp.6.000.000,-
The second runner up will receive Rp.4.000.000,-

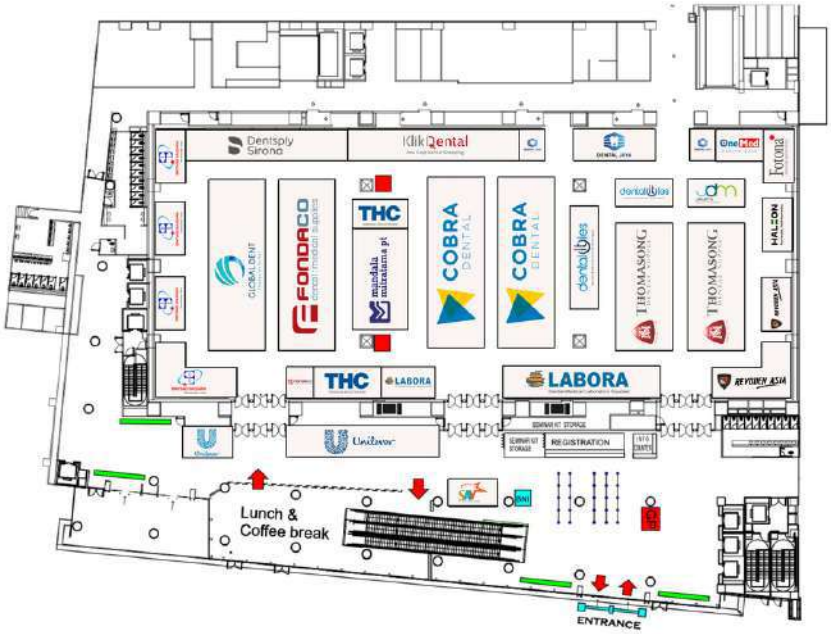
HOW TO PARTICIPATE?

- Participants can choose to enter award competition during abstract submission. Choose the correct category (Student/Dentist).
- Once the abstract is accepted, participants are expected to complete the registration/publishing payment and submit full paper. It is strongly recommended for the participant to proofread the manuscript using manuscript editor (Enago, etc) before full paper submission.
- The judges will review the abstract and full paper based on the originality and writing methods of the research/case report/literature review.
- The award finalists will be announced to present their paper to a panel of judges at the venue (offline session).
- The judging session will be held during the Pre-Foril session at Faculty of Dentistry Universitas Trisakti, Jakarta.

FLOOR PLAN

JIEXPO Convention Centre

GRAND BALLROOM



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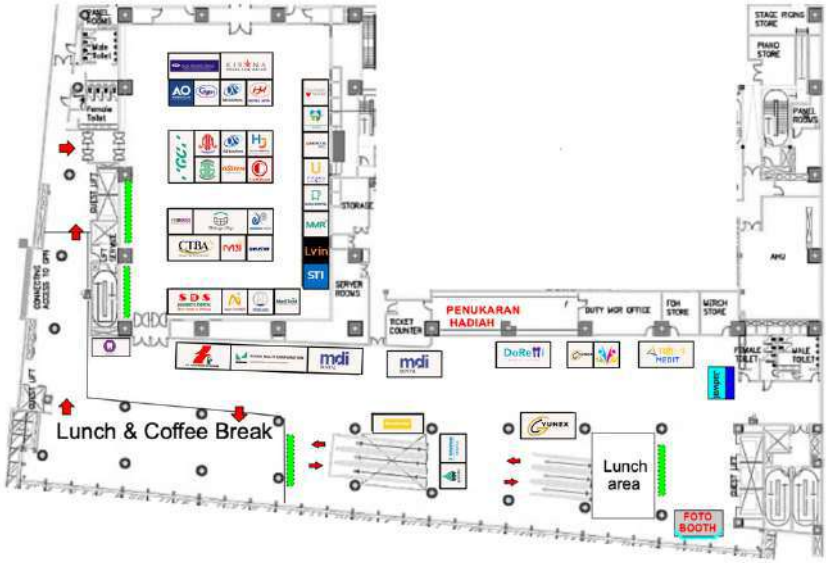

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Arumanis mango leaves (*Mangifera indica* L.) extract efficacy on *Porphyromonas gingivalis* biofilm *in-vitro*

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ABSTRACT: Arumanis mango leaves (*Mangifera indica* L.) have the potential to be antibacterial and antibiofilm agents as they contain mangiferin, flavonoid, and tannin that might inhibit the growth of *P. gingivalis* and its biofilm formation. The objective of this study is to determine the antibacterial and antibiofilm effects of *Mangifera indica* L. leaves ethanol extract against *P. gingivalis*. An *in-vitro* laboratory experiment was performed with a post-test only control group design. The present study used 3.125%, 6.25%, 12.5%, 25%, 50%, and 100% concentrations of *Mangifera indica* L ethanol extract; dimethyl sulfoxide (DMSO) was used as negative control and amoxicillin as positive control. The plate count method was performed for the antibacterial test and microtiter plate biofilm assay for the antibiofilm test. One-way ANOVA was used for the statistical analysis, with $p < 0.05$ considered as significant level. The result showed the most effective antibacterial activity against *P. gingivalis* was 100% extract concentration compared to negative control ($p<0.05$). Moreover, the most effective concentration against *P. gingivalis* biofilm formation was 100% extract in 3 hours incubation period compared to negative control ($p<0.05$). It can be concluded that *Mangifera indica* L. leaves ethanol extract inhibited *P. gingivalis* growth and biofilm formation.

Keywords: antibacterial, antibiofilm, periodontitis, Porphyromonas gingivalis, Mangifera indica L

1 INTRODUCTION

According to the 2018 Basic Health Research (*Riset Kesehatan Dasar*/RISKESDAS), periodontitis is one of the most common periodontal diseases in Indonesia with a prevalence of 74.1% (Kemenkes 2018). Periodontitis is an inflammatory condition that occurs in periodontium, such as gingiva, cementum, periodontal ligament, and alveolar bone. It begins with poor oral hygiene, which leads to the accumulation of biofilm in the gingiva and tooth surface, and as the biofilm grows thicker and more complex, the more severe the periodontitis (Mehrotra & Singh 2020).

Porphyromonas gingivalis is the etiology of periodontitis. This opportunistic bacterium colonizes the biofilm as the second colonizer whose main habitat is in the subgingival area (Kinane et al. 2017). In treating periodontitis, the administration of antibiotics (amoxicillin, tetracycline, clindamycin, and ciprofloxacin) is one of the treatments in the etiotropic phase to reduce the growth of pathogenic bacteria in the oral cavity (Ciancio & Mariotti 2019). However, the use of antibiotics such as amoxicillin can have negative effects on the body, including hypersensitivity, vomiting, nausea, gastrointestinal disturbances, and opportunistic infections, while the use of tetracyclines can cause diarrhea, vomiting, dizziness, and discoloration of teeth (Akhavan et al. 2020).

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In addition, bacteria in a biofilm also have greater resistance to antibiotics and some antibiotics are unable to penetrate the biofilm due to its matrix that prevents the diffusion of antibiotics, expresses multiantibiotic efflux pumps, and reduces the permeability of the bacteria. Thus, antibiotics are unable to penetrate the biofilm (Bat *et al.* 2021). Therefore, other alternative materials, such as herbal products with minimal side effects, in treating periodontal disease are indispensable (Joshua & Takudzwa 2013).

Mangifera indica L., also known as mango arumanis plant, is a plant that grows in tropical and subtropical countries, especially in the Asian region. Mangiferin is the main polyphenolic compound that is often found in all parts of the *M. indica* L. plant, including fruit, bark, tree, and leaves (Kulkarni & Rathod 2014). This compound has a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, such as *Streptococcus mutans, Staphylococcus aureus*, and *Enterococcus faecalis* (Kurniasih 2016). The leaves of the *M. indica* L. arumanis variety were proven to have the highest percentage of mangiferin content and the most potent antibacterial power against *S. aureus* when compared to other varieties (Utami et al. 2020). Other than mangiferin, the leaves of *M. indica* L. also contain flavonoid compounds, tannins, alkaloids, steroids, and saponins, which also contribute to antibacterial activity (Jhaumeer et al. 2018).

To the knowledge of the authors, to this date, research on antibacterial and antibiofilm effects of ethanolic extract of *M. indica* L. leaves against *P. gingivalis* is yet to be conducted. To cover this research gap, this study aimed to determine the effect of ethanolic extract of *M. indica* L. leaves on the growth and formation of *P. gingivalis* biofilms. The utilization of mango arumanis leaves has the potential to exhibit antibacterial and antibiofilm properties for the treatment of periodontitis.

2 METHODS

2.1 *Preparation of M. indica L. leaf ethanol extract*

The sample used was an ethanol extract of the leaves of mango arumanis (*Mangifera indica* L.) made by the Indonesian Research Institute for Spices and Medicinal Plants (*Balai Penelitian Tanaman Rempah dan Obat*/BALITTRO). As much as 1,500 g of *M. indica* L. leaves were cleaned and dried at 40°C. Then the leaves were blended and the powder was mixed with 70% ethanol solvent in a ratio of 1:5, and macerated for 2-3 hours. Next, the mixture was filtered to get macerate, which was evaporated with a rotary evaporator, thus the thick ethanol extract of *M. indica* L. leaves with a concentration of 100% was obtained. Moreover, several dilutions were made with 10% dimethyl sulfoxide (DMSO) to obtain concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125%.

2.2 *Bacterial culture*

Porphyromonas gingivalis ATCC 33277 bacteria were cultured on Tryptic Soy Broth (TSB) (Oxoid, Hampshire, UK) media, which had been enriched with hemin (5 mg/L), vitamin K1 (10 mg/L), 0.5% yeast extract, and L-cystine (400 mg/L), then incubated under anaerobic conditions at 37°C. After 24 hours, the bacterial suspension was measured with a microplate reader until absorbance was equivalent to 0.5 McFarland (1.5×10^8 CFU/mL) or $OD_{600} \pm 0.132$.

2.3 *Antibacterial test with plate count method*

Antibacterial testing was performed using the microdilution method. A total of 100 µL suspension of *P. gingivalis* ATCC 33277 was distributed into a 96-well-plate well using micropipette. A total of 100 µL of each test solution was added to the wells and incubated at 37°C under anaerobic conditions.

After incubation for 24 hours, the microdilution results from each treatment were taken and diluted 10,000 times. Moreover, 5 µL was taken to be placed on Brain Heart Infusion

Agar (BHI-A) media in a petri dish. The growth of bacterial colonies was calculated after incubation for 24 hours at 37°C.

2.4 *Antibiofilm test with microtiter plate biofilm assay*

A total of 200 µL suspension of *P. gingivalis* ATCC 33277 was inserted into a 96-well-plate well with a micropipette and incubated at 37°C under anaerobic conditions. After incubation for 48 hours, the supernatant was removed from the wells leaving a layer of biofilm at the bottom, and the wells were washed with phosphate-buffered saline (PBS).

Ethanol extract of *M. indica* L. Leaves, with different concentrations (3.125%, 6.25%, 12.5%, 25%, 50%, and 100%), was added into the well as much as 200 µL using a micropipette. Biofilm without treatment was used as the negative control and amoxicillin 200 µg/mL as the positive control. Moreover, the wells were incubated for 1, 3, and 24 hours at 37°C. The wells were washed again with PBS and fixed over the fire. To measure the density of *P. gingivalis* biofilm, the wells were given 200 µL of crystal violet stain (0.05% w/v), then left for 15 minutes. Then, the wells were washed with PBS twice and 200 µL of 96% ethanol was added. Optical density (OD) measurement of biofilm was performed with a microplate reader (SAFAS MP96, SAFAS, Monaco) at the wavelength of 490 nm.

3 RESULTS

The results of the antibacterial test using the plate count method can be seen in Figure 1. In this study, ethanol extract of *M. indica* L. leaves with various concentrations is shown to inhibit the growth of *P. gingivalis* (Figure 2).

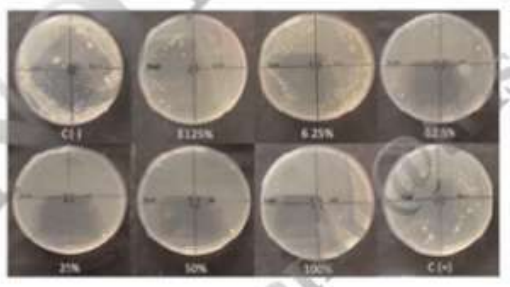

Figure 1. The results of the growth inhibition test of *P.* gingivalis with plate count method.

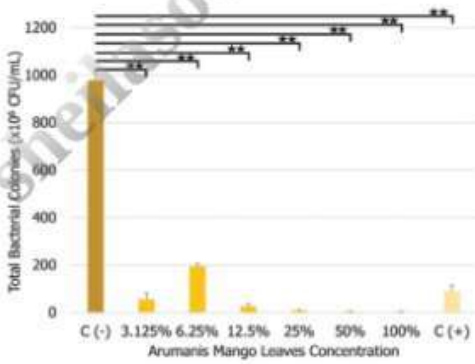

Figure 2. Graphic of total bacterial colonies of *P. gingivalis* by plate count method. DMSO 10% as negative control and amoxicillin 200 µg/mL as positive control.

