



## Research Article

# Antimicrobial Effect of Rosella Flower Extract (*Hibiscus sabdariffa* L.) Against Multispecies *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*

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## Abstract

Periodontal disease has a high prevalence in the world and Indonesia. There are 2 types of periodontal disease, such as gingivitis and periodontitis, which can be caused by pathogenic bacteria such as *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*. Minimizing periodontal disease can be done by brushing, flossing, scaling, and using mouthwash that contains antibacterial. Rosella (*Hibiscus sabdariffa* L.) is an herbal plant that contains antibacterial properties, so it can be used as an additional therapy to minimize periodontal disease. The research was conducted in the MiCore laboratory FKG Usakti with the method in this study using an in vitro laboratory experiment using a biofilm assay test with a concentration of 1 g/mL, 0.50 g/mL, 0.25 g/mL, 0.125 g/mL, 0.0625 g/mL, 0.0312 g/mL, 0.015 g/mL. The extract was distributed into well plates, stained with crystal violet, incubated for 1 hour, 6 hours, and 24 hours, and then counted using a microplate reader. The results showed that extract concentrations starting from 0.015 g/mL at an incubation period of 1 hour, concentrations starting from 0.125 g/mL at an incubation period of 6 hours, and concentrations starting from 0.0625 g/mL at an incubation period of 24 hours were proven to inhibit bacterial growth and proved to be more effective than 0.2% chlorhexidine with significantly different results ( $p < 0.05$ ). It can be concluded that Rosella extract can inhibit the growth of multispecies bacteria *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*.

**Keywords:** periodontal disease; rosella (*Hibiscus sabdariffa* L.); *Fusobacterium nucleatum*; *Aggregatibacter actinomycetemcomitans*; multispecies *Fusobacterium nucleatum*; *Aggregatibacter actinomycetemcomitans*

## INTRODUCTION

Oral health is an indicator of health because the oral cavity is the first place or "entrance" and plays a key role in maintaining oral and systemic health.<sup>1</sup> According to Basic Health Research (RISKESDAS) data, 74.1% of periodontitis cases in Indonesia show that the prevalence of periodontitis in Indonesia is high.<sup>2,3</sup>

Periodontal disease is inflammation of the supporting tissues. The supporting tissue comprises the gingiva, cementum, periodontal ligament, and alveolar bone.<sup>4</sup> There are 2 types of periodontal disease, namely gingivitis and periodontitis.<sup>5</sup> In

general, periodontal disease is caused by bacterial plaque on the tooth surface. Plaque is a thin biofilm layer containing a collection of pathogenic microorganisms.<sup>6</sup> If plaque is not cleaned regularly, it will mineralize and calculus formed. Plaque containing many microorganisms will then cause inflammation of the gingiva.<sup>7</sup> Bacteria that can cause periodontitis include *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*.<sup>8-10</sup>

Periodontitis can be minimized by mechanical plaque control such as tooth brushing with fluoride, cleaning the

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interdental areas using dental floss, and visiting the dentist at least 2 times a year.<sup>7,11</sup> The usual mouthwash used is *Chlorhexidine* (CHX), but the use of CHX causes side effects such as staining of the teeth, hypersensitivity, irritation of the mucous membrane, and taste disturbances.<sup>10,12-14</sup> Therefore, natural materials are developing, which are used as alternatives to inhibit plaque in the oral cavity more safely.<sup>15</sup>

One of the most widely used natural ingredients is *Hibiscus sabdariffa* L. (Rosella). Rosella flower petals have benefits such as antibacterial as they contain flavonoids, polyphenols, saponins, and alkaloids.<sup>9,13,15-17</sup>

