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):

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[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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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

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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

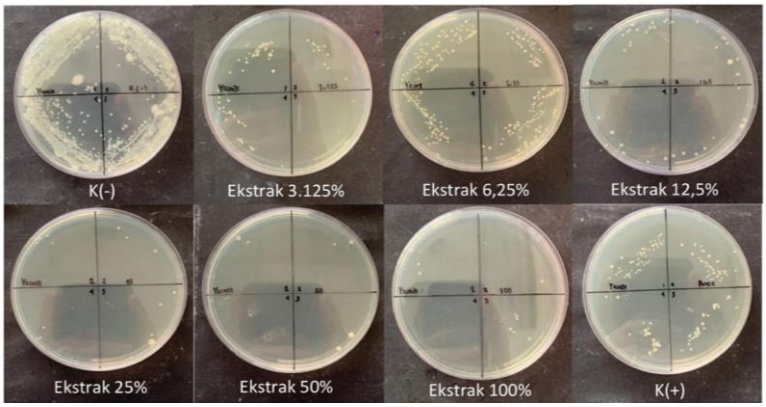

Figure 1. [The results of the growth inhibition test of *P. gingivalis* with plate count method.]

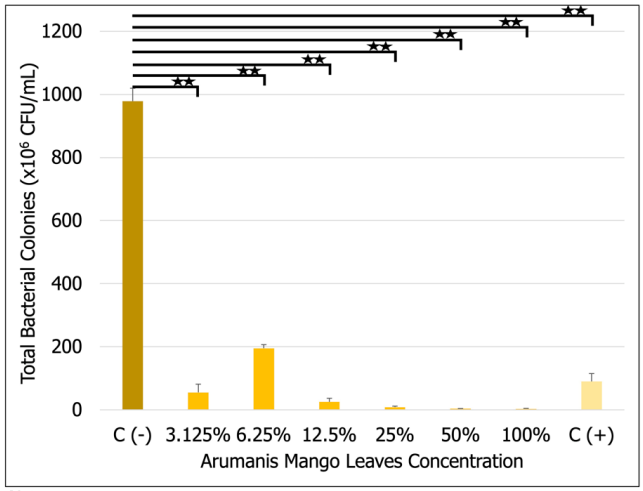

Figure 2. [Graphic of total bacterial colonies of *P. gingivalis* by plate count method.]

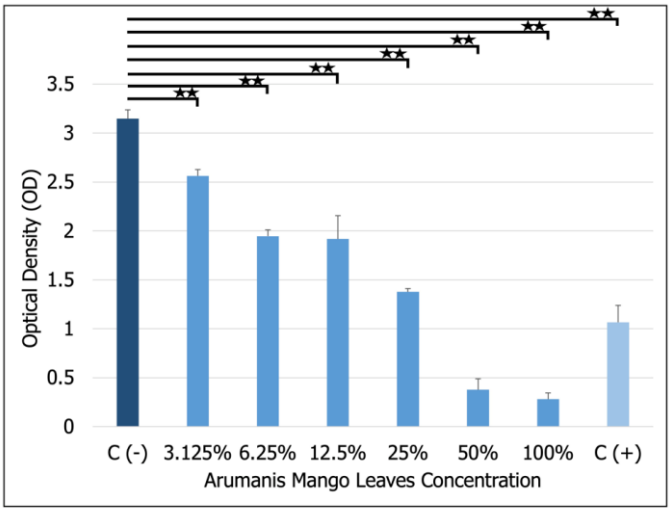

Figure 3. [Graphic of mean OD of *P. gingivalis* biofilm with 1 hour incubation period]

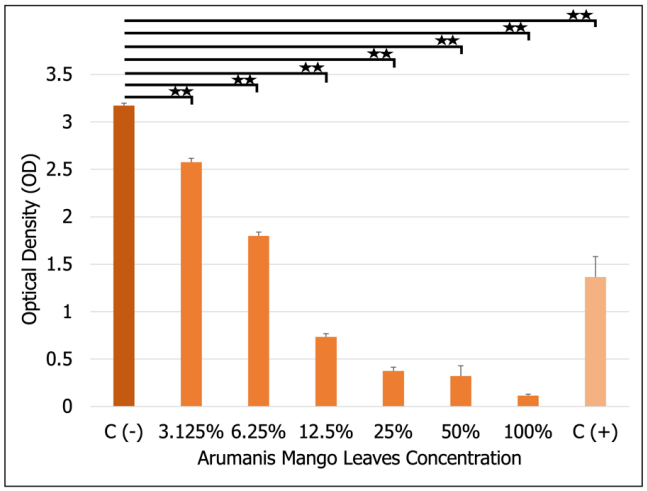

Figure 4. [Graphic of mean OD of *P. gingivalis* biofilm with 3 hours incubation period]