Ethanol extract of *M. indica* L. leaves with a concentration of 100% produced the best antibacterial activity against *P. gingivalis* with a total colony of *P. gingivalis* (3.33 ± 1.15) x 10^6 CFU/mL (Table 1).

Table 1. The result of the mean total colony of *P. gingivalis* by plate count method.

Treatment	Mean (CFU/mL)
K(-)	(978,67 ± 41,05) × 10^6
3,125%	(55,33 ± 26,1) × 10^6
6,25%	(195,33 ± 11,37) × 10^6
12,5%	(25,33 ± 11,37) × 10^6
25%	(8,67 ± 3,06) × 10^6
50%	(4 ± 0,00) × 10^6
100%	(3,33 ± 1,15) × 10^6
K(+)	(90 ± 24,98) × 10^6

The results of the antibiofilm test using microtiter plate biofilm assay showed that ethanol extract of *M. indica* L. leaves with different concentrations had an antibiofilm effect on *P. gingivalis* at incubation periods of 1, 3, and 24 hours (Figures 3, 4, and5).

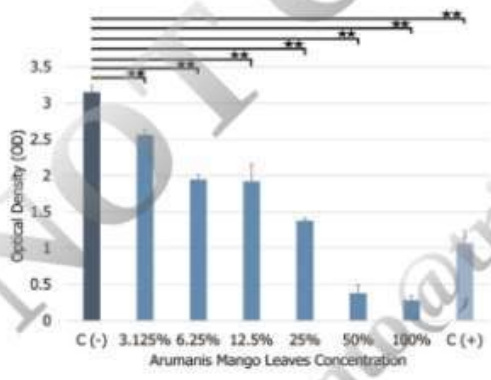

Figure 3. Graphic of mean OD of *P. gingivalis* biofilm with 1 hour incubation period. Biofilm without treatment as negative control and amoxicillin 200 μg/mL as positive control.

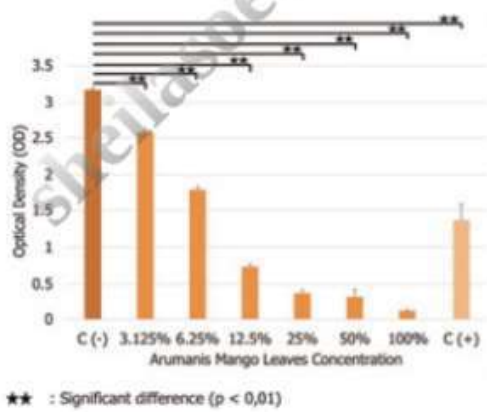

Figure 4. Graphic of mean OD of *P. gingivalis* biofilm with 3 hours incubation period. Biofilm without treatment as the negative control and amoxicillin 200 μg/mL as positive control.

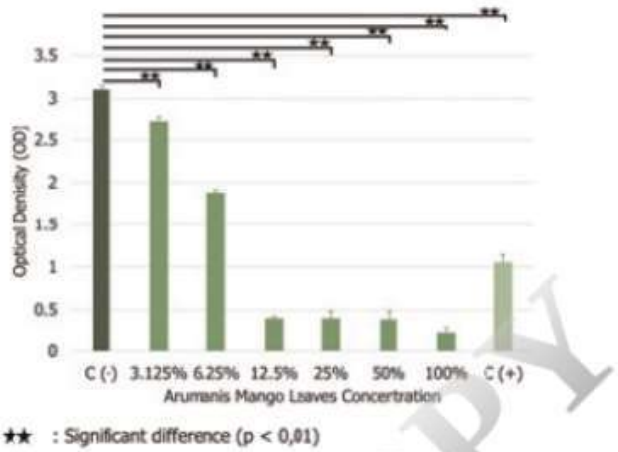

Figure 5. Graphic of mean OD of *P. gingivalis* biofilm with 24 hours incubation period. Biofilm without treatment as the negative control and amoxicillin 200 μg/mL as the positive control.

The extract with 100% concentration was the most effective in inhibiting *P. gingivalis* biofilm during the incubation period of 3 hours due to the smallest OD value, namely OD 0.115 ± 0.015 (Table 2).

Table 2. Average OD ± SD biofilm *P. Gingivalis.*

Treatment	OD 1 hr	OD 3 hr	OD 24 hr
K (-)	3,148 ± 0,089	3,172 ± 0,026	3,104 ± 0,044
3,125%	2,563 ± 0,065	2,575 ± 0,042	2,738 ± 0,051
6,25%	1,947 ± 0,064	1,798 ± 0,04	1,884 ± 0,029
12,5%	1,918 ± 0,238	0,735 ± 0,033	0,404 ± 0,016
25%	1,377 ± 0,034	0,376 ± 0,039	0,402 ± 0,086
50%	0,377 ± 0,112	0,321 ± 0,108	0,389 ± 0,098
100%	0,281 ± 0,063	0,115 ± 0,015	0,214 ± 0,054
K(+)	1,066 ± 0,173	1,365 ± 0,215	1,055 ± 0,090

4 DISCUSSION

This study showed that ethanol extract of *M.indica* L. leaves is effective in antibacterial activity against *P. gingivalis* biofilm. The ethanolic extract of *M. indica* L. leaves is known to contain alkaloids, saponins, tannins, phenolics, flavonoids, and steroids, which contribute to antibacterial and antibiofilm activity against *P. gingivalis* (Ningsih 2017). The mechanism of bacterial death by alkaloid compounds occurs due to its compound, which inhibits peptidoglycan from bacterial cells, thus the cell wall is not fully formed and leads to lysis (Sylvana et al. 2021). Saponin compound plays a role in inhibiting bacterial growth by damaging bacterial cell membranes and disrupting the balance of intra- and extra-cellular substances (Sebastian & Widyarman 2021).

Phenolic compounds have high antimicrobial power, therefore they can damage cell structure membranes, interfere with bacterial protein synthesis, and change bacterial DNA genes (Tirado *et al.* 2021). Tannin compound form complex bonds with proline proteins thus

cell walls are damaged. Flavonoid compounds exhibit antibacterial properties by interfering with the formation of cell walls, nucleic acids, and bacterial proteins (Sylvana et al. 2021). These compounds also exhibit antibiofilm properties by inhibiting the formation of quorum-sensing signals, thus communication between bacteria during biofilm formation is disrupted (Federika et al. 2020). The ability of steroid compounds to cause liposomes to leak on the phospholipid membrane can result in bacterial cell lysis (Hassan & Ullah 2019).

The most effective antibacterial effect on the growth of *P. gingivalis* was ethanol extract of *M. indica* L. leaves with a concentration of 100%, which produced the least total bacterial colonies of *P. gingivalis*, namely $(3.33 \pm 1.15) \times 10^6$ CFU/mL. This is in accordance with a study by Kurniasih on the effectiveness of the concentration of mango arumanis leaf extract on the growth of *S. mutans* with the disc diffusion method. Based on previous research, the ethanol extract of *M. indica* L. leaves with the highest concentration, which was 80% concentration, showed the largest zone of inhibition against *S. mutans* (Kurniasih 2016).

The incubation period used in antibiofilm assay in this study was 1 hour, 3 hours, and 24 hours. This incubation period was adjusted to the stage of biofilm formation. In the first few seconds to minutes, biofilm begins with the formation of a pellicle on the tooth surface. 2-4 hours later, the adhesion phase of the bacterial colony occurs. If after 24 hours, the bacteria on the tooth surface are still attached, the biofilm will enter the maturation phase (Bjarnsholt 2013).

This proves that the extract was most effective in inhibiting the formation of *P. gingivalis* biofilm in the adhesion phase. As the concentration of extract increased, it showed a lower OD value, which means an increase in inhibitory effect against *P. gingivalis* biofilm formation, hence this extract is dose-dependent. This study is in accordance with previous studies, which showed that ethanolic extract of *M. indica* L. leaves could reduce the attachment of mature biofilm of *S. aureus* (Manzur et al. 2020). This study demonstrates that ethanolic extract of *M. indica* L. leaves has the potential for antibacterial and antibiofilm activity against *P. gingivalis* in vitro.

5 CONCLUSION

The ethanol extract of *M. indica* L. leaves proved effective in inhibiting the growth and formation of *P. gingivalis* biofilms in vitro. Ethanol extract of *M. indica* L. leaves with a concentration of 100% was the most effective concentration as antibacterial and antibiofilm against *P. gingivalis*, especially during the incubation period of 3 hours.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

ACKNOWLEDGMENT

The authors thank the Faculty of Dentistry, Trisakti University, for their invaluable support in this study. The authors also would like to thank Mario Richi, S.Si from the Microbiology Center of Research and Education (MiCORE) laboratory for his laboratory assistance.

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by Sheila Soesanto FKG

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ARUMANIS MANGO LEAVES (*Mangifera indica* L.) EXTRACT EFFICACY on *Porphyromonas gingivalis* BIOFILM *in-vitro*

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ABSTRACT

Background(s):

[The prevalence of periodontitis in Indonesia was 74.1%. The etiology of periodontitis is pathogen bacteria within biofilm, like *Porphyromonas gingivalis*. Antibiotics such as amoxicillin may be prescribed in etiotropic phase of periodontitis treatment. However, amoxicillin may develop unwanted side effects as well as antibiotic resistances, hence the use of natural ingredients with antibacterial activity and minimal side effects are needed. Arumanis mango leaves (*Mangifera indica* L.) has the potential to be antibacterial and antibiofilm agents as they contain mangiferin, flavonoid, and tannin that might inhibit the growth of *P. gingivalis* and its biofilm formation.]

Objective(s):

[To determine antibacterial and antibiofilm effects of *Mangifera indica* L. leaves ethanol extract against *P. gingivalis*.]

Methods:

[An in-vitro laboratory experiment was performed with post test only control group design. The present study used dimethyl sulfoxide (DMSO) as negative control, amoxicillin as positive control, and 3,125%, 6,25%, 12,5%, 25%, 50%, 100% concentrations of *Mangifera indica* L. leaves ethanol extract. Plate count method was performed for antibacterial test and microtiter plate biofilm assay for antibiofilm test. One way ANOVA was used for the statistical analysis with $P< 0.05$ was considered as significant level.]

Result(s):

[The most effective antibacterial activity against *P. gingivalis* was 100% extract concentration compared to negative control ($p<0,05$). Moreover, the most effective concentration against *P. gingivalis* biofilm formation was 100% extract in 3 hours incubation period compared to negative control ($p<0,05$).]

Conclusion(s):

[*Mangifera indica* L. leaves ethanol extract inhibited *P. gingivalis* growth and its biofilm formation.]

Keywords:

[Antibacterial, antibiofilm, periodontitis, *Porphyromonas gingivalis*, *Mangifera indica* L.,]

BACKGROUND(s)

[According to the 2018 Basic Health Research (*Riset Kesehatan Dasar* / RISKESDAS), periodontitis is one of the most common periodontal diseases in Indonesia with prevalence of 74.1%.[1] Periodontitis is an inflammatory condition that occurs in periodontium, such as gingiva, cementum, periodontal ligament, and alveolar bone. It begins with poor oral hygiene which leads to accumulation of biofilm in gingiva and tooth surface, and as the biofilm grows thicker and more complex, the more severe the periodontitis. [3,4]

Porphyromonas gingivalis is the etiology of periodontitis. This opportunistic bacterium colonize in biofilm as the second colonizer whose main habitat is in the subgingival area. *P. gingivalis* virulence factors, such as lipopolysaccharides, outer membrane proteins, capsules, proteases, fimbriae, and enzymes, can trigger inflammatory response in tissues surrounding the teeth resulting in gingivitis. If the inflammation progresses to deeper tissues, the periodontal ligament and alveolar bone will be damaged and become periodontitis which ultimately leads to tooth loss.[2,5]

In treating periodontitis, administration of antibiotics (amoxicillin, tetracycline, clindamycin, and ciprofloxacin) is one of treatments in etiotropic phase to reduce the growth of pathogenic bacteria in oral cavity.[6] However, the use of antibiotics such as amoxicillin can have negative effects on the body, including hypersensitivity, vomiting, nausea, gastrointestinal disturbances, and opportunistic infections, while the use of tetracyclines can cause diarrhea, vomiting, dizziness, and discoloration of teeth.[7,8] Irrational use of antibiotics can trigger emergence of bacterial resistance, where mild infections tends to be difficult to be controlled by antibiotics.[9]

In addition, bacteria in biofilm also have greater resistance to antibiotics and some antibiotics unable to penetrate biofilm due to its matrix that prevents the diffusion of antibiotics, express multi-antibiotic efflux pumps, and reduce permeability of the bacteria. Thus, antibiotics are unable to penetrate the biofilm.[10] Therefore, other alternative materials, such as herbal products with minimal side effects in treating periodontal disease are indispensible.[9]

Mangifera indica L., also known as mango arumanis plant, is a plant that grows in tropical and subtropical countries, especially Asian region. Arumanis mango has the characteristics of, namely, sweet taste, fragrant, and appearance that is enough to attract the attention of the whole world, so it is known as the king of fruits.[11-13] This variety of mango plants is often cultivated due to its type which is the most demanded by people of Indonesia.[14] However, along with the increase in number of *M. indica* L. plants, there was an increase in amount of waste from leaves of this plant, even though the leaves of *M. indica* L. are known to have bioactive potential compounds as antibacterial and antibiofilm.[15,16]

Mangiferin is the main polyphenolic compound that is often found in all parts of *M. indica* L. plant, including fruit, bark, tree, and leaves.[17] This compound has broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, such as *Streptococcus mutans, Staphylococcus aureus*, and *Enterococcus faecalis*.[18,19] The leaves of *M. indica* L. arumanis variety were proven to have the highest percentage of mangiferin content and the most potent antibacterial power against *S. aureus* when compared to other varieties.[20,21] Other than mangiferin, the leaves of *M. indica* L. also contain flavonoid compounds, tannins, alkaloids, steroids, and saponins, which also contribute to antibacterial activity.[22]

To the knowledge of the authors, to this date, research on antibacterial and antibiofilm effects of ethanolic extract of *M. indica* L. leaves against *P. gingivalis* has yet to be performed. To cover this research gap, this study aimed to determine the effect of ethanolic extract of *M. indica* L. leaves on the growth and formation of *P. gingivalis* biofilms. Utilization of mango arumanis leaves can be a potential antibacterial and antibiofilm properties to treat periodontitis.]