## MATERIALS AND METHODS

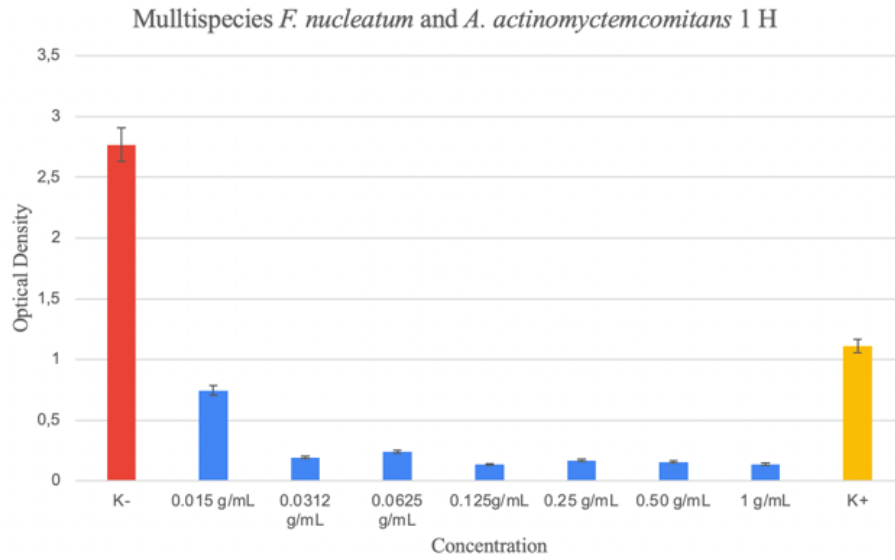
The type of research is laboratory experimental in vitro to identify the effect of ethanol extract of rosella petals on multispecies biofilm density of *F. nucleatum* and *A. actinomycetemcomitans* using the biofilm assay method. The research design used was a post-test-only control group design. This research was conducted at the MiCORE Laboratory (Microbiology Center of Research and Education) Faculty of Dentistry Trisakti from August to November 2022. The research sample used was the ethanol extract of *Hibiscus sabdariffa* L. obtained from Balitro, *F. nucleatum* ATCC 2558, and *A. actinomycetemcomitans* ATCC 29522. Brain heart infusion (BHI) as a negative control, and 0.2% chlorhexidine as a positive control. Rosella flower ethanol extract was obtained from 1 kg of dried rosella flowers obtained from Balitro plantations, mashed and extracted using the maceration method using 96% ethanol as a solvent. Evaporated using a rotary evaporator to obtain a concentration of 1 g/mL, 0.50 g/mL, 0.25g/mL, 0.125 g/mL, 0.0625 g/mL, 0.0312 g/mL, 0.015 g/mL. The multispecies biofilm density of *F. nucleatum* and *A. actinomycetemcomitans* was cultured using agar medium for 24

hours, then incubated using the Gaspack jar system, and then the colonies were put into BHI broth and incubated for 3x24. The bacterial culture was diluted 100 times until the concentration reached  $1.5 \times 10^6$  CFU/mL, and then it was distributed in a 96-well flat-bottom microplate. The two bacterial cultures were inoculated in BHI broth for 48 hours at 37°C in an anaerobic atmosphere. The next procedure was to supply roselle extract with concentrations 1g/mL, 0.50 g/mL, 0.25g/mL, 0.125 g/mL, 0.0625 g/mL, 0.0312 g/mL, 0.015 g/mL into the well-plate then incubated for 1 hour, 6 hours, and 24 hours at 37°C. The extract was discarded and rinsed then fixed over the fire. Bacteria were stained using crystal violet, and then the biofilms were counted using a microplate reader after adding 200µL of 96% ethanol. Statistical tests were performed using SPSS at a significance level of 95%. Shapiro-Wilk for normality test, and One Way Anova parametric test were employed for all treatment groups, followed by the Post Hoc LSD test to determine the significant differences in each group ( $P < 0.05$ ).