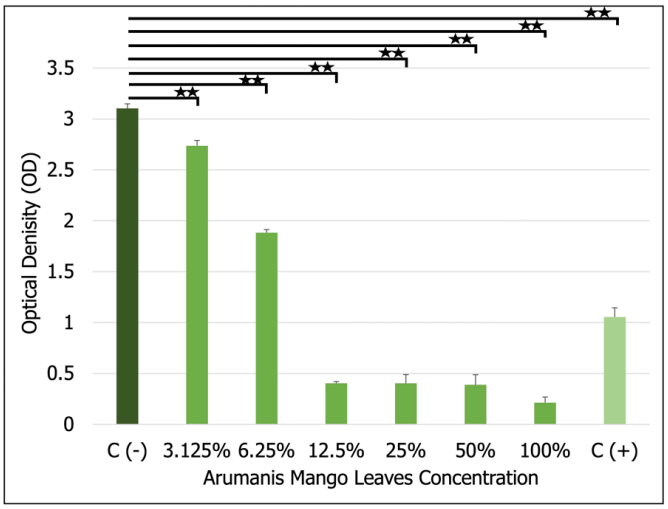

Figure 5. [Graphic of mean OD of *P. gingivalis* biofilm with 24 hours incubation period]

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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(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).

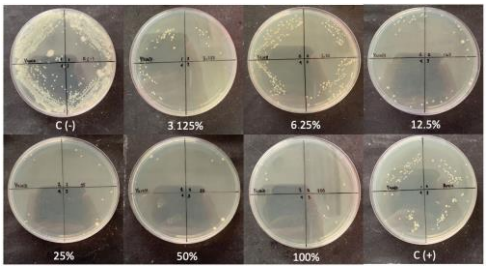

Figure 1. The results of the growth inhibition test of *P. gingivalis* with plate count method.

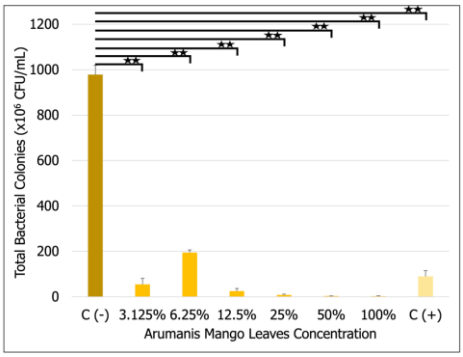

Figure 2. Graphic of total bacterial colonies of *P. gingivalis* by plate count method. DMSO 10% as negative control and amoxicillin 200 µg/mL as positive control

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).

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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).

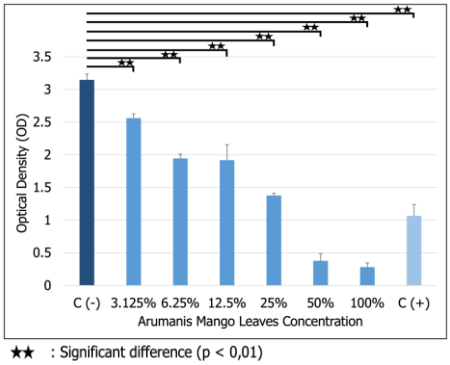

Figure 3. Graphic of mean OD of *P. gingivalis* biofilm with 1 hour incubation period. Biofilm without treatment as negative control and amoxicillin 200 µg/mL as positive control.

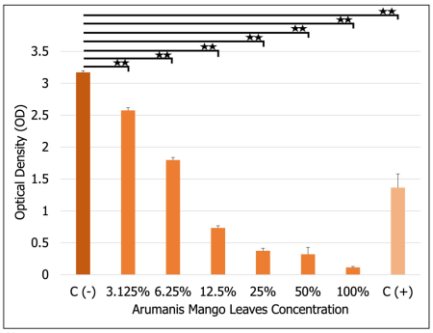

Figure 4. Graphic of mean OD of *P. gingivalis* biofilm with 3 hours incubation period. Biofilm without treatment as negative control and amoxicillin 200 µg/mL as positive control.