METHODS

[This research is experimental laboratory in vitro with post-test only control group design. This research was performed at Microbiology Center of Research and Education (MiCORE) laboratory, Faculty of Dentistry, Trisakti University. This study used 10% Dimethyl Sulfoxide (DMSO) solution as negative control, amoxicillin as positive control, and ethanol extract of *M. indica* L. leaves with concentrations of 3.125%, 6.25%, 12.5%, 25 %, 50%, and 100%.]

Preparation of *M. indica* L. Leaf ethanol extract

[The sample used was ethanol extract of the leaves of mango arumanis (*Mangifera indica* L.) made by Indonesian Research Institute for Spices and Medicinal Plants (*Balai Penelitian Tanaman Rempah dan Obat* / BALITTRO). As much as 1.500 g of *M. indica* L. leaves were cleaned and dried at 40°C. Moreover, the leaves of *M. indica* L. were blended and the powder was mixed with 70% ethanol solvent in ratio of 1:5, and macerated for 2-3 hours. Next, the mixture was filtered to get maserate which was evaporated with rotary evaporator, thus the thick ethanol extract of *M. indica* L. leaves with concentration of 100% was obtained.

Moreover, several dilutions were made with 10% dimethyl sulfoxide (DMSO) to obtain concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125%.]

Preparation of positive control

[Positive control used amoxicillin 200 µg/mL solution, made by crushing 500 mg amoxicillin tablets into fine powder using mortar and pestle. Moreover, as much as 1.2 mg of amoxicillin powder was taken and 6 mL of sterile distilled water was added and mixed until homogeneous.]

Bacterial culture

[*P. gingivalis* ATCC 33277 bacteria were cultured on Tryptic Soy Broth (TSB) (Oxoid, Hampshire, UK) media which had been enriched with hemin (5 mg/L), vitamin K1 (10 mg/L), 0.5% yeast extract, and L-cystine (400 mg/L), then incubated under anaerobic conditions at 37°C. After 24 hours, the bacterial suspension was measured with microplate reader until absorbance was equivalent to 0.5 McFarland (1.5×10^8 CFU/mL) or $OD_{600} \pm 0.132$.]

Antibacterial Test with Plate Count Method

[Antibacterial testing was performed using microdilution method. A total of 100 µL suspension of *P. gingivalis* ATCC 33277 was distributed into 96-well-plate well using micropipette. A total of 100 µL of each test solution was added to the wells and incubated at 37°C under anaerobic conditions.

After incubation for 24 hours, the microdilution results from each treatment were taken and diluted 10,000 times. Moreover, 5 µL was taken to be placed on Brain Heart

Infusion Agar (BHI-A) media in petri dish. The growth of bacterial colonies was calculated after incubation for 24 hours at 37°C. The results of measurement of total bacterial colonies were obtained by the following formula:

$$\text{CFU / ml} = \frac{\textit{Bacterial colonies x dilution}}{\textit{volume pipetted (ml)}}]$$

Antibiofilm Test with Microtiter Plate Biofilm Assay

[A total of 200 µL suspension of *P. gingivalis* ATCC 33277 was inserted into 96-well-plate well with micropipette and incubated at 37°C under anaerobic conditions. After incubation for 48 hours, supernatant was removed from the wells leaving a layer of biofilm at the bottom and the wells were washed with phosphate-buffered saline (PBS).

Ethanol extract of *M. indica* L. leaves with different concentrations (3.125%, 6.25%, 12.5%, 25%, 50%, and 100%), TSB as negative control, and amoxicillin as positive control were added into the well as much as 200 µL using a micropipette. Moreover, the wells were incubated for 1 hour, 3 hours, and 24 hours at 37°C. The wells were washed again with PBS and fixed over the fire. To measure density of *P. gingivalis* biofilm, the wells were given 200 µL of crystal violet stain (0.05% w/v), then left for 15 minutes. Then, the wells were washed with PBS twice and 200 µL of 96% ethanol was added. Optical Density (OD) measurement of biofilm was performed with microplate reader (SAFAS MP96, SAFAS, Monaco) at the wavelength of 490 nm.]

Statistic analysis

[The Statistical Product and Service Solution (SPSS) program version 26 (IBM, Armonk, NY) was used to process data from this research. Normality test was performed using

Shapiro-wilk method. If the data was normally distributed ($p>0.05$), then proceed with one-way Analysis of Variance (ANOVA) test. The group with significant difference ($p<0.05$) will be continued with Post Hoc test using Tukey Honestly Significance Difference (HSD) method to see which treatment group was significantly different.]

RESULT(s)

[The results of antibacterial test using plate count method can be seen in Figure 1. In this study, ethanol extract of *M. indica* L. leaves with various concentrations was shown to inhibit the growth of *P. gingivalis* (Figure 2). Ethanol extract of *M. indica* L. leaves with concentration of 100% produced the best antibacterial activity against *P. gingivalis* with total colony of *P. gingivalis* $(3.33 \pm 1.15) \times 10^6$ CFU/mL (Table 1).

The results of antibiofilm test using microtiter plate biofilm assay showed that ethanol extract of *M. indica* L. leaves with different concentrations had antibiofilm effect on *P. gingivalis* at incubation periods of 1, 3 and 24 hours (Figures 3, 4, and 5). The extract with 100% concentration was the most effective in inhibiting *P. gingivalis* biofilm during incubation period of 3 hours due to the smallest OD value, namely OD 0.115 ± 0.015 (Table 2).]

Statistic analysis

[The results of normality test showed that all data on antibacterial test and antibiofilm test with incubation periods of 1, 3, and 24 hours were normally distributed ($p>0.05$). The results of one-way ANOVA test proved that there was significant difference ($p<0.05$) in all groups, while the results of Post Hoc Tukey HSD test showed that ethanol extract of *M. indica* L. leaves in all concentrations was significantly different ($p<0.05$) with negative control.]

DISCUSSION

[The ethanolic extract of *M. indica* L. leaves is known to contain alkaloids, saponins, tannins, phenolics, flavonoids, and steroids which contribute to antibacterial and antibiofilm activity against *P. gingivalis*.[23] The mechanism of bacterial death by alkaloid compounds occurs due to its compound which inhibit peptidoglycan from bacterial cells, thus the cell wall is not fully formed and leads to lysis.[24] Saponin compound plays a role in inhibiting bacterial growth by damaging bacterial cell membranes, and disrupting the balance of intra and extracellular substances.[25]

Phenolic compounds have high antimicrobial power due to its compounds can damage cell structure membranes, interfere with bacterial protein synthesis, and change bacterial DNA genes.[26] Tannin compound form complex bonds with proline proteins thus cell walls are damaged.[24] Flavonoid compound is antibacterial by interfering the formation of cell walls, nucleic acids, and bacterial proteins.[24] These compounds are also antibiofilms by inhibiting the formation of quorum sensing signals, thus communication between bacteria during biofilm formation is disrupted.[27] The ability of steroid compound to cause liposomes to leak on phospholipid membrane can result in bacterial cell lysis.[28]

All secondary metabolites contained in *M. indica* L. leaves were extracted using ethanol as solvent. Ethanol was chosen due to its lower toxicity than other solvents, and the polarity is almost close to polyphenol compound, where mangiferin is part of the most dominant polyphenol compound.[26,29] In this study, 10% DMSO was used as extract diluent due to its hydrophobic compounds, thus it is unable to dissolve completely in distilled water. 10% DMSO is still within the safe concentration limit, nontoxic to body, and will not interfere the results of study.[30,31]

In antibacterial test, all concentrations of ethanol extract of *M. indica* L. leaves had fewer colonies and significantly different ($p<0.05$) from negative control. This indicates effectiveness of extract in inhibiting the growth of *P. gingivalis* in vitro. The most effective antibacterial effect on the growth of *P. gingivalis* was ethanol extract of *M. indica* L. leaves with concentration of 100% which produced the least total bacterial colonies of *P. gingivalis*, namely $(3.33 \pm 1.15) \times 10^6$ CFU/mL. This is in accordance with study by Kurniasih on effectiveness of concentration of mango arumanis leaves extract on the growth of *S. mutans* with disc diffusion method. Based on previous research, ethanol extract of *M. indica* L. leaves with the highest concentration, which was 80% concentration, showed the largest zone of inhibition against *S. mutans*.[19]

The incubation period used in antibiofilm assay in this study was 1 hour, 3 hours, and 24 hours. This incubation period was adjusted to the stage of biofilm formation. In first few seconds to minutes, biofilm begins with formation of pellicle on tooth surface. At 2-4 hours later, adhesion phase of bacterial colony occurs. If after 24 hours the bacteria on tooth surface are still attached, biofilm will enter maturation phase.[32]

In antibiofilm assay, results of this study showed that all concentrations of ethanol extract of *M. indica* L. leaves during incubation period of 1, 3, and 24 hours had lower OD value and significantly different ($p<0.05$) against negative control, which was it means that there is inhibitory effect on formation of *P. gingivalis* biofilm in vitro. The extract with 100% concentration during incubation period of 3 hours had the smallest OD value, namely OD 0.115 ± 0.015. This proves that the extract was most effective in inhibiting formation of *P. gingivalis* biofilm in adhesion phase. As concentration of extract increased, it showed lower OD value, which means an increase in inhibitory effect against *P. gingivalis* biofilm formation, hence this extract is dose dependent. This study is in accordance with previous

studies, which showed that ethanolic extract of *M. indica* L. leaves could reduce attachment of mature biofilm of *S. aureus*.[33] In this study, ethanolic extract of *M. indica* L. leaves has potential as antibacterial and antibiofilm against *P. gingivalis* in vitro.]

CONCLUSION(s)

[The ethanol extract of *M. indica* L. leaves proved effective in inhibiting the growth and formation of *P. gingivalis* biofilms in vitro. Ethanol extract of *M. indica* L. leaves with concentration of 100% was the most effective concentration as antibacterial and antibiofilm against *P. gingivalis*, especially during incubation period of 3 hours.]

ACKNOWLEDGMENT

[The authors thank Faculty of Dentistry, Trisakti University, for invaluable support in this study. Authors also would like to thank Mario Richi, S.Si from the Microbiology Center of Research and Education (MiCORE) laboratory for his laboratory assistances.]

