The effect of Sarang Semut (Myrmecodia pendens and Myrmecodia tuberosa jack) as Antibacterial for Periodontal Pocket Therapy

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Abstract

Porphyromonas gingivalis is one of the bacteria that causes periodontal disease. Periodontal treatment primarily aims to remove supra- and subgingival biofilm from the root surface to remove pathogenic bacteria. Currently, people have turned to using medicinal plants as therapeutic agents instead of drugs. Myrmecodia pendens and Myrmecodia tuberosa Jack (Sarang S emut) are considered to have medicinal value due to the various active compounds they contain.

This study is experimental research with a post-test only control group design to compare the antibacterial activity of Myrmecodia tuberosa Jack and Myrmecodia pendens against the growth of Porphyromonas gingivalis bacteria. The effectiveness test of Myrmecodia tuberosa Jack and Myrmecodia pendens was carried out using 20 different concentrations with 4 repetitions for each concentration. We used Chlorhexidine gluconate 0.2% as positive control and DMSO 10% as negative control.

To determine the minimum inhibitory levels, we used microdilution method. Data were analyzed using the Fisher and McNemar tests. There was no significant difference in antibacterial effectiveness between the two types of Sarang Semut plants (p=1.000). Sarang Semut plant Myrmecodia tuberosa Jack and Myrmecodia pendens have antibacterial effectiveness against Porphyromonas gingivalis bacteria.

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Introduction

Periodontal disease is a disease caused by inflammation of bacteria which results in progressive damage to the supporting tissues of the teeth so that it can cause the teeth to be released from their sockets[1]. The most common periodontal tissue diseases are gingivitis and periodontitis[1]. Globally, 796 million people experience periodontitis worldwide[2]. The prevalence of periodontal disease in Indonesia has reached 60% which indicates that Indonesian people are at risk of developing periodontal disease[3]. The main cause of periodontitis is related to the overactivity of the host's immune-inflammatory response to plaque pathogenic bacteria[3].

One of the bacteria that often occurs in cases of periodontitis is *Porphyromonas gingivalis* with periodontitis with a prevalence of 40-100%. This bacteria has the characteristics of being gram-negative, rod-shaped, non-motile, anaerobic, and *assacharolytic*, which uses amino acids as an energy source, so that it can live in deep periodontal pockets, where there is little sugar for energy. As much as 85.75% of these bacteria were found in the subgingival plaque area in chronic periodontitis patients[4].

Periodontal treatment primarily aims to remove supra- and subgingival biofilm from the root surface to remove pathogenic bacteria, which initiate and lead to the development of periodontal disease[5]. Currently, the use of drugs as therapeutic agents has been minimized because they have side effects such as bacterial resistance to antibiotics as well as taste disturbances and tooth discoloration. Instead,

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people have turned to using medicinal plants as therapeutic agents[6].

Indonesia is one of the countries with tropical forests which are known as a source of raw materials for medicines and can be used to treat various diseases. Like India and China, Indonesia is one of the largest users of medicinal plants in the world. Medicinal plants have been used for thousands of years as raw materials for medicines, but their use has not been well documented[7]. One example of a plant that is useful and can be found in several regions in Indonesia is Sarang Semut.

The Sarang Semut plant is a type of epiphytic plant that often grows on larger branches and other tree trunks. This plant does not live as a parasite that sucks food from its host, but only as a place to attach and ride on to grow[8] . This plant is named "semut (ants)" because the ants use this plant as their nest, so there is a connection between the ants and this plant.

Previous research revealed that Sarang Semut plants have various active compounds contained therein, such as flavonoids, tannins, and polyphenols[9] . Flavonoids, as the most widely distributed phenolic compounds in nature, have been reported to exhibit three different mechanisms of antimicrobial activity. They inhibit microbial nucleic acid synthesis, damage the microbial cytoplasmic membrane causing cell perforation, and sabotage metabolic processes in many microorganisms[10].

There are two general of Sarang Semut plants that are related to ants, namely the genera Myrmecodia and Hydnophytum. Of the various species of Sarang Semut, only *Hypnophytum formicarum, Myrmecodia pendens* and *Myrmecodia tuberosa* Jack are considered to have medicinal value. However, the scientific literature on this plant is still very limited[11]. There are 26 species of the genus *Myrmecodia* found in Indonesia, and 80% of them grow in swamps and wilderness areas[12]. This study compared the antibacterial activity of *Myrmecodia tuberosa Jack* and *Myrmecodia pendens* against the growth of *Porphyromonas gingivalis* bacteria.