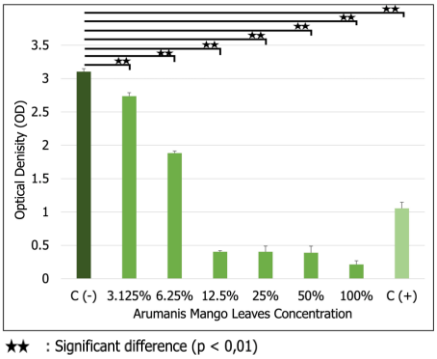

Figure 5. Graphic of mean OD of *P. gingivalis* biofilm with 24 hours incubation period. Biofilm without treatment as negative control and amoxicillin 200 µg/mL as positive control.

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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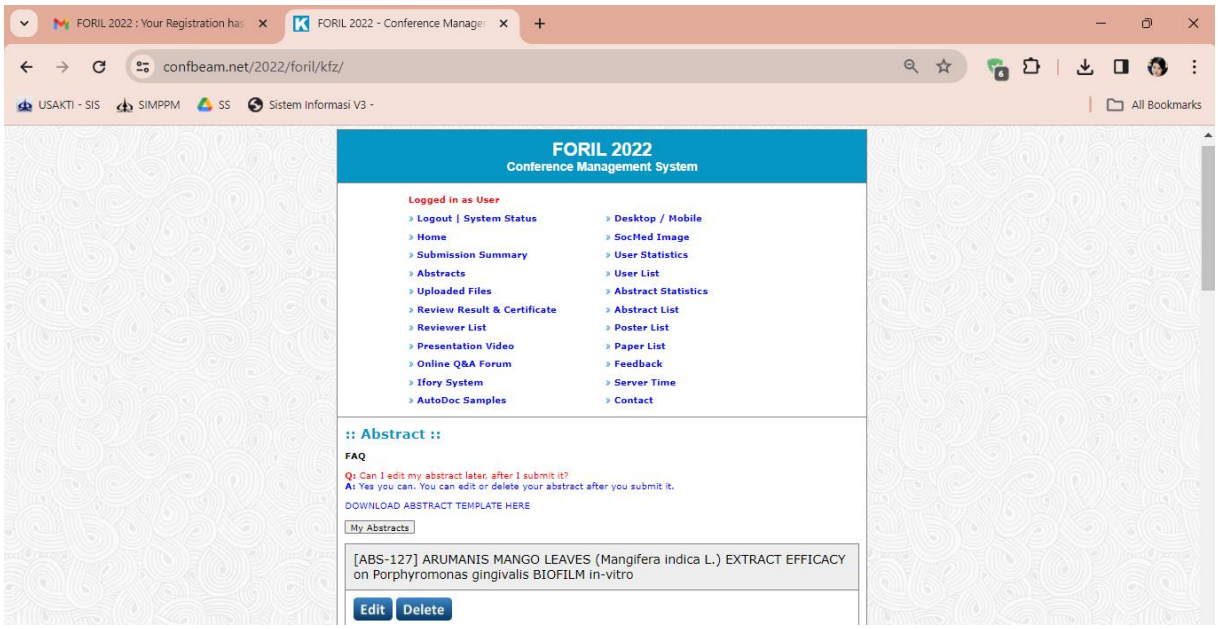
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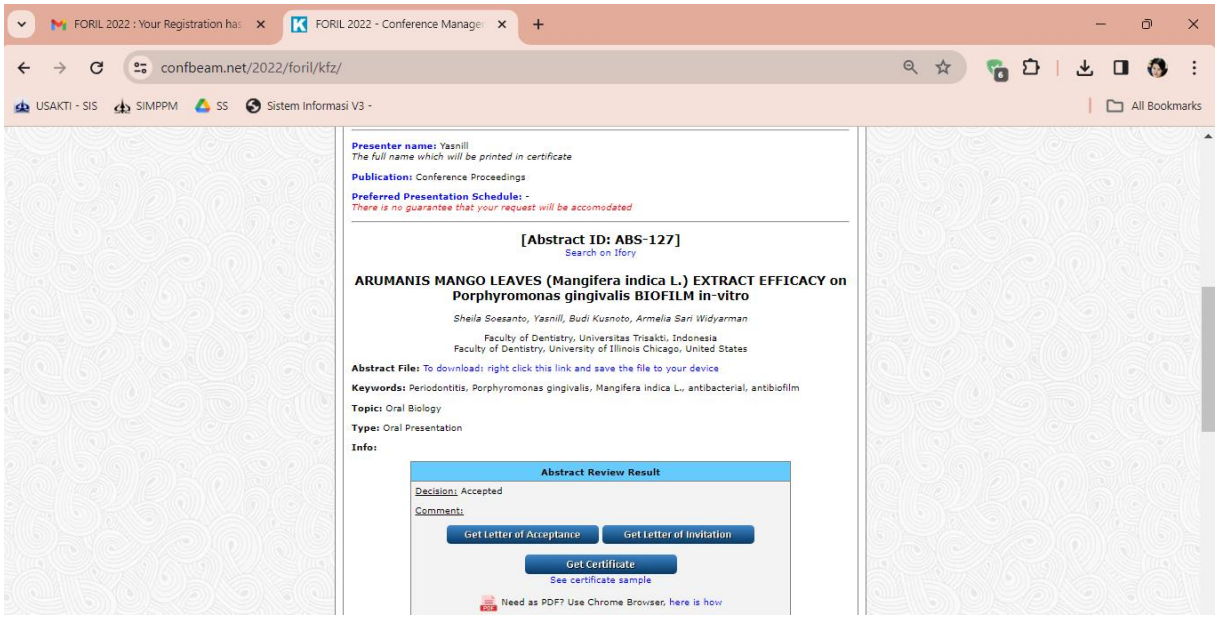
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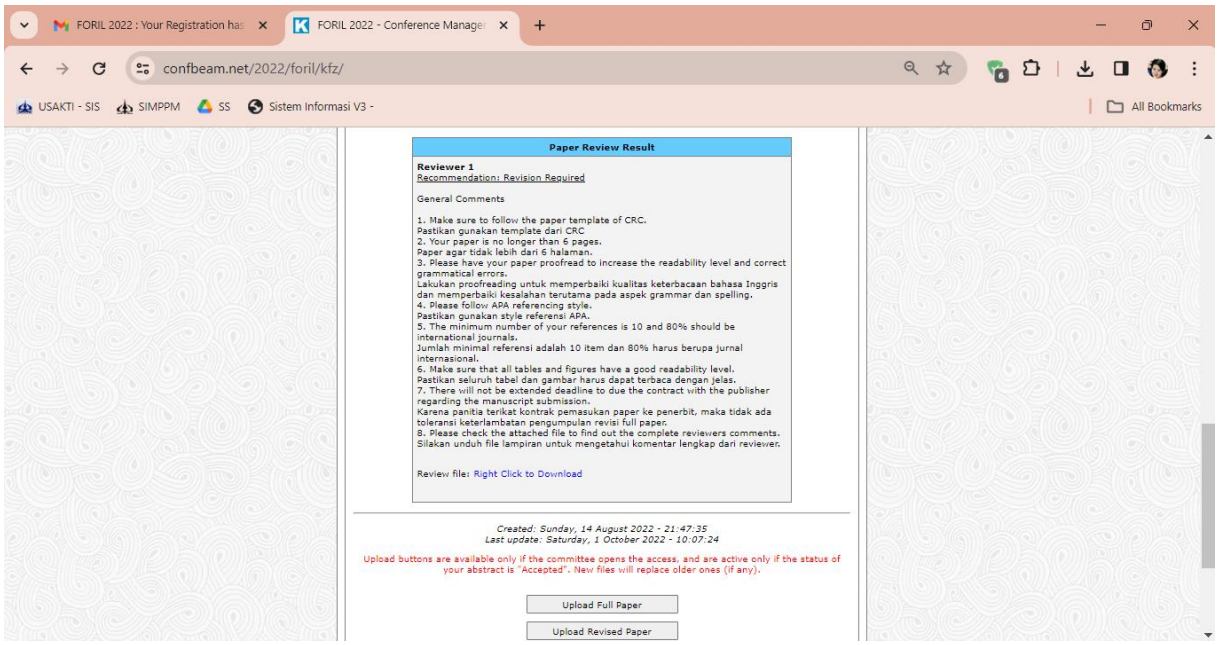
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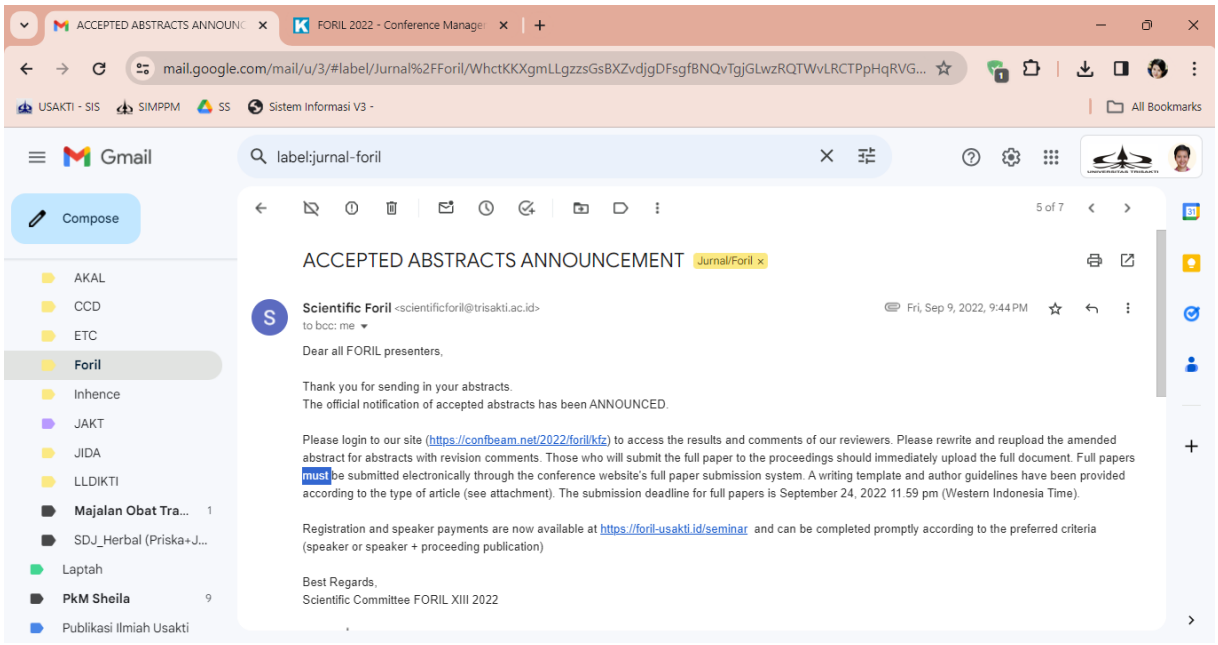
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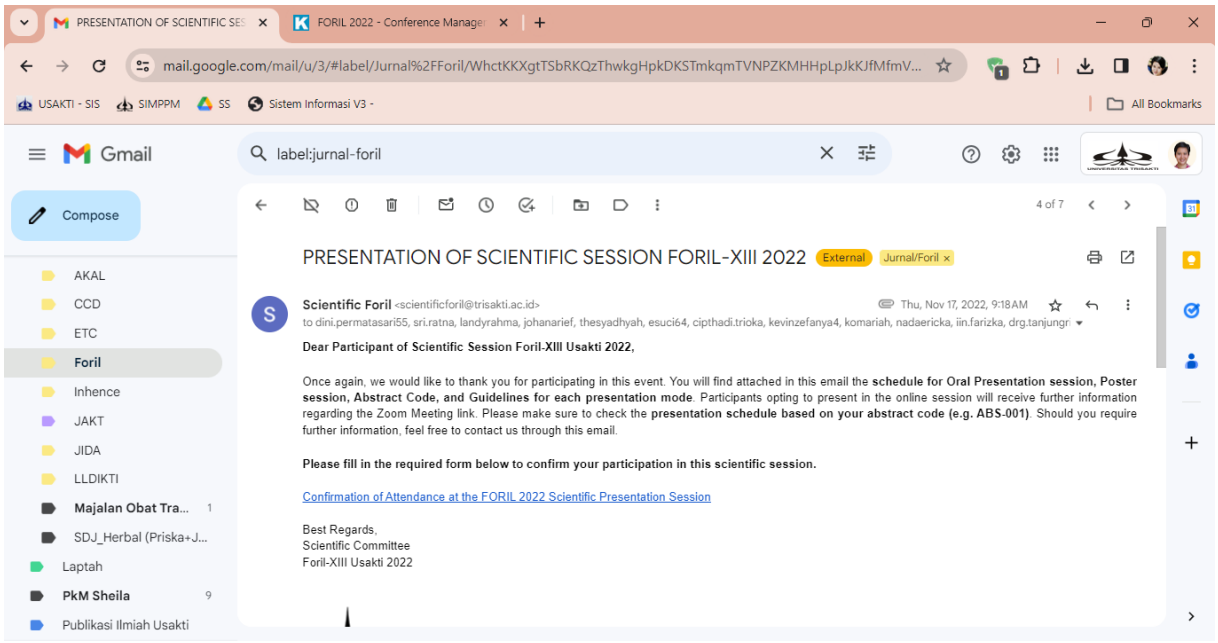