CONFLICT OF INTEREST

[Authors have no conflict of interest to declare]

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TABLES

Table 1. [The result of mean total colony of *P. gingivalis* by plate count method]

Treatment	Mean (CFU/mL)
K(-)	$(978{,}67 \pm 41{,}05) \times 10^6$
3,125%	$(55{,}33 \pm 26{,}1) \times 10^6$
6,25%	$(195{,}33 \pm 11{,}37) \times 10^6$

12,5%	(25,33 ± 11,37) x 10^6
25%	(8,67 ± 3,06) x 10^6
50%	(4 ± 0,00) x 10^6
100%	(3,33 ± 1,15) x 10^6
K(+)	(90 ± 24,98) x 10^6

Table 2. [Result mean OD ± SD biofilm P. gingivalis]

Treatment	OD 1 hr	OD 3 hr	OD 24 hr
K (-)	3,148 ± 0,089	3,172 ± 0,026	3,104 ± 0,044
3,125%	2,563 ± 0,065	2,575 ± 0,042	2,738 ± 0,051
6,25%	1,947 ± 0,064	1,798 ± 0,04	1,884 ± 0,029
12,5%	1,918 ± 0,238	0,735 ± 0,033	0,404 ± 0,016
25%	1,377 ± 0,034	0,376 ± 0,039	0,402 ± 0,086
50%	0,377 ± 0,112	0,321 ± 0,108	0,389 ± 0,098
100%	0,281 ± 0,063	0,115 ± 0,015	0,214 ± 0,054
K(+)	1,066 ± 0,173	1,365 ± 0,215	1,055 ± 0,090

FIGURES

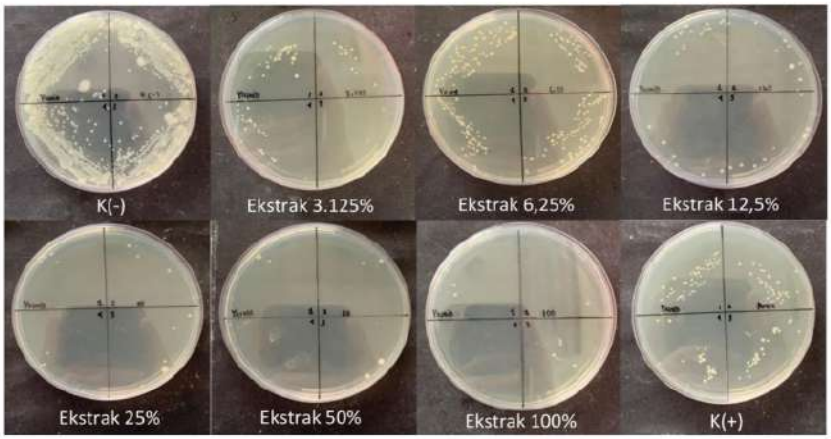
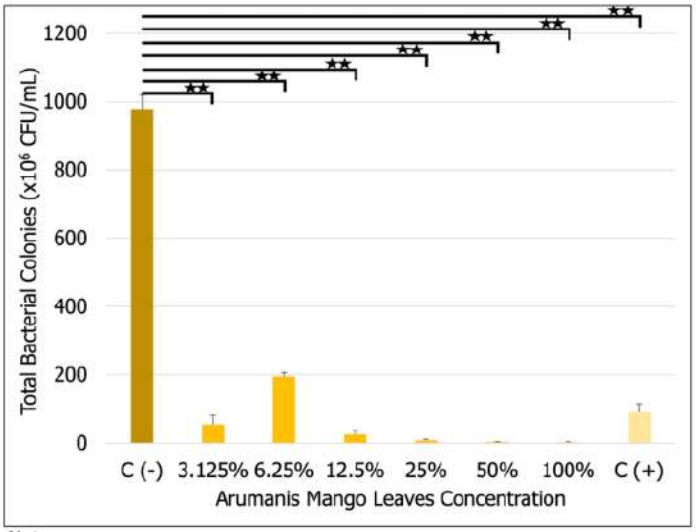
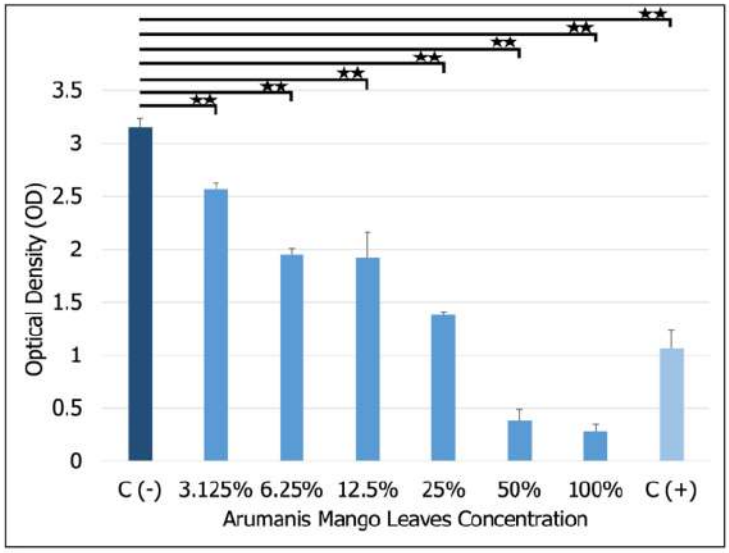


Figure 1. [The results of the growth inhibition test of *P. gingivalis* with plate count method.]



Notes :
★★ : Significant difference ($p < 0,01$)
C (-) : DMSO 10%
C (+) : amoxicillin

Figure 2. [Graphic of total bacterial colonies of *P. gingivalis* by plate count method.]



Notes :
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C (-) : BHI-B
C (+) : amoxicillin

Figure 3. [Graphic of mean OD of *P. gingivalis* biofilm with 1 hour incubation period]

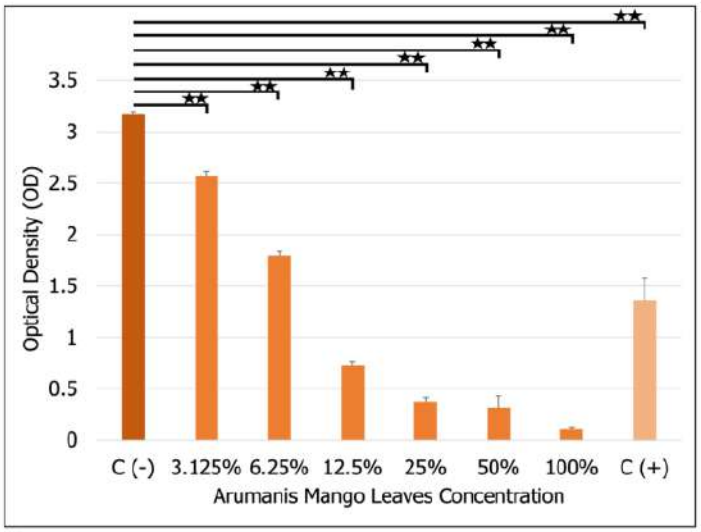

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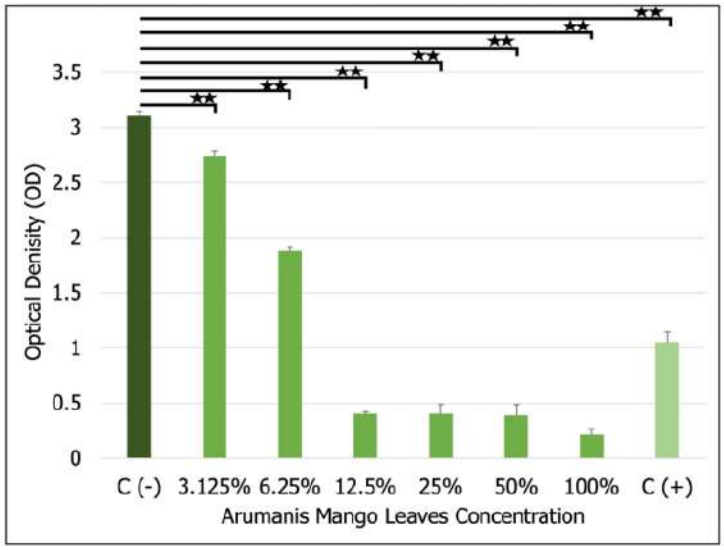
Figure 4. [Graphic of mean OD of *P. gingivalis* biofilm with 3 hours incubation period]



Notes :
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C (-) : BHI-B
C (+) : amoxicillin

Figure 5. [Graphic of mean OD of *P. gingivalis* biofilm with 24 hours incubation period]

