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RESEARCH ARTICLE

Formulation and Antibacterial Potential of Sarang Semut (*Myrmecodia pendans*) against Oral Pathogenic Bacteria: An *In Vitro* Study

Ameta Primasari^{1,*}, Eti Apriyanti², Nurul Ambardhani², Mieke Hemiawati Satari³, Yetty Herdiyati⁴ and Dikdik Kurnia²

¹Department of Oral Biology, Faculty Dentistry, Universitas Sumatera Utara, Medan, Indonesia

²Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang, Indonesia

³Department of Oral Biology, Faculty of Dentistry, Universitas Padjadjaran, Sumedang, Indonesia

⁴Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia

Abstract:

Background:

Dental diseases are generally caused by oral bacteria such as *Enterococcus faecalis*, *Streptococcus mutans*, and *Streptococcus sanguinis*. These bacteria have resistance to synthetic drugs; thus, it is required to discover new antibacterial agents. Sarang Semut (*Myrmecodia pendans*) has been empirically used as a medicinal plant to treat various conditions, including those caused by pathogenic bacteria.

Objective:

The present study was aimed to investigate the antibacterial activity of Sarang Semut extracts against *E. faecalis*, *S. mutans*, and *S. sanguinis*.

Materials and Methods:

Sarang Semut was extracted with several solvents to yield *n*-hexane, ethyl acetate, methanol, and water extracts. Each extract and combination were adjusted for assay with chlorhexidine, fosfomycin, and quercetin and used as positive controls.

Results:

The *n*-hexane extract showed activity with inhibition zone values of 7.15 and 10.45 ppm against *E. faecalis* and *S. mutans* at 1%, respectively. All combination extracts could inhibit the growth of *E. faecalis* and *S. sanguinis*. The synergistic effects resulting from the combination of extract-fosfomycin were also presented in this evaluation, with the strongest shown by water-fosfomycin against *S. mutans*, with inhibition zones of 28.5 mm at 1%.

Conclusion:

Sarang Semut extracts demonstrated antibacterial activity against oral pathogenic bacteria. These results offer alternative natural sources for the new antibacterial drug candidate.

Keywords: Sarang Semut, *Myrmecodia pendans*, Antibacterial activity, *E. faecalis*, *S. mutans*, *S. sanguinis*.

Article History

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1. INTRODUCTION

Oral health problems continue to be a prevalent disease of humankind, affecting all age groups [1]. Tooth decay and periodontitis are types of dental diseases caused by oral bacteria. Tooth decay leads to the formation of cavities, as known as dental caries, resulting from the growth of microbial

biofilms that produce acid, infecting the enamel and dentine [2]. Periodontal disease is defined as an endogenous microbial disease that defects the tooth structure and periodontium [3]. The activity of oral microorganisms may lead to the destruction of the periodontal ligament, which is characterized by loss of the ligament, and ruin surrounding alveolar bone [4].

Caries and periodontitis are associated with certain bacteria, including *Streptococcus sanguinis*, *Streptococcus mutans*, and *Enterococcus faecalis*, which together cause dental infection [5]. Two of these bacteria, *S. mutans* and *S.*

* Address correspondence to this author at the Department of Oral Biology, Faculty Dentistry, Universitas Sumatera Utara, Medan, Indonesia;
E-mail: ameta.primasari@usu.ac.id

sanguinis, are responsible for the formation of dental plaque. This cariogenic bacterium breaks down sugars and produces lactic acids, resulting in tooth demineralization and initiating caries lesions on the teeth [6]. *E. faecalis* is one of the oral bacteria causing root canal infection. This pathogen is not only found in the root canal but can also live in both root canals and saliva [7]. *E. faecalis* is able to survive in unfavorable environments, as well as in low pH levels, and then establish a biofilm [8]. This enables the pathogen to break through dentinal tubules [9, 10].

Due to increasing drug tolerance as a result of bacterial resistance, researchers are still exploring new antibacterial agents to combat oral pathogens. Natural herbal remedies have been known for folk treatment of all kinds of oral infections and diseases [11]. Because of fewer side effects, researchers have been more concerned with medicinal plants, which were also found to have synergistic effects [12]. Bioactive secondary metabolites from natural sources are agents that are promising new candidates for treating oral diseases caused by oral pathogenic bacteria [13 - 15]. One potential and prospective herbal medicine that can be used for treatment is Sarang Semut.