Materials and methods

The type of research used is experimental research (true experiment). Research design: post test only control group. The research was conducted in July - December 2022 in the Microbiology laboratory of the Faculty of Medicine, Mulawarman University. This study used two different groups of each Sarang Semut plant. The first group (treatment group) is a group of bacteria treated using 20 concentrations of Sarang Semut plant extract 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.81 mg/mL, 3.90 mg/mL, 1.95 mg/mL, 0.97 mg/mL, 0.48 mg/mL, 0.24 mg/mL, 0.12 mg/mL, 0.06 mg/mL, 0.03 mg/mL, 0.015 mg/mL, 0.007 mg/mL, 0.003 mg/mL, 0.0019 mg/mL, 0.0009 mg/mL, 0.0004 mg/mL, 0.0002 mg/mL respectively.. The second group (control group) consists of positive control and negative control, positive control using chlorhexidine gluconate 0.2% and negative control using DMSO 10%.

Figure 1. Sarang Semut plants (source: primary data by researchers).

Sarang Semut plants *Myrmecodia tuberosa* Jack and *Myrmecodia Pendens* were dried and then pulverized. Next, each of the powder of the Sarang Semut plant was put into a maceration container and maceration extraction was carried out for 3x24 hours. The extracted filtrate was then evaporated using a rotary evaporator to produce a thick extract of the Sarang Semut plant. Then the extracted filtrate was filtered into an Elernmeyer flask. Weighing the extract is done according to the required concentration. Each concentration solution of

Sarang Semut plants of both types was mixed with 10% DMSO solvent. The concentration solution was filtered using Laminar Air Flow. Test the antibacterial activity of the Sarang Semut plant using the microdilution method.

Visual observation of minimum inhibitory concentration was done by adding Microtetrazolium dye into each well to see the inhibition of bacteria marked by changes in the color of the wells. Extract solutions that can inhibit bacterial growth are characterized by clear wells and changes in the color of the wells to purple are considered solutions that cannot inhibit bacterial growth or solutions overgrown with bacteria.

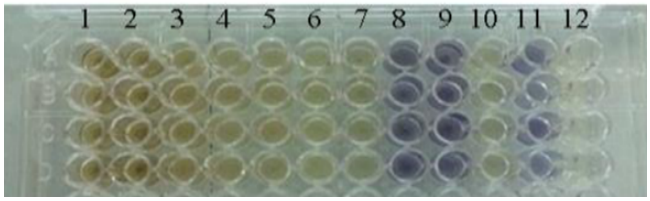
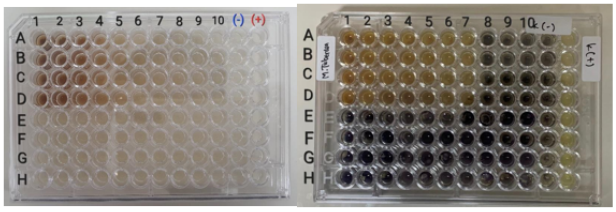


Figure 2. Indicator of bacterial inhibition.

Results

In this study, the antibacterial activity test was carried out using concentrations of the Sarang Semut plant extracts *Myrmecodia tuberosa* Jack and *Myrmecodia pendens* in 4 different columns with each plant being repeated 4 times with 20 concentrations including 125 mg/mL (1A-1D), 62.5 mg/mL (2A-2D), 31.25 mg/mL (3A-3D), 15.625 mg/mL (4A-4D), 7.81 mg/mL (5A-5D), 3.90 mg/mL (6A-6D), 1.95 mg/mL (7A-7D), 0.97 mg/mL (8A-8D), 0.48 mg/mL (9A-9D), 0.24 mg/mL (10A-10D), 0.12 mg/mL (1E-1H), 0.06 mg/mL (2E-2H), 0.03 mg/mL (3E-3H), 0.015 mg/mL (4E-4H), 0.007 mg/mL (5E-5H), 0.003 mg/mL (6E-6H), 0.0019 mg/mL (7E-7H), 0.0009 mg/mL (8E-8H), 0.0004 mg/mL (9E-9H) and 0.0002 mg/mL (10E-10H).