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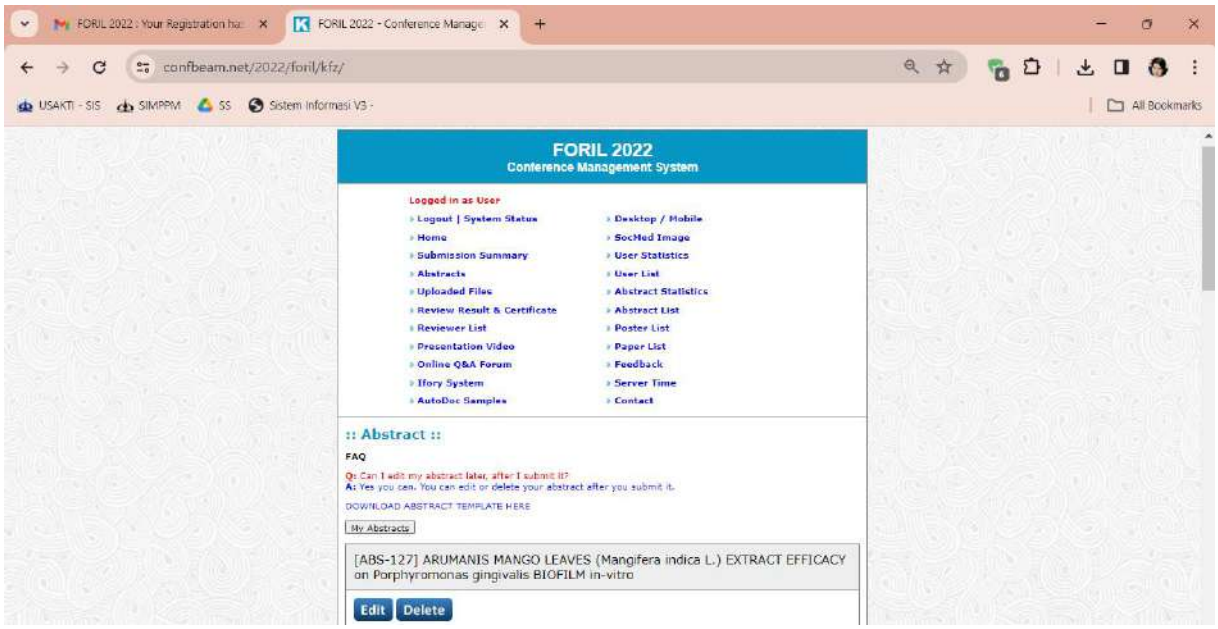
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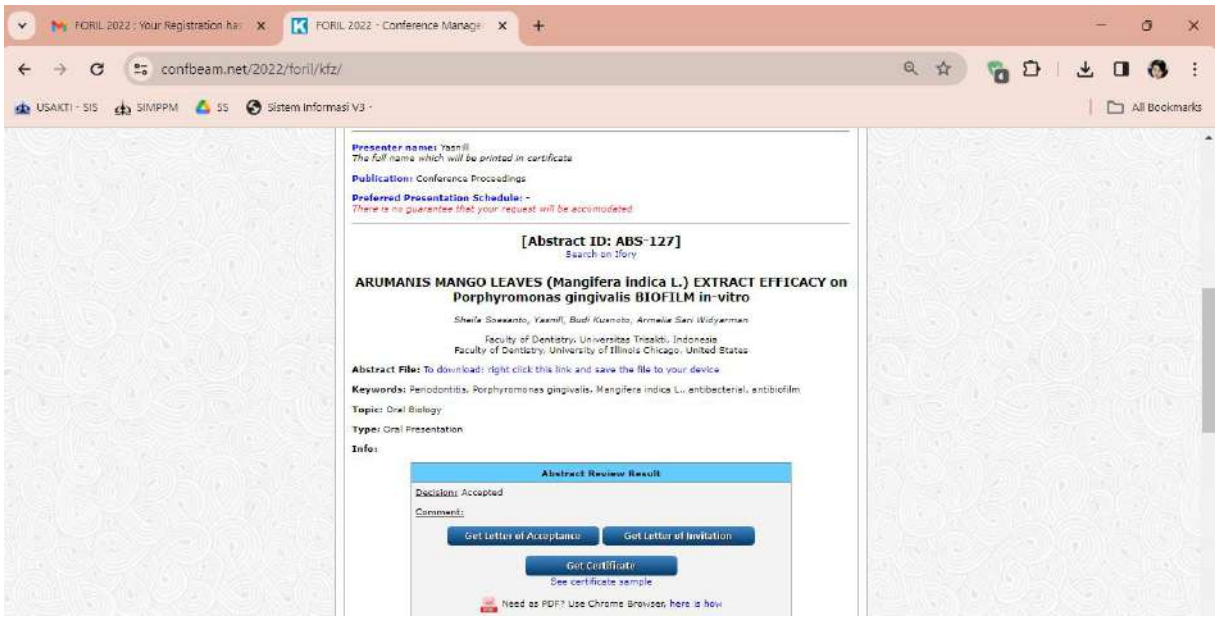
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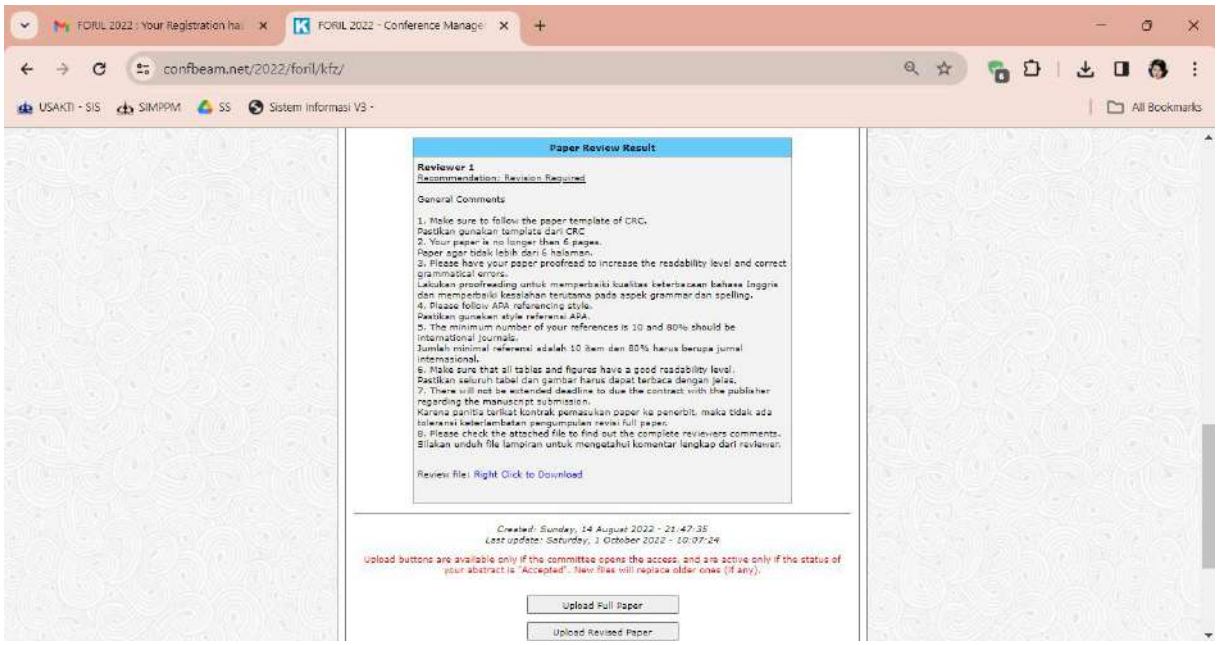
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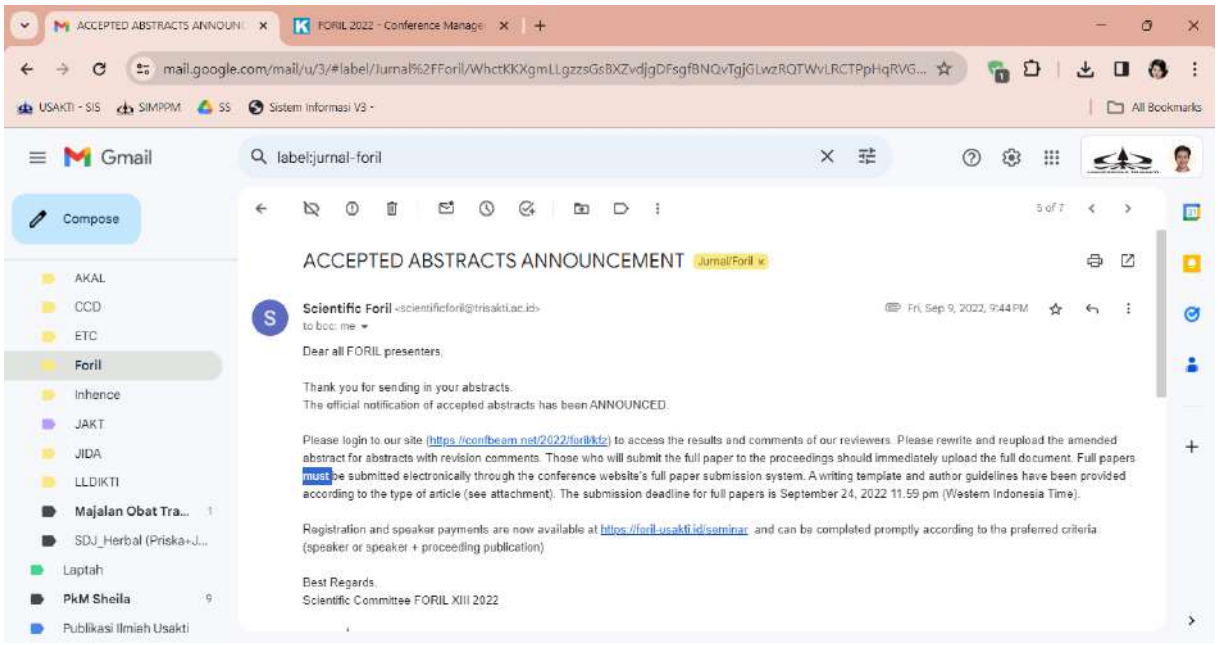
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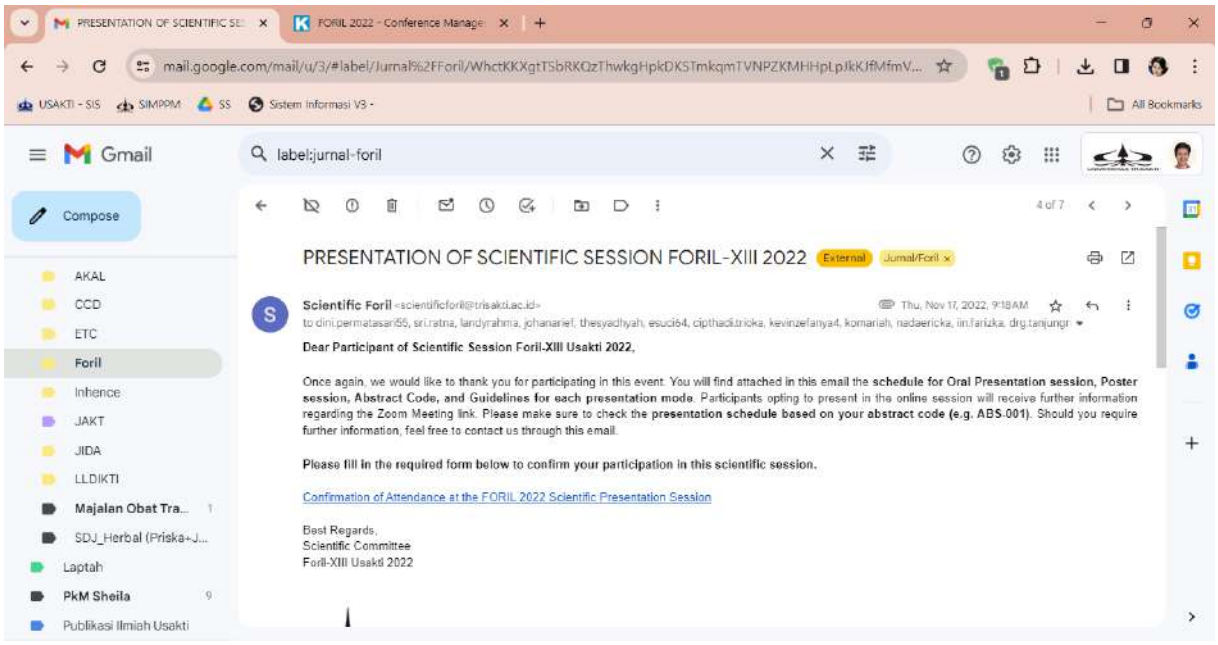
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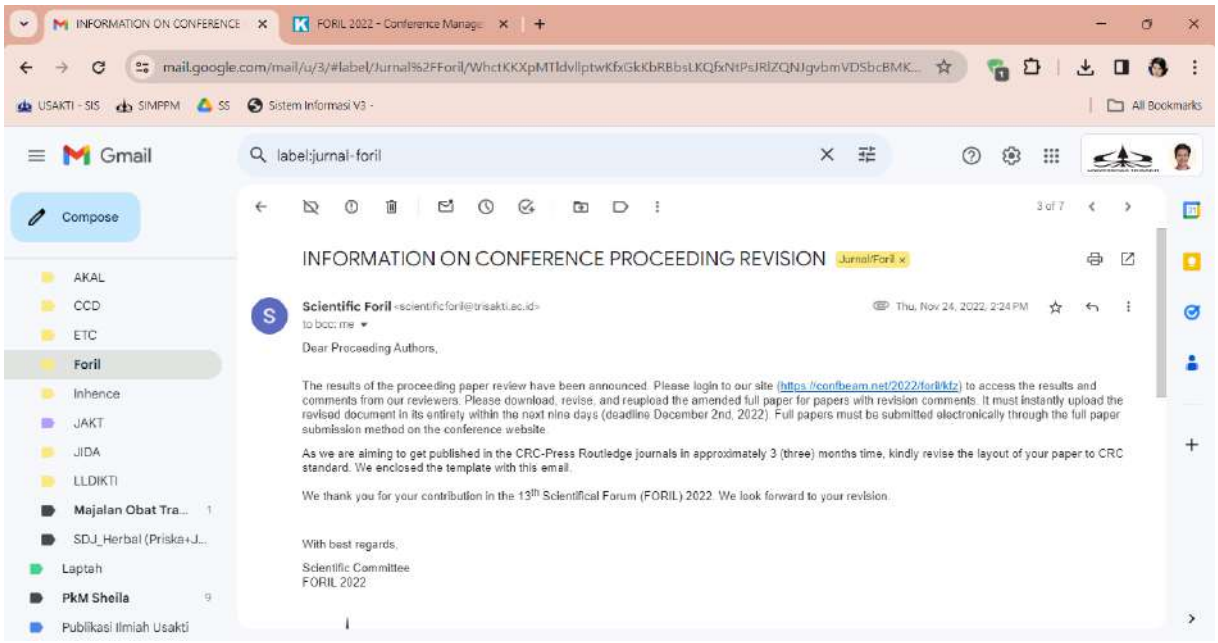
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ARUMANIS MANGO LEAVES (*Mangifera indica* L.) EXTRACT EFFICACY on *Porphyromonas gingivalis* BIOFILM *in-vitro*

Commented [DZ1]: Pastikan aturan penulisan judul penggunaan huruf besar dan kecilnya.

Sheila Soesanto[1], Yasnill[2], Budi Kusnoto[3], Armelia Sari Widyarman[4§]

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ABSTRACT

Background(s):

[The prevalence of periodontitis in Indonesia was 74.1%. The etiology of periodontitis is pathogen bacteria within biofilm, like *Porphyromonas gingivalis*. Antibiotics such as amoxicillin may be prescribed in etiotropic phase of periodontitis treatment. However, amoxicillin may develop unwanted side effects as well as antibiotic resistances, hence the use of natural ingredients with antibacterial activity and minimal side effects are needed. Arumanis mango leaves (*Mangifera indica* L.) has the potential to be antibacterial and antibiofilm agents as they contain mangiferin, flavonoid, and tannin that might inhibit the growth of *P. gingivalis* and its biofilm formation.]

Objective(s):

[To determine antibacterial and antibiofilm effects of *Mangifera indica* L. leaves ethanol extract against *P. gingivalis*.]

Methods:

[An in-vitro laboratory experiment was performed with post test only control group design. The present study used dimethyl sulfoxide (DMSO) as negative control, amoxicillin as positive control, and 3,125%, 6,25%, 12,5%, 25%, 50%, 100% concentrations of *Mangifera indica* L. leaves ethanol extract. Plate count method was performed for antibacterial test and microtiter plate biofilm assay for antibiofilm test. One way ANOVA was used for the statistical analysis with P< 0.05 was considered as significant level.]

Result(s):

Commented [DZ2]: Pointer backgrounds, objective, method dll, mohon dihapus saja.

Commented [DZ3]: Background cukup dalam 2 kalimat saja.

Commented [DZ4]: Abstrak dibuat secara berkesinambungan kalimatnya.

[The most effective antibacterial activity against *P. gingivalis* was 100% extract concentration compared to negative control ($p<0,05$). Moreover, the most effective concentration against *P. gingivalis* biofilm formation was 100% extract in 3 hours incubation period compared to negative control ($p<0,05$).]

Conclusion(s):

[*Mangifera indica* L. leaves ethanol extract inhibited *P. gingivalis* growth and its biofilm formation.]