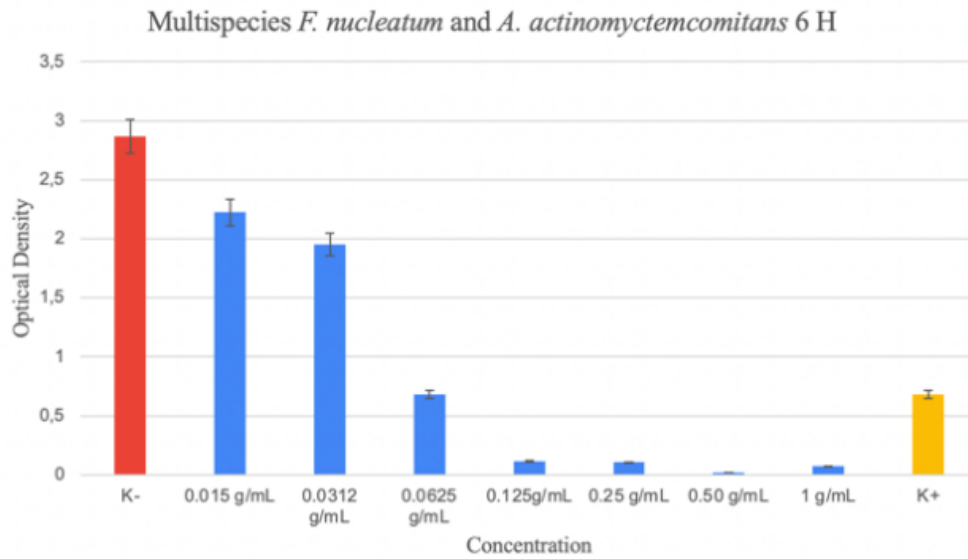
## RESULT

Phytochemical was conducted at the Balitro, Bogor. The test results found that rosella extract contained alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides.

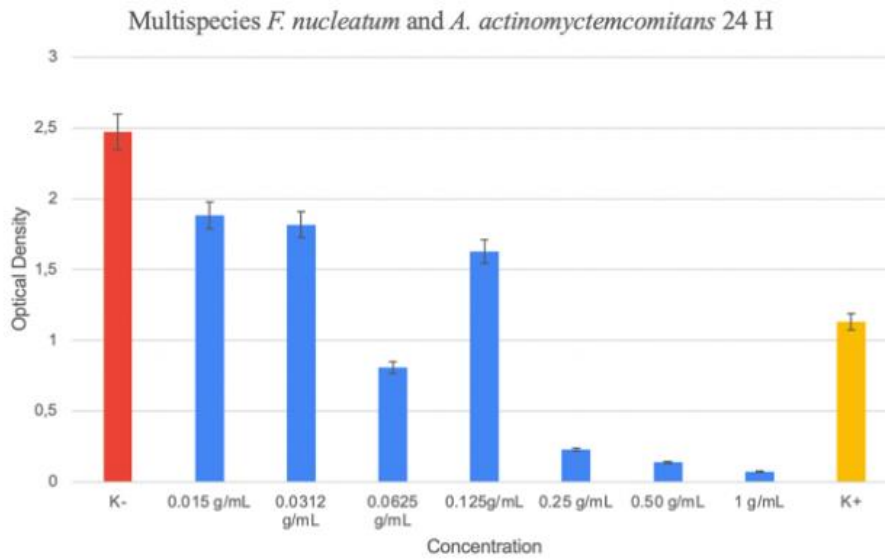
The normality test with *Sapshiro-Wilk* showed that the data were normally distributed  $p > 0,05$  (Table 1). Further analysis by One-Way ANOVA revealed that the results were significantly different  $p < 0.05$  (Table 2). Based on the results of the data and biofilm test, it was known from research data (average value  $OD \pm SD$ ) Rosella flower ethanol extract affected reducing biofilm density on multispecies *F. nucleatum* and *A. actinomycetemcomitans* seen from the decreasing values in all incubation periods, 1 hour, 6 hours and 24 hours (Table 3, Figure 1, 2, and 3).



**Figure 1.** Biofilm OD graph multispecies *F. nucleatum* and *A. actinomycetemcomitans* 1 Hour



**Figure 2.** Multispecies biofilm OD graph *F. nucleatum* and *A. actinomycetemcomitans* 6 Hours



**Figure 3.** 24 Hour OD graph of multispecies biofilms *F. nucleatum* and *A. actinomycetemcomitans*.

**Table 1.** Test results of Shapiro-Wilk

Variable	Test Type	p.s
Multispecies <i>F. nucleatum</i> and <i>A. actinomycetemcomitans</i> 1 Hour	Shapiro-Wilk	.050
Multispecies <i>F. nucleatum</i> and <i>A. actinomycetemcomitans</i> 6 Hours	Shapiro-Wilk	.707
Multispecies <i>F. nucleatum</i> and <i>A. actinomycetemcomitans</i> 24 Hours	Shapiro-Wilk	.967