After 24 hours of incubation, the test results showed that the ethanol extract of Sarang Semut plant (*Myrmecodia tuberosa Jack*) was proven to have antibacterial activity against *Porphyromonas gingivalis* bacteria, at concentrations of 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.81 mg/mL, 3.90 mg/mL, and 1.95 mg/mL as evidenced by the test results, which show that the initial 7 wells of the microplate with 4 repetitions did not change color or remained clear with MIC at a concentration of 1.95 mg/mL. It can also be seen that there was a color change in all the negative controls indicating that all the wells in the negative control were overgrown with *Porphyromonas gingivalis* bacteria. Whereas the positive control (*Chlorhexidine gluconate* 0.2%) had battery inhibition according to its nature which was indeed used as a mouthwash which was characterized by no color change in all wells of the positive control (*Chlorhexidine gluconate* 0.2%).



(a) (b)

Test Preparations	Concentration (mg/mL)											
	1	2	3	4	5	6	7	8	9	10	K(-)	K(+)
A	-	-	-	-	-	-	-	+	+	+	+	-
B	-	-	-	-	-	-	-	+	+	+	+	-
C	-	-	-	-	-	-	-	+	+	+	+	-
D	-	-	-	-	-	-	-	+	+	+	+	-
E	+	+	+	+	+	+	+	+	+	+	+	-
F	+	+	+	+	+	+	+	+	+	+	+	-
G	+	+	+	+	+	+	+	+	+	+	+	-
H	+	+	+	+	+	+	+	+	+	+	+	-

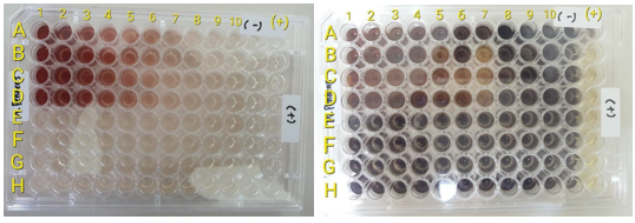
(c)

Figure 3. (a) Test the antibacterial activity of Sarang Semut plants (*Myrmecodia tuberosa* Jack) against *Porphyromonas gingivalis* by microdilution method before incubation, **(b)** 24 hours after incubation **(c)** Interpretation of anti-bacterial activity test results.

There were 7 concentrations where there was no visible bacterial growth (-) from 4 repetitions after being given MTT staining, namely at concentrations of 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.81 mg/mL, 3.90 mg/mL, and 1.95 mg/mL, while at 13 other concentrations there was visible growth of (+) bacteria, namely at concentrations of 0.97 mg/mL, 0.48 mg/mL, 0.24 mg/mL, 0.12 mg/mL, 0.06 mg/mL, 0.03 mg/mL, 0.015 mg/mL, 0.007 mg/mL, 0.003 mg/mL, 0.0019 mg/mL, 0.0009 mg/mL, 0.0004 mg/mL and 0.0002 mg/mL.

In Figure 4 below it can be seen that the ethanol extract of the Sarang Semut (*Myrmecodia pendans*) on a microplate with the same concentration as the ethanol extract of the

Sarang Semut (*Myrmecodia tuberosa* Jack), namely 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL , 15.625 mg/mL, 7.81 mg/mL, 3.90 mg/mL, 1.95 mg/mL, 0.97 mg/mL, 0.48 mg/mL, 0.24 mg/mL, 0, 12 mg/mL, 0.06 mg/mL, 0.03 mg/mL, 0.015 mg/mL, 0.007 mg/mL, 0.003 mg/mL, 0.0019 mg/mL, 0.0009 mg/mL, 0, 0004 mg/mL, 0.0002 mg/mL. The control group that will be used is Chlorhexidine gluconate 0.2% (positive control) and 10% DMSO (negative control).



(a) (b)

Test Preparations	Concentration (mg/mL)											
	1	2	3	4	5	6	7	8	9	10	K(-)	K(+)
A	-	-	-	-	-	-	-	+	+	+	+	-
B	-	-	-	-	-	-	-	+	+	+	+	-
C	-	-	-	-	-	-	-	+	+	+	+	-
D	-	-	-	-	-	-	-	+	+	+	+	-
E	+	+	+	+	+	+	+	+	+	+	+	-
F	+	+	+	+	+	+	+	+	+	+	+	-
G	+	+	+	+	+	+	+	+	+	+	+	-
H	+	+	+	+	+	+	+	+	+	+	+	-

(c)

Figure 4. (a) Test the antibacterial activity of Sarang Semut plants (*Myrmecodia Pendens*) against *Porphyromonas gingivalis* by microdilution method before incubation, **(b)** 24 hours after incubation **(c)** Interpretation of anti-bacterial activity test results.