Keywords:

[Antibacterial, antibiofilm, periodontitis, *Porphyromonas gingivalis*, *Mangifera indica* L.,]

BACKGROUND(s)

[According to the 2018 Basic Health Research (*Riset Kesehatan Dasar* / RISKESDAS), periodontitis is one of the most common periodontal diseases in Indonesia with prevalence of 74.1%.[1] Periodontitis is an inflammatory condition that occurs in periodontium, such as gingiva, cementum, periodontal ligament, and alveolar bone. It begins with poor oral hygiene which leads to accumulation of biofilm in gingiva and tooth surface, and as the biofilm grows thicker and more complex, the more severe the periodontitis. [3,4]

Porphyromonas gingivalis is the etiology of periodontitis. This opportunistic bacterium colonize in biofilm as the second colonizer whose main habitat is in the subgingival area. *P. gingivalis* virulence factors, such as lipopolysaccharides, outer membrane proteins, capsules, proteases, fimbriae, and enzymes, can trigger inflammatory response in tissues surrounding the teeth resulting in gingivitis. If the inflammation progresses to deeper tissues, the periodontal ligament and alveolar bone will be damaged and become periodontitis which ultimately leads to tooth loss.[2,5]

In treating periodontitis, administration of antibiotics (amoxicillin, tetracycline, clindamycin, and ciprofloxacin) is one of treatments in etiotropic phase to reduce the growth of pathogenic bacteria in oral cavity.[6] However, the use of antibiotics such as amoxicillin can have negative effects on the body, including hypersensitivity, vomiting, nausea, gastrointestinal disturbances, and opportunistic infections, while the use of tetracyclines can cause diarrhea, vomiting, dizziness, and discoloration of teeth.[7,8] Irrational use of antibiotics can trigger emergence of bacterial resistance, where mild infections tends to be difficult to be controlled by antibiotics.[9]

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Contoh: pada teks ditulis (Thiruvoth, 2015)
Pada references ditulis:
Thiruvoth, F. M., Mohapatra, D. P., Kumar, D., Chittoria, S. R. K., & Nandhagopal, V. (2015). Current concepts in the physiology of adult wound healing. *Plastic and Aesthetic Research, 2, 250-256.*

In addition, bacteria in biofilm also have greater resistance to antibiotics and some antibiotics unable to penetrate biofilm due to its matrix that prevents the diffusion of antibiotics, express multi-antibiotic efflux pumps, and reduce permeability of the bacteria. Thus, antibiotics are unable to penetrate the biofilm.[10] Therefore, other alternative materials, such as herbal products with minimal side effects in treating periodontal disease are indispensible.[9]

Mangifera indica L., also known as mango arumanis plant, is a plant that grows in tropical and subtropical countries, especially Asian region. Arumanis mango has the characteristics of, namely, sweet taste, fragrant, and appearance that is enough to attract the attention of the whole world, so it is known as the king of fruits.[11-13] This variety of mango plants is often cultivated due to its type which is the most demanded by people of Indonesia.[14] However, along with the increase in number of *M. indica* L. plants, there was an increase in amount of waste from leaves of this plant, even though the leaves of *M. indica* L. are known to have bioactive potential compounds as antibacterial and antibiofilm.[15,16]

Mangiferin is the main polyphenolic compound that is often found in all parts of *M. indica* L. plant, including fruit, bark, tree, and leaves.[17] This compound has broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, such as *Streptococcus mutans*, *Staphylococcus aureus*, and *Enterococcus faecalis*.[18,19] The leaves of *M. indica* L. arumanis variety were proven to have the highest percentage of mangiferin content and the most potent antibacterial power against *S. aureus* when compared to other varieties.[20,21] Other than mangiferin, the leaves of *M. indica* L. also contain flavonoid compounds, tannins, alkaloids, steroids, and saponins, which also contribute to antibacterial activity.[22]

To the knowledge of the authors, to this date, research on antibacterial and antibiofilm effects of ethanolic extract of *M. indica* L. leaves against *P. gingivalis* has yet to be performed. To cover this research gap, this study aimed to determine the effect of ethanolic extract of *M. indica* L. leaves on the growth and formation of *P. gingivalis* biofilms. Utilization of mango arumanis leaves can be a potential antibacterial and antibiofilm properties to treat periodontitis.]

METHODS

[This research is experimental laboratory in vitro with post-test only control group design. This research was performed at Microbiology Center of Research and Education (MiCORE) laboratory, Faculty of Dentistry, Trisakti University. This study used 10% Dimethyl Sulfoxide (DMSO) solution as negative control, amoxicillin as positive control, and ethanol extract of *M. indica* L. leaves with concentrations of 3.125%, 6.25%, 12.5%, 25 %, 50%, and 100%.]

Preparation of *M. indica* L. Leaf ethanol extract

[The sample used was ethanol extract of the leaves of mango arumanis (*Mangifera indica* L.) made by Indonesian Research Institute for Spices and Medicinal Plants (*Balai Penelitian Tanaman Rempah dan Obat* / BALITTRO). As much as 1.500 g of *M. indica* L. leaves were cleaned and dried at 40^{o}C. Moreover, the leaves of *M. indica* L. were blended and the powder was mixed with 70% ethanol solvent in ratio of 1:5, and macerated for 2-3 hours. Next, the mixture was filtered to get maserate which was evaporated with rotary evaporator, thus the thick ethanol extract of *M. indica* L. leaves with concentration of 100% was obtained.

Moreover, several dilutions were made with 10% dimethyl sulfoxide (DMSO) to obtain concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125%.]

Preparation of positive control

[Positive control used amoxicillin 200 μg/mL solution, made by crushing 500 mg amoxicillin tablets into fine powder using mortar and pestle. Moreover, as much as 1.2 mg of amoxicillin powder was taken and 6 mL of sterile distilled water was added and mixed until homogeneous.]

Bacterial culture

[*P. gingivalis* ATCC 33277 bacteria were cultured on Tryptic Soy Broth (TSB) (Oxoid, Hampshire, UK) media which had been enriched with hemin (5 mg/L), vitamin K1 (10 mg/L), 0.5% yeast extract, and L-cystine (400 mg/L), then incubated under anaerobic conditions at 37ºC. After 24 hours, the bacterial suspension was measured with microplate reader until absorbance was equivalent to 0.5 McFarland (1.5 x 10^8 CFU/mL) or OD_{600} ± 0.132.]

Antibacterial Test with Plate Count Method

[Antibacterial testing was performed using microdilution method. A total of 100 μL suspension of *P. gingivalis* ATCC 33277 was distributed into 96-well-plate well using micropipette. A total of 100 μL of each test solution was added to the wells and incubated at 37ºC under anaerobic conditions.

After incubation for 24 hours, the microdilution results from each treatment were taken and diluted 10,000 times. Moreover, 5 μL was taken to be placed on Brain Heart

Infusion Agar (BHI-A) media in petri dish. The growth of bacterial colonies was calculated after incubation for 24 hours at 37°C. The results of measurement of total bacterial colonies were obtained by the following formula:

$$\text{CFU / ml} = \frac{Bacterial\ colonies\ x\ dilution}{volume\ pipetted\ (ml)}$$

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Antibiofilm Test with Microtiter Plate Biofilm Assay

[A total of 200 µL suspension of *P. gingivalis* ATCC 33277 was inserted into 96-well-plate well with micropipette and incubated at 37°C under anaerobic conditions. After incubation for 48 hours, supernatant was removed from the wells leaving a layer of biofilm at the bottom and the wells were washed with phosphate-buffered saline (PBS).

Ethanol extract of *M. indica* L. leaves with different concentrations (3.125%, 6.25%, 12.5%, 25%, 50%, and 100%), TSB as negative control, and amoxicillin as positive control were added into the well as much as 200 µL using a micropipette. Moreover, the wells were incubated for 1 hour, 3 hours, and 24 hours at 37°C. The wells were washed again with PBS and fixed over the fire. To measure density of *P. gingivalis* biofilm, the wells were given 200 µL of crystal violet stain (0.05% w/v), then left for 15 minutes. Then, the wells were washed with PBS twice and 200 µL of 96% ethanol was added. Optical Density (OD) measurement of biofilm was performed with microplate reader (SAFAS MP96, SAFAS, Monaco) at the wavelength of 490 nm.]

Statistic analysis

[The Statistical Product and Service Solution (SPSS) program version 26 (IBM, Armonk, NY) was used to process data from this research. Normality test was performed using

Shapiro-wilk method. If the data was normally distributed ($p>0.05$), then proceed with one-way Analysis of Variance (ANOVA) test. The group with significant difference ($p<0.05$) will be continued with Post Hoc test using Tukey Honestly Significance Difference (HSD) method to see which treatment group was significantly different.]

RESULT(s)

[The results of antibacterial test using plate count method can be seen in Figure 1. In this study, ethanol extract of *M. indica* L. leaves with various concentrations was shown to inhibit the growth of *P. gingivalis* (Figure 2). Ethanol extract of *M. indica* L. leaves with concentration of 100% produced the best antibacterial activity against *P. gingivalis* with total colony of *P. gingivalis* $(3.33 \pm 1.15) \times 10^6$ CFU/mL (Table 1).

The results of antibiofilm test using microtiter plate biofilm assay showed that ethanol extract of *M. indica* L. leaves with different concentrations had antibiofilm effect on *P. gingivalis* at incubation periods of 1, 3 and 24 hours (Figures 3, 4, and 5). The extract with 100% concentration was the most effective in inhibiting *P. gingivalis* biofilm during incubation period of 3 hours due to the smallest OD value, namely OD 0.115 ± 0.015 (Table 2).]

Statistic analysis

[The results of normality test showed that all data on antibacterial test and antibiofilm test with incubation periods of 1, 3, and 24 hours were normally distributed ($p>0.05$). The results of one-way ANOVA test proved that there was significant difference ($p<0.05$) in all groups, while the results of Post Hoc Tukey HSD test showed that ethanol extract of *M. indica* L. leaves in all concentrations was significantly different ($p<0.05$) with negative control.]

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DISCUSSION

[The ethanolic extract of *M. indica* L. leaves is known to contain alkaloids, saponins, tannins, phenolics, flavonoids, and steroids which contribute to antibacterial and antibiofilm activity against *P. gingivalis.*[23] The mechanism of bacterial death by alkaloid compounds occurs due to its compound which inhibit peptidoglycan from bacterial cells, thus the cell wall is not fully formed and leads to lysis.[24] Saponin compound plays a role in inhibiting bacterial growth by damaging bacterial cell membranes, and disrupting the balance of intra and extracellular substances.[25]

Phenolic compounds have high antimicrobial power due to its compounds can damage cell structure membranes, interfere with bacterial protein synthesis, and change bacterial DNA genes.[26] Tannin compound form complex bonds with proline proteins thus cell walls are damaged.[24] Flavonoid compound is antibacterial by interfering the formation of cell walls, nucleic acids, and bacterial proteins.[24] These compounds are also antibiofilms by inhibiting the formation of quorum sensing signals, thus communication between bacteria during biofilm formation is disrupted.[27] The ability of steroid compound to cause liposomes to leak on phospholipid membrane can result in bacterial cell lysis.[28]

All secondary metabolites contained in *M. indica* L. leaves were extracted using ethanol as solvent. Ethanol was chosen due to its lower toxicity than other solvents, and the polarity is almost close to polyphenol compound, where mangiferin is part of the most dominant polyphenol compound.[26,29] In this study, 10% DMSO was used as extract diluent due to its hydrophobic compounds, thus it is unable to dissolve completely in distilled water. 10% DMSO is still within the safe concentration limit, nontoxic to body, and will not interfere the results of study.[30,31]

In antibacterial test, all concentrations of ethanol extract of *M. indica* L. leaves had fewer colonies and significantly different ($p<0.05$) from negative control. This indicates effectiveness of extract in inhibiting the growth of *P. gingivalis* in vitro. The most effective antibacterial effect on the growth of *P. gingivalis* was ethanol extract of *M. indica* L. leaves with concentration of 100% which produced the least total bacterial colonies of *P. gingivalis*, namely (3.33 ± 1.15) x 10^6 CFU/mL. This is in accordance with study by Kurniasih on effectiveness of concentration of mango arumanis leaves extract on the growth of *S. mutans* with disc diffusion method. Based on previous research, ethanol extract of *M. indica* L. leaves with the highest concentration, which was 80% concentration, showed the largest zone of inhibition against *S. mutans*.[19]

The incubation period used in antibiofilm assay in this study was 1 hour, 3 hours, and 24 hours. This incubation period was adjusted to the stage of biofilm formation. In first few seconds to minutes, biofilm begins with formation of pellicle on tooth surface. At 2-4 hours later, adhesion phase of bacterial colony occurs. If after 24 hours the bacteria on tooth surface are still attached, biofilm will enter maturation phase.[32]

In antibiofilm assay, results of this study showed that all concentrations of ethanol extract of *M. indica* L. leaves during incubation period of 1, 3, and 24 hours had lower OD value and significantly different ($p<0.05$) against negative control, which was it means that there is inhibitory effect on formation of *P. gingivalis* biofilm in vitro. The extract with 100% concentration during incubation period of 3 hours had the smallest OD value, namely OD 0.115 ± 0.015. This proves that the extract was most effective in inhibiting formation of *P. gingivalis* biofilm in adhesion phase. As concentration of extract increased, it showed lower OD value, which means an increase in inhibitory effect against *P. gingivalis* biofilm formation, hence this extract is dose dependent. This study is in accordance with previous

studies, which showed that ethanolic extract of *M. indica* L. leaves could reduce attachment of mature biofilm of *S. aureus*.[33] In this study, ethanolic extract of *M. indica* L. leaves has potential as antibacterial and antibiofilm against *P. gingivalis* in vitro.]