PRESENTATION OF SCIENTIFIC SESSION FORIL-XIII 2022
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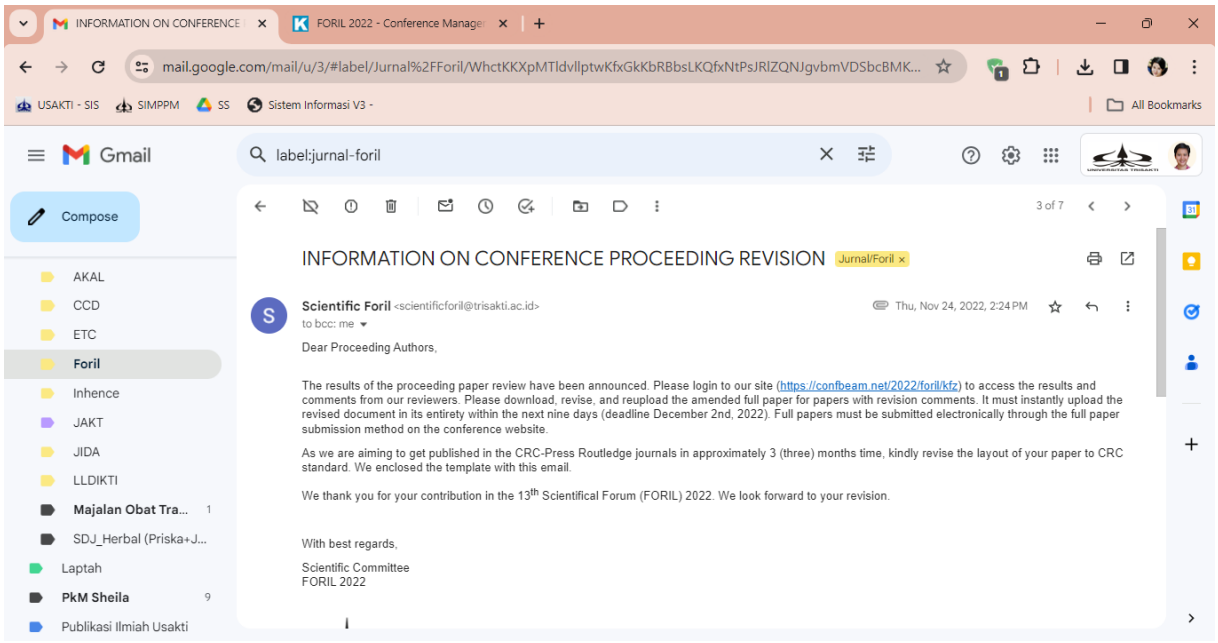
Scientific Foril <scientificforil@trisakti.ac.id>
Thu, Nov 17, 2022, 9:18 AM
Dear Participant of Scientific Session Foril-XIII Usakti 2022,
Once again, we would like to thank you for participating in this event. You will find attached in this email the schedule for Oral Presentation session, Poster session, Abstract Code, and Guidelines for each presentation mode. Participants opting to present in the online session will receive further information regarding the Zoom Meeting link. Please make sure to check the presentation schedule based on your abstract code (e.g. ABS-001). Should you require further information, feel free to contact us through this email.
Please fill in the required form below to confirm your participation in this scientific session.
Confirmation of Attendance at the FORIL 2022 Scientific Presentation Session
Best Regards,
Scientific Committee
Foril-XIII Usakti 2022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Scientific Foril <scientificforil@trisakti.ac.id>
Thu, Nov 24, 2022, 2:24 PM
Dear Proceeding Authors,
The results of the proceeding paper review have been announced. Please login to our site (https://confbeam.net/2022/foril/kfz) to access the results and comments from our reviewers. Please download, revise, and reupload the amended full paper for papers with revision comments. It must instantly upload the revised document in its entirety within the next nine days (deadline December 2nd, 2022). Full papers must be submitted electronically through the full paper submission method on the conference website.
As we are aiming to get published in the CRC-Press Routledge journals in approximately 3 (three) months time, kindly revise the layout of your paper to CRC standard. We enclosed the template with this email.
We thank you for your contribution in the 13th Scientifical Forum (FORIL) 2022. We look forward to your revision.
With best regards,
Scientific Committee
FORIL 2022