After incubation for 24 hours, the test results showed that the ethanol extract of Sarang Semut plant (*Myrmecodia pendans*) had antibacterial activity against *Porphyromonas gingivalis* bacteria at several concentrations, namely 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.81 mg/mL, 3.90 mg/mL and 1.95 mg/mL. At the other 13 concentrations, there was a change in color which meant that *Porphyromonas gingivalis* had grown at the other 13 concentrations. The negative control (DMSO 10%) showed a change in color and the positive control (Chlorhexidine gluconate 0.2%) did not show any color change indicating no bacterial growth (Figure 4).

Table 1 below shows that there were 13 concentrations that experienced (+) bacterial growth at concentrations of 0.97 mg/mL, 0.48 mg/mL, 0.24 mg/mL, 0.12 mg/mL, 0. 06 mg/mL, 0.03 mg/mL, 0.015 mg/mL, 0.007 mg/mL, 0.003 mg/mL, 0.0019 mg/mL, 0.0009 mg/mL, 0.0004 mg/mL, and 0 .0002 mg/mL. and had 7 concentrations where bacterial growth did not occur (-) after being given MTT staining, namely at concentrations of 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.81 mg/mL, 3.90 mg/mL, and 1.95 mg/mL.

The results of the analysis of the statistical test will be shown in the next table, Fisher's statistical test was carried out to determine the relationship between Sarang Semut *Myrmecodia tuberosa* Jack and *Myrmecodia pendens* with bacterial growth *Porphyromonas gingivalis* and the most effective concentration as an anti-bacterial.

		Test results Activity Bacteria				p value
		Does not grow bacteria		Growing bacteria		
		n	%	n	%	
Myrmecodia tuberosa Jack	(125 mg/mL-0.24 mg/mL)	7	35%	3	15%	
	(0.12 mg/mL-0.0002 mg.mL)	0	0	10	50%	0.002
Total		7	35%	13	65%	
Myrmecodia pendens	(125 mg/mL-0.24 mg/mL)	7	35%	3	15%	
	(0.12 mg/mL-0.0002 mg.mL)	0	0	10	50%	0.002
Total		7	35%	13	65%	

Table 1. Fisher hypothesis test results.

Test results on table 1 above shows statistically and clinically *Myrmecodia tuberosa* Jack and *Myrmecodia pendens* associated with the growth of *Porphyromonas gingivalis* bacteria (p=0.002), when viewed from the average, the most effective concentration as an anti-bacterial was at concentration (125 mg/mL-0.24 mg/mL).

		Myrmecodia pendens				Total	p value
		Does not grow bacteria		Growing bacteria			
		n	%	n	%		
Myrmecodia tuberosa Jack	Does not grow bacteria	7	35	0	0	7(35%)	1,000
	Growing bacteria	0	0	13	65	13(65%)	
		7	35	13	65	20(100)	

Table 2. MC Nemar's statistical analysis above show statistically and clinically.

The results of MC Nemar's statistical analysis above show statistically and clinically there was no significant difference between *Myrmecodia tuberosa* Jack and *Myrmecodia pendans* on the growth of *Porphyromonas gingivalis* (p=1,000). (Table 2).

		Negative control			
		Does not grow bacteria	Growing bacteria	Total	p value
		n	n		
Myrmecodia tuberosa Jack	Does not grow bacteria	0	7	7	0,016
	Growing bacteria	0	13	13	
	Total	0	20	20	
		Positive control			
Myrmecodia tuberosa Jack	Does not grow bacteria	7	0	7	0,000
	Growing bacteria	13	0	13	
	Total	20	0	20	
		Positive control			
Negative Control	Does not grow bacteria	0	0	0	0,000
	Growing bacteria	20	0	20	
	Total	20	0	20	

Table 3. Mc Nemar's analysis which is a post hoc analysis from Cochran (*Myrmecodia tuberosa* Jack and *Porphyromonas gingivalis* bacteria).

Table 3 above shows that the *Myrmecodia tuberosa* Jack extract has a significant difference with the negative control (p=0.016) where the *Myrmecodia tuberosa* Jack extract has much better antibacterial effectiveness than the negative control (DMSO 10%). *Myrmecodia tuberosa* Jack extract had a significant difference with the positive control (Chlorhexidine gluconate 0.2%) (p=0.000) and there was also a significant difference between the positive control and negative control (p=0.000).

		Negative control			
		Does not grow bacteria	Growing bacteria	Total	p value
		n	n		
Myrmecodia pendens	Does not grow bacteria	0	7	7	0,016
	Growing bacteria	0	13	13	
	Total	0	20	20	
		Positive control			
Myrmecodia pendens	Does not grow bacteria	7	0	7	0,000
	Growing bacteria	13	0	13	
	Total	20	0	20	
		Positive control			
Negative control	Does not grow bacteria	0	0	0	0,000
	Growing bacteria	20	0	20	
	Total	20	0	20	

Table 4. Mc Nemar's analysis is a post hoc analysis from Cochran (*Myrmecodia pendens* and *Porphyromonas gingivalis* bacteria).