CONCLUSION(s)

[The ethanol extract of *M. indica* L. leaves proved effective in inhibiting the growth and formation of *P. gingivalis* biofilms in vitro. Ethanol extract of *M. indica* L. leaves with concentration of 100% was the most effective concentration as antibacterial and antibiofilm against *P. gingivalis*, especially during incubation period of 3 hours.]

ACKNOWLEDGMENT

[The authors thank Faculty of Dentistry, Trisakti University, for invaluable support in this study. Authors also would like to thank Mario Richi, S.Si from the Microbiology Center of Research and Education (MiCORE) laboratory for his laboratory assistances.]

CONFLICT OF INTEREST

[Authors have no conflict of interest to declare]

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TABLES

Table 1. [The result of mean total colony of *P. gingivalis* by plate count method]

Treatment	Mean (CFU/mL)
K(-)	(978,67 $\pm$ 41,05) x 10^6
3,125%	(55,33 $\pm$ 26,1) x 10^6
6,25%	(195,33 $\pm$ 11,37) x 10^6

12,5%	(25,33 ± 11,37) x 10^6
25%	(8,67 ± 3,06) x 10^6
50%	(4 ± 0,00) x 10^6
100%	(3,33 ± 1,15) x 10^6
K(+)	(90 ± 24,98) x 10^6

Table 2. [Result mean OD ± SD biofilm P. gingivalis]

Treatment	OD 1 hr	OD 3 hr	OD 24 hr
K (-)	3,148 ± 0,089	3,172 ± 0,026	3,104 ± 0,044
3,125%	2,563 ± 0,065	2,575 ± 0,042	2,738 ± 0,051
6,25%	1,947 ± 0,064	1,798 ± 0,04	1,884 ± 0,029
12,5%	1,918 ± 0,238	0,735 ± 0,033	0,404 ± 0,016
25%	1,377 ± 0,034	0,376 ± 0,039	0,402 ± 0,086
50%	0,377 ± 0,112	0,321 ± 0,108	0,389 ± 0,098
100%	0,281 ± 0,063	0,115 ± 0,015	0,214 ± 0,054
K(+)	1,066 ± 0,173	1,365 ± 0,215	1,055 ± 0,090

FIGURES

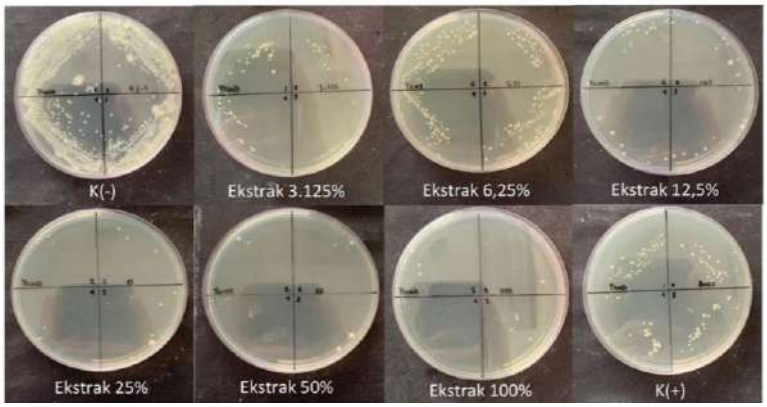

Figure 1. [The results of the growth inhibition test of *P. gingivalis* with plate count method.]

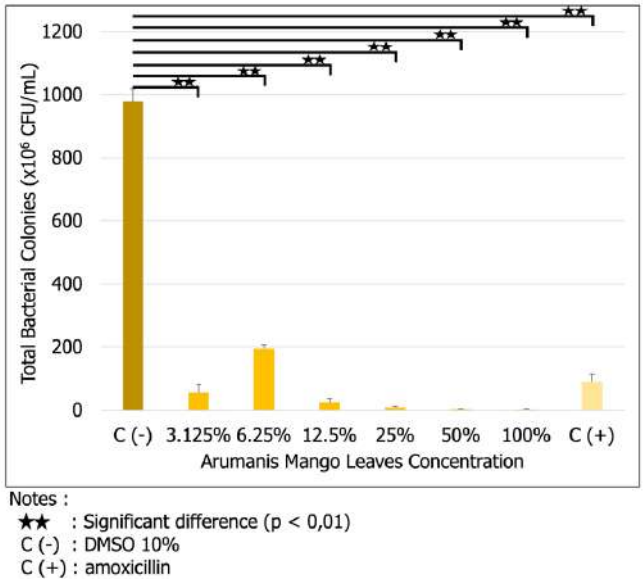

Figure 2. [Graphic of total bacterial colonies of *P. gingivalis* by plate count method**.**]

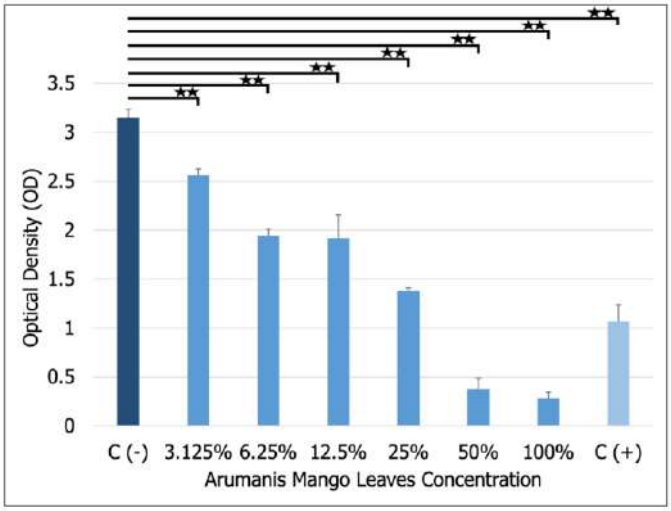

Figure 3. [Graphic of mean OD of *P. gingivalis* biofilm with 1 hour incubation period]

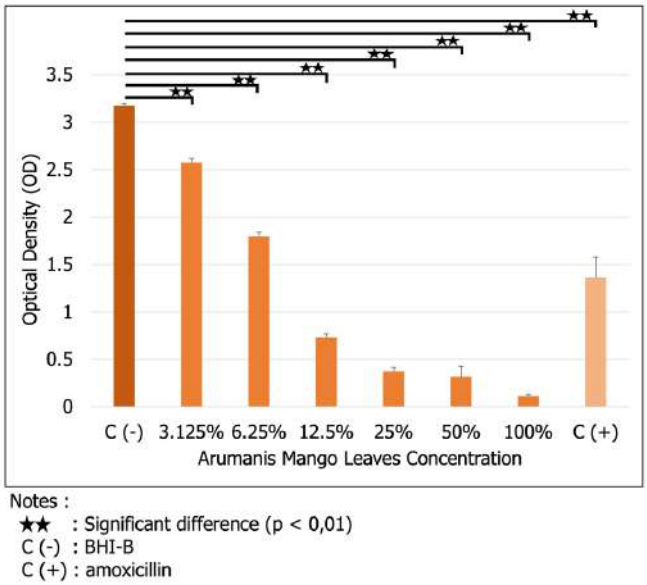

Figure 4. [Graphic of mean OD of *P. gingivalis* biofilm with 3 hours incubation period]

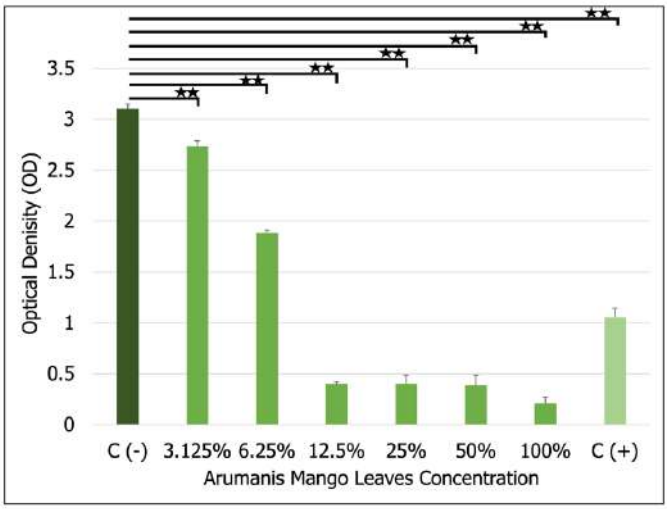

Figure 5. [Graphic of mean OD of *P. gingivalis* biofilm with 24 hours incubation period]

Arumanis mango leaves (*Mangifera indica* L.) extract efficacy on *Porphyromonas gingivalis* biofilm *in-vitro*

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ABSTRACT :Arumanis mango leaves (*Mangifera indica* L.) has the potential to be antibacterial and antibiofilm agents as they contain mangiferin, flavonoid, and tannin that might inhibit the growth of *P. gingivalis* and its biofilm formation. The objective of this study is to determine antibacterial and antibiofilm effects of *Mangifera indica* L. leaves ethanol extract against *P. gingivalis*. An in-vitro laboratory experiment was performed with post test only control group design. The present study used 3.125%, 6.25%, 12.5%, 25%, 50%, 100% concentrations of *Mangifera indica* L ethanol extract, dimethyl sulfoxide (DMSO) was used as negative control, and amoxicillin as positive control. Plate count method was performed for antibacterial test and microtiter plate biofilm assay for antibiofilm test. One way ANOVA was used for the statistical analysis with $p < 0.05$ was considered as significant level. Result showed the most effective antibacterial activity against *P. gingivalis* was 100% extract concentration compared to negative control ($p<0.05$). Moreover, the most effective concentration against *P. gingivalis* biofilm formation was 100% extract in 3 hours incubation period compared to negative control ($p<0.05$). It can be concluded that *Mangifera indica* L. leaves ethanol extract inhibited *P. gingivalis* growth and its biofilm formation. Keywords: antibacterial, antibiofilm, periodontitis, *Porphyromonas gingivalis*, *Mangifera indica* L.

1 INTRODUCTION

According to the 2018 Basic Health Research (*Riset Kesehatan Dasar*/RISKESDAS), periodontitis is one of the most common periodontal diseases in Indonesia with prevalence of 74.1% (Kemenkes, 2018). Periodontitis is an inflammatory condition that occurs in periodontium, such as gingiva, cementum, periodontal ligament, and alveolar bone. It begins with poor oral hygiene which leads to accumulation of biofilm in gingiva and tooth surface, and as the biofilm grows thicker and more complex, the more severe the periodontitis (Mehrotra & Singh, 2020).

Porphyromonas gingivalis is the etiology of periodontitis. This opportunistic bacterium colonize in biofilm as the second colonizer whose main habitat is in the subgingival area

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Pada references ditulis:
Thiruvoth, F. M., Mohapatra, D. P., Kumar, D., Chittoria, S. R. K., & Nandhagopal, V. (2015). Current concepts in the physiology of adult wound healing. *Plastic and Aesthetic Research, 2, 250-256.*

(Kinane et al., 2017). In treating periodontitis, administration of antibiotics (amoxicillin, tetracycline, clindamycin, and ciprofloxacin) is one of treatments in etiotropic phase to reduce the growth of pathogenic bacteria in oral cavity (Ciancio & Mariotti, 2019). However, the use of antibiotics such as amoxicillin can have negative effects on the body, including hypersensitivity, vomiting, nausea, gastrointestinal disturbances, and opportunistic infections, while the use of tetracyclines can cause diarrhea, vomiting, dizziness, and discoloration of teeth (Akhavan et al., 2020).

In addition, bacteria in biofilm also have greater resistance to antibiotics and some antibiotics unable to penetrate biofilm due to its matrix that prevents the diffusion of antibiotics, express multi-antibiotic efflux pumps, and reduce permeability of the bacteria. Thus, antibiotics are unable to penetrate the biofilm (Bat et al., 2021). Therefore, other alternative materials, such as herbal products with minimal side effects in treating periodontal disease are indispensible (Joshua & Takudzwa, 2013).

Mangifera indica L., also known as mango arumanis plant, is a plant that grows in tropical and subtropical countries, especially Asian region. Mangiferin is the main polyphenolic compound that is often found in all parts of *M. indica* L. plant, including fruit, bark, tree, and leaves (Kulkarni & Rathod, 2014). This compound has broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, such as *Streptococcus mutans, Staphylococcus aureus*, and *Enterococcus faecalis* (Kurniasih, 2016). The leaves of *M. indica* L. arumanis variety were proven to have the highest percentage of mangiferin content and the most potent antibacterial power against *S. aureus* when compared to other varieties (Utami et al., 2020). Other than mangiferin, the leaves of *M. indica* L. also contain flavonoid compounds, tannins, alkaloids, steroids, and saponins, which also contribute to antibacterial activity (Jhaumeer et al., 2018).