**Table 2.** Test results of One Way ANOVA

Variable	p.s
Multispecies <i>F. nucleatum</i> and <i>A. actinomycetemcomitans</i> 1 Hour	.000
Multispecies <i>F. nucleatum</i> and <i>A. actinomycetemcomitans</i> 6 Hours	.000
Multispecies <i>F. nucleatum</i> and <i>A. actinomycetemcomitans</i> 24 Hours	.000

**Table 3.** Average OD ± Sd Values of Biofilm *F. Nucleatum* and *A. Actinomycetemcomitans*

Concentration	N	1 Hour	6 Hour	24 Hour
K-	3	2.768±0.114	2.869±0.035	2.472±0.033
0.015 g/mL	3	0.746±0.063	2.223±0.244	1.884±0.073
0.0312 g/mL	3	0.194±0.035	1.952±0.020	1.817±0.065
0.0625 g/mL	3	0.242±0.130	0.679±0.034	0.808±0.058
0.125g/mL	3	0.136±0.072	0.113±0.029	1.628±0.059
0.25 g/mL	3	0.170±0.067	0.105±0.023	0.229±0.016
0.50 g/mL	3	0.157±0.103	0.018±0.097	0.138±0.084
1 g/mL	3	0.139±0.014	0.070±0.016	0.072±0.62
K+	3	1.112±0.064	0.679±0.108	1.131±0.146

The 1 hour incubation period showed that the concentration 0.125g/mL received the lowest OD value with an OD value 0.136±0.072 (Figure 1). For an incubation period of 6 hours, the lowest OD value was 0.50 g/mL with an OD value of

0.018±0.097 (Figure 2). During the 24-hour incubation period, the lowest concentration was found at a concentration of 1 g/mL with an OD value of 0.072±0.62 (Figure 3).

## DISCUSSION

The results of the phytochemical tests in this study stated that rosella flowers contained alkaloids, saponins, tannins, flavonoids, phenolics, triterpenoids, and glycosides. The results of these data are supported by research conducted by Suniarti et al. that yielded the same phytochemical result.<sup>16</sup>

Flavonoid has three different mechanisms as a microbial agent: it inhibits nucleic acid synthesis, cell membrane functioning, and energy metabolism. Flavonoid compounds can inhibit bacterial growth by forming complex compounds with proteins through hydrogen bonds. Phenol can change cell proteins and damage the bacterial plasma membrane. Saponins can release proteins and enzymes from inside the bacterial cells. Anthocyanins can inhibit glucose oxidation and bind iron, thereby inhibiting bacterial metabolism. Tannins can inhibit enzyme production.<sup>18</sup> Alkaloid exerts an antibacterial effect by interrupting the formation of peptidoglycan bacterial cells, affecting their formation and causing cell death through the rupture of cell walls.<sup>10</sup> Rosella extract is more effective than CHX, supported by research conducted by Ratnasari Dyah (2015) that showed that a 70% concentration of rosella flowers had a larger inhibitory zone diameter than chlorhexidine.<sup>19</sup>

Based on the results of the biofilm measurement study, it was shown that the highest average OD (Optical Density) was in the 24-hour incubation period, and the lowest average OD value was in the 1-hour incubation period. It relates to the biofilm formation process, which is divided into several stages, namely, adhesion occurs in a few minutes, then adheres firmly and forms microcolonies within 2 to 4 hours, and 6 hours to 12 hours, the biofilm becomes resistant to antiseptics and antibiotics and will mature within 24 hours.<sup>20</sup>

The results of rosella extract treatment against multispecies *F.*

*nucleatum* and *A. actinomycetemcomitans* at an incubation period of 1 hour showed that the concentration of 1 g/mL, 0.50 g/mL, 0.25g/mL, 0.125g/mL, 0.0625 g/mL, 0.0312 g/mL, and 0.015 g/mL could inhibit the growth of *F. nucleatum* and *A. actinomycetemcomitans* biofilm. It can be seen from the lower OD value than the positive control with the lowest OD at a concentration of 0.125g/mL with an OD value of  $0.136 \pm 0.072$ . In the post-hoc test results, all extract concentrations differed significantly from the positive control ( $p < 0.05$ ). It revealed that the ethanol extract of rosella flowers at an incubation period of 1 hour with a concentration of 0.015 g/mL already has a significant inhibitory effect on biofilms compared to 0.2% chlorhexidine as a positive control.