Discussion

The results of the MIC (Minimal Inhibitory Concentration) observation of the Sarang Semut plants extracts of *Myrmecodia pendens* and *Myrmecodia tuberosa* Jack types show that the smallest concentration of antimicrobial agents needed to inhibit microbial growth is at a concentration of 1.95 mg / mL[17]. Liquid microdilution is a suitable method used for screening antimicrobial activity because it is a sensitive method with a relatively short testing time[18]. The wells in the test results are clear on the microplate that has been given Sarang Semut plant extract indicating the antibacterial activity possessed by Sarang Semut plant extract against bacteria that cause periodontitis at several concentrations. When viewed from the average, the most effective concentration as antibacterial in both types of plants is at a concentration of 125 mg/mL-0.24 mg/mL. The greater the concentration, the greater the active substance components or secondary metabolites contained therein so that the inhibition produced is also different for each concentration[19].

The antibacterial activity of the two Sarang Semut plants, *Myrmecodia pendens* and *Myrmecodia tuberosa* Jack, does not have a significant difference, presumably because both still have the phytochemical content of the *Myrmecodia* genus such as terpenoids and phenolics. Another study mentioned that both types of Sarang Semut plants increased lymphocyte proliferation and macrophage phagocytosis activity even though the phytochemical analysis of *M. tuberosa* indicated the absence of alkaloids, polyhydroxy flavonoids, and components with carbonyl groups, but there were positive results on the content of terpenoids and phenolics[20]. *Myrmecodia pendens* and *Myrmecodia tuberosa* Jack belong to the genus *Myrmecodia* which explains the Sarang Semut plant has potential antimicrobial activity to be utilized in medicine[21].

The *Rubiaceae* family contains many phenolic components such as flavonoids, tannins, saponins, phenolics, alkaloids, triterpenoids, and glycosides[22]. The antibacterial activity of Sarang Semut plants *Myrmecodia pendens* and *Myrmecodia tuberosa* Jack against *Porphyromonas gingivalis* bacteria can be explained by the phenolic content of Sarang Semut plants[17]. Flavonoids have the ability as antibiotics directly by disrupting the function of microorganisms or viruses, and as antioxidants against free radicals. Flavonoids not only suppress the growth of bacterial plaque, but have the potential to support successful periodontal disease treatment because they can suppress the body's immunity, accelerate the healing of injured tissues such as bleeding gums, postoperative wounds, or the healing process after periodontal treatment[23]. Flavonoids will inhibit the synthesis of microbial nucleic acids,

damage the microbial cytoplasmic membrane causing cell perforation, and sabotage metabolic processes. Plant phenolics also mostly have antibiofilm effects that interfere with quorum sensing, affecting bacterial growth. Terpenoids can damage the planktonic cells of biofilms and disrupt bacterial cell integrity[22].

Previous studies explained that flavonoids can inhibit the growth of *Streptococcus mutans* bacteria by increasing cell osmotics or inhibiting the Glucosyltransferase enzyme activity of bacteria so that the bacteria are lysed and the number of bacteria decreases[23]. *Myrmecodia tuberosa* ethanol extract has active components that have the potential to be developed as antimicrobials against *Staphylococcus aureus*, as well as *Enterococcus faecalis* which could potentially be used for alternative endodontic treatment irrigation[17, 23]. Another study on M. pendans extract was found to have the effect of inhibiting biofilms of *S. sanguinis* and *T. denticola* both as mono and multispecies. There is antibacterial in the ethanol extract of *Myrmecodia pendens* against the growth of *Fusobacterium nucleatum* in concentrations of 20%, 40%, 60%, and 80%[24]. Research on the genus *Myrmecodia* Sarang Semut plant has conducted many antimicrobial activity studies and found that the Sarang Semut plant has a variety of compounds that are very useful in the health sector, namely it can inhibit gram-positive and negative types of bacteria.

Conclusions

Myrmecodia tuberosa Jack and Myrmecodia Pendens have the same antibacterial effectiveness against Porphyromonas gingivalis bacteria, namely at concentrations of 1.95 mg/mL -125 mg/mL. There was no significant difference in antibacterial effectiveness between the two types of Sarang Semut plants.

Declaration of Interest

The authors report no conflict of interest.

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