To the knowledge of the authors, to this date, research on antibacterial and antibiofilm effects of ethanolic extract of *M. indica* L. leaves against *P. gingivalis* has yet to be performed. To cover this research gap, this study aimed to determine the effect of ethanolic extract of *M. indica* L. leaves on the growth and formation of *P. gingivalis* biofilms. Utilization of mango arumanis leaves can be a potential antibacterial and antibiofilm properties to treat periodontitis.

2 METHODS

2.1 *Preparation of M. indica* L. *leaf ethanol extract*

The sample used was ethanol extract of the leaves of mango arumanis (*Mangifera indica* L.) made by Indonesian Research Institute for Spices and Medicinal Plants (*Balai Penelitian Tanaman Rempah dan Obat*/BALITTRO). As much as 1.500 g of *M. indica* L. leaves were cleaned and dried at 40^{o}C. Moreover, the leaves of *M. indica* L. were blended and the powder was mixed with 70% ethanol solvent in ratio of 1:5, and macerated for 2-3 hours. Next, the mixture was filtered to get maserate which was evaporated with rotary evaporator, thus the thick ethanol extract of *M. indica* L. leaves with concentration of 100% was obtained. Moreover, several dilutions were made with 10% dimethyl sulfoxide (DMSO) to obtain concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125%.

2.2 *Bacterial culture*

Porphyromonas gingivalis ATCC 33277 bacteria were cultured on Tryptic Soy Broth (TSB) (Oxoid, Hampshire, UK) media which had been enriched with hemin (5 mg/L), vitamin K1

(10 mg/L), 0.5% yeast extract, and L-cystine (400 mg/L), then incubated under anaerobic conditions at 37°C. After 24 hours, the bacterial suspension was measured with microplate reader until absorbance was equivalent to 0.5 McFarland (1.5 x 10^8 CFU/mL) or OD_{600} ± 0.132.

2.3 *Antibacterial test with plate count method*

Antibacterial testing was performed using microdilution method. A total of 100 µL suspension of *P. gingivalis* ATCC 33277 was distributed into 96-well-plate well using micropipette. A total of 100 µL of each test solution was added to the wells and incubated at 37°C under anaerobic conditions.

After incubation for 24 hours, the microdilution results from each treatment were taken and diluted 10,000 times. Moreover, 5 µL was taken to be placed on Brain Heart Infusion Agar (BHI-A) media in petri dish. The growth of bacterial colonies was calculated after incubation for 24 hours at 37°C.

2.4 *Antibiofilm test with microtitter plate biofilm assay*

A total of 200 µL suspension of *P. gingivalis* ATCC 33277 was inserted into 96-well-plate well with micropipette and incubated at 37°C under anaerobic conditions. After incubation for 48 hours, supernatant was removed from the wells leaving a layer of biofilm at the bottom and the wells were washed with phosphate-buffered saline (PBS).

Ethanol extract of *M. indica* L. leaves with different concentrations (3.125%, 6.25%, 12.5%, 25%, 50%, and 100%), were added into the well as much as 200 µL using a micropipette. Biofilm without treatment was used as negative control and amoxicillin 200 µg/mL as positive control. Moreover, the wells were incubated for 1, 3, and 24 hours at 37°C. The wells were washed again with PBS and fixed over the fire. To measure density of *P. gingivalis* biofilm, the wells were given 200 µL of crystal violet stain (0.05% w/v), then left for 15 minutes. Then, the wells were washed with PBS twice and 200 µL of 96% ethanol was added. Optical Density (OD) measurement of biofilm was performed with microplate reader (SAFAS MP96, SAFAS, Monaco) at the wavelength of 490 nm.

3 RESULTS

The results of antibacterial test using plate count method can be seen in Figure 1. In this study, ethanol extract of *M. indica* L. leaves with various concentrations ware shown to inhibit the growth of *P. gingivalis* (Figure 2).

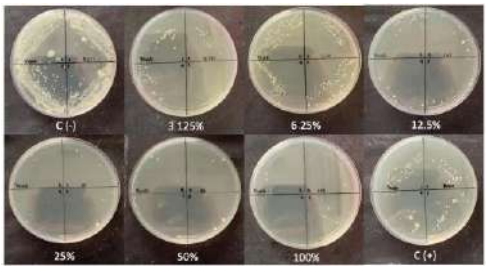

Figure 1. The results of the growth inhibition test of *P. gingivalis* with plate count method.

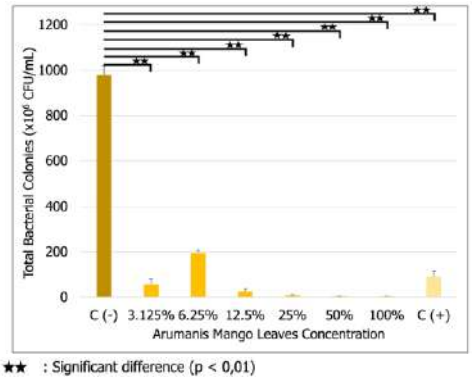

Figure 2. Graphic of total bacterial colonies of *P. gingivalis* by plate count method. DMSO 10% as negative control and amoxicillin 200 µg/mL as positive control

Ethanol extract of *M. indica* L. leaves with concentration of 100% produced the best antibacterial activity against *P. gingivalis* with total colony of *P. gingivalis* (3.33 ± 1.15) x 10^6 CFU/mL (Table 1).

Commented [DZ4]: Tabel dan gambar disimpan setelah dipanggil dalam teks.

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Table 1. The result of mean total colony of *P. gingivalis* by plate count method

Treatment	Mean (CFU/mL)
K(-)	(978,67 ± 41,05) x 10^6
3,125%	(55,33 ± 26,1) x 10^6
6,25%	(195,33 ± 11,37) x 10^6
12,5%	(25,33 ± 11,37) x 10^6
25%	(8,67 ± 3,06) x 10^6
50%	(4 ± 0,00) x 10^6
100%	(3,33 ± 1,15) x 10^6
K(+)	(90 ± 24,98) x 10^6

The results of antibiofilm test using microtiter plate biofilm assay showed that ethanol extract of *M. indica* L. leaves with different concentrations had antibiofilm effect on *P. gingivalis* at incubation periods of 1, 3, and 24 hours (Figures 3, 4, and 5).

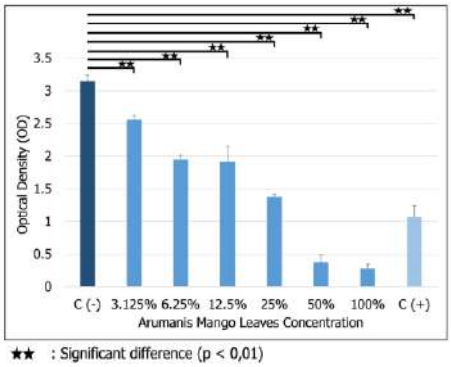

Figure 3. Graphic of mean OD of *P. gingivalis* biofilm with 1 hour incubation period. Biofilm without treatment as negative control and amoxicillin 200 µg/mL as positive control.

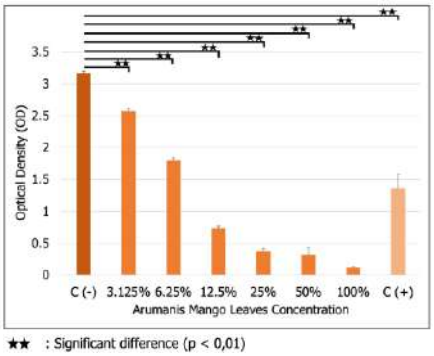

Figure 4. Graphic of mean OD of *P. gingivalis* biofilm with 3 hours incubation period. Biofilm without treatment as negative control and amoxicillin 200 µg/mL as positive control.

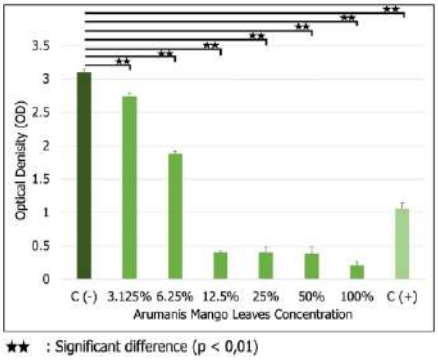

Figure 5. Graphic of mean OD of *P. gingivalis* biofilm with 24 hours incubation period. Biofilm without treatment as negative control and amoxicillin 200 µg/mL as positive control.

The extract with 100% concentration was the most effective in inhibiting *P. gingivalis* biofilm during incubation period of 3 hours due to the smallest OD value, namely OD 0.115 ± 0.015 (Table 2).

Table 2. Result mean OD ± SD biofilm *P. gingivalis*

Treatment	OD 1 hr	OD 3 hr	OD 24 hr
K (-)	3,148 ± 0,089	3,172 ± 0,026	3,104 ± 0,044
3,125%	2,563 ± 0,065	2,575 ± 0,042	2,738 ± 0,051
6,25%	1,947 ± 0,064	1,798 ± 0,04	1,884 ± 0,029
12,5%	1,918 ± 0,238	0,735 ± 0,033	0,404 ± 0,016
25%	1,377 ± 0,034	0,376 ± 0,039	0,402 ± 0,086
50%	0,377 ± 0,112	0,321 ± 0,108	0,389 ± 0,098
100%	0,281 ± 0,063	0,115 ± 0,015	0,214 ± 0,054
K(+)	1,066 ± 0,173	1,365 ± 0,215	1,055 ± 0,090

4 DISCUSSION

This study showed that ethanol extract of *M.indica* L. leaves is effective as antibacterial activity against *P. gingivalis* biofilm. The ethanolic extract of *M. indica* L. leaves are known to contain alkaloids, saponins, tannins, phenolics, flavonoids, and steroids which contribute to antibacterial and antibiofilm activity against *P. gingivalis* (Ningsih, 2017). The mechanism of bacterial death by alkaloid compounds occurs due to its compound which inhibit peptidoglycan from bacterial cells, thus the cell wall is not fully formed and leads to lysis (Sylvana et al., 2021). Saponin compound plays a role in inhibiting bacterial growth by damaging bacterial cell membranes and disrupting the balance of intra and extracellular substances (Sebastian & Widyarman, 2021).

Phenolic compounds have high antimicrobial power due to its compounds can damage cell structure membranes, interfere with bacterial protein synthesis, and change bacterial DNA genes (Tirado et al., 2021). Tannin compound form complex bonds with proline proteins thus cell walls are damaged. Flavonoid compound is antibacterial by interfering the formation of cell walls, nucleic acids, and bacterial proteins (Sylvana et al., 2021). These compounds are also antibiofilms by inhibiting the formation of quorum sensing signals, thus communication between bacteria during biofilm formation is disrupted (Federika et al., 2020). The ability of steroid compound to cause liposomes to leak on phospholipid membrane can result in bacterial cell lysis (Hassan & Ullah, 2019).

The most effective antibacterial effect on the growth of *P. gingivalis* was ethanol extract of *M. indica* L. leaves with concentration of 100% which produced the least total bacterial colonies of *P. gingivalis*, namely $(3.33 \pm 1.15) \times 10^6$ CFU/mL. This is in accordance with study by Kurniasih on effectiveness of concentration of mango arumanis leaves extract on the growth of *S. mutans* with disc diffusion method. Based on previous research, ethanol extract of *M. indica* L. leaves with the highest concentration, which was 80% concentration, showed the largest zone of inhibition against *S. mutans* (Kurniasih, 2016).

The incubation period used in antibiofilm assay in this study was 1 hour, 3 hours, and 24 hours. This incubation period was adjusted to the stage of biofilm formation. In first few seconds to minutes, biofilm begins with formation of pellicle on tooth surface. At 2-4 hours later, adhesion phase of bacterial colony occurs. If after 24 hours the bacteria on tooth surface are still attached, biofilm will enter maturation phase (Bjarnsholt, 2013).

This proves that the extract was most effective in inhibiting formation of *P. gingivalis* biofilm in adhesion phase. As concentration of extract increased, it showed lower OD value, which means an increase in inhibitory effect against *P. gingivalis* biofilm formation, hence this extract is dose dependent. This study is in accordance with previous studies, which showed that ethanolic extract of *M. indica* L. leaves could reduce attachment of mature biofilm of *S. aureus* (Manzur et al., 2020). In this study, ethanolic extract of *M. indica* L. leaves has potential as antibacterial and antibiofilm against *P. gingivalis* in vitro.

5 CONCLUSIONS

The ethanol extract of *M. indica* L. leaves proved effective in inhibiting the growth and formation of *P. gingivalis* biofilms in vitro. Ethanol extract of *M. indica* L. leaves with concentration of 100% was the most effective concentration as antibacterial and antibiofilm against *P. gingivalis*, especially during incubation period of 3 hours.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare

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