The 6-hour incubation period showed that the rosella extract, starting with a concentration of 0.125 g/mL, can inhibit the growth of *F. nucleatum* and *A. actinomycetemcomitans* biofilms. It can be seen from the lower OD value than the positive control with the lowest OD at a concentration of 0.50 g/mL, with an OD value of  $0.018 \pm 0.097$ . The post hoc test results differed significantly from the positive control ( $p < 0.05$ ). However, a 0.0625 g/mL concentration did not show significantly different results from the positive control,  $p = .893$ . It showed that the ethanol extract of rosella flowers at an incubation period of 1 hour with a concentration of 1 g/mL, 0.50 g/mL, 0.25g/mL, and 0.125g/mL has a significantly inhibiting effect on biofilms compared to 0.2% chlorhexidine as a positive control. In contrast, a concentration of 0.0625 g/mL has an inhibition equivalent to 0.2% chlorhexidine.

The 24-hour incubation period showed that the concentration of rosella extract starting from 0.0625 g/mL can inhibit the biofilm growth of *F. nucleatum* and *A. actinomycetemcomitans*. It can be seen from the lower OD value than the positive control, which has the lowest OD at a concentration of 1 g/mL and an OD

value of  $0.072 \pm 0.62$ . At a concentration of 0.125g/mL, there was an increase in OD of  $1.628 \pm 0.059$  of a concentration of 0.0625 g/mL with an OD of  $0.808 \pm 0.058$ . This deviation may occur due to errors when researching so that bacterial contamination or errors during pipetting during rinsing of extracts may occur. In the post-hoc test results, all concentrations differed significantly from the positive control ( $p < 0.05$ ). The result demonstrated that the ethanol extract of rosella flowers at an incubation period of 24 hours with a concentration of 1 g/mL, 0.50 g/mL, 0.25g/mL, 0.125g/mL, and 0.0625 g/mL already had an inhibiting effect on biofilms significantly compared to 0.2% chlorhexidine as the positive control.

This research is supported by research conducted by Andrietta G (2019), that rosella extract was able to inhibit the density of the monospecies *A. actinomycetemcomitans* biofilm with a concentration of 1.5% at an incubation period of 1 hour and a concentration of 3.12% at an incubation period of 6 hours and 24 hours which proved to be more effective than chlorhexidine 0.2%.<sup>8</sup> Sebastian J (2019) also stated that rosella extract could inhibit the density of the monospecies *F. nucleatum*, which had the greatest inhibition and the lowest OD at the highest concentration of 1 g/mL during the 24-hour incubation period.<sup>9</sup> Moreover, Suwandi et al. (2013), in their research on the effect of rosella flower ethanol extract on *Streptococcus sanguinis* bacteria with the same research method with an incubation period of 20 hours and 24 hours with a concentration of 0.8%, 10 %, and 20% showed almost the same OD values as 0.1% and 0.2% chlorhexidine, proving that rosella extract was able to reduce the density of biofilms.<sup>15</sup>

## CONCLUSION

The results showed that the ethanol extract of rosella flowers (*Hibiscus sabdariffa* L.) was able to inhibit or reduce the density of multispecies *Fusobacterium*

*nucleatum* and *Aggregatibacter actinomycetemcomitans* biofilms in vitro. Extract concentrations ranging from 0.015 g/mL at an incubation period of 1 hour, concentrations starting at 0.125 g/mL at an incubation period of 6 hours, and concentrations starting at 0.0625 g/mL at an incubation period of 24 hours were proven to inhibit bacterial growth and more effective than chlorhexidine 0.2%.

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