Sarang Semut (*Myrmecodia pendans*) is an epiphytic plant belonging to the family Rubiaceae, comprising five genera. Local people in West Papua, Indonesia, consumed this plant by boiling the dried part into the water and using it as an herb [16]. Sarang Semut is believed to relieve various systemic diseases such as leukemia, heart diseases, tuberculosis, kidney and prostate diseases, various allergies, migraine, rheumatism, hemorrhoid, and infectious diseases [17]. Phytochemical studies reveal that Sarang Semut contains active compounds, including polyphenols, flavonoids, tannins, and glycosides [18, 19]. Sarang Semut extract has been reported to inhibit the growth of *P. gingivalis* effectively, which are the gram-negative oral bacteria that cause biofilm formation [20]. It also exhibited antioxidant activities [16, 21], with water fraction EC₅₀ value of 30.66 and ethanol extract value of 3.6830 µg/mL [22]. The water extract also showed cytotoxic activity against both HeLa human cervix cancer and MCM-B2 canine mammary tumor cell lines, with IC₅₀ values of 27.61 and 54.57 ppm, respectively [23]. The *n*-hexane fraction was observed to induce cell death actively and inhibit cell proliferation of HCT-116 and Caco-2 human colon cancer cell lines at 33 and 24 ppm, respectively [24].

Therefore, Sarang Semut could be an alternative bioactive source plant. Further study regarding the antibacterial activities of this plant against other oral bacteria is still necessary. As part of ongoing research for antibacterial drugs from natural resources, the present study focused on investigating the overall potency and antibacterial effects of epiphytic plant Sarang Semut against oral pathogenic bacteria *E. faecalis*, *S. mutans*, and *S. sanguinis*.

2. MATERIALS AND METHODS

2.1. Materials

The air-dried Sarang Semut (*M. pendans*) was collected in

June 2018 from Papua, Indonesia. The extracts were prepared by an extraction method with several organic solvents based on polarities, such as methanol, *n*-hexane, and ethyl acetate.

2.2. Instruments

The instruments used were as follows: Laminar airflow, incubator (Memmert, IN55), anaerobic jar (Oxoid, AG0025A), autoclave (HVE-50 Hirayama), microplate reader (Biochrom EZ read 400, 80-4001-40), micropipette (Eppendorf, 3120000062 and 3120000054), and colony counter (Schuett-Biotec, 3081502).

2.3. Preparation of Sarang Semut Extracts

The dried part of Sarang Semut was extracted with methanol (1:3 m/v) for 3 × 24 hours, filtered, and then evaporated *in vacuo* at 40°C, yielding crude extracts. The methanol extract was subsequently partitioned between *n*-hexane-water and ethyl acetate-water, resulting in *n*-hexane, ethyl acetate, and water extracts, respectively.

2.4. Preparation of the Combination Extracts and Reference Compounds

Each extract and fraction of Sarang Semut was carefully prepared, and the concentrations were adjusted according to inhibition zone data values [25, 26].

2.5. Preliminary Phytochemical Screening

Screening for alkaloids, flavonoids, phenolics, saponins, steroids, and triterpenoids secondary metabolites was carried out for all extracts, including methanol, *n*-hexane, ethyl acetate, and water extracts, following published standard procedures [27, 28].

2.6. Microorganism Assay

The bacteria of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10566 were used for antibacterial test on Muller Hinton broth and Muller Hinton agar as a medium, chlorhexidine (purchased from Merck Co. Ltd. and Sigma-Aldrich) as a positive control, Brain Heart Infusion broth (Oxoid, CM1135), Muller Hinton agar (Oxoid, CM0337), paper disc 6 mm (Sigma-Aldrich, Z741310), aqua dest (Ikapharmindo Putramas), microplate 96 well (Iwaki, 3820 024), filter tips (Biologix, code 22-0019, 22-0200, and 22-1000), and parafilm (Sigma-Aldrich P7688-1EA).

2.7. Antibacterial Activity Assay

Antibacterial effects of Sarang Semut extracts against *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10566 were evaluated using Kirby-Bauer disk diffusion. The determination of the sensitivity or resistance of *E. faecalis*, *S. mutans*, and *S. sanguinis* to compounds was based on CLSI protocols [29, 30]. All samples were diluted with methanol except chlorhexidine (control) with water. The concentrations used for all samples and control were 40, 20, and 10%. Paper discs (6 mm) were impregnated with 20 µL of each sample and then placed on the surface of the agar. Tests were performed in duplicate.

Table 1. Phytochemical screening data of Sarang Semut (*M. pendans*) extracts.

Secondary Metabolites	Reagent	Extracts			
		MeOH	<i>n</i> -Hexane	EtOAc	H ₂ O
Phenolic	FeCl ₃ 5%	+	-	+	+
Tannin	FeCl ₃ 1%	+	-	+	+
Flavonoid	HCl (p.a) + Mg	+	-	+	+
	H ₂ SO ₄ 2N	+	-	+	+
	NaOH 10%	+	-	+	+
Triterpenoid	Lieberman-Burchard	+	+	+	+
Steroid	Lieberman-Burchard	+	+	+	-
Saponin	HCl 2N+ H ₂ O	+	-	+	-
Alkaloid	Dragendorff	+	-	+	-

Note: MeOH: methanol, EtOAc: ethyl acetate, H₂O: water, +: positive results according to the procedure, -: negative results not according to the procedure.

3. RESULTS

3.1. Phytochemical Screening of Sarang Semut (*M. pendans*) Extracts

Phytochemical analysis data, as shown in Table 1, revealed that the secondary metabolite constituents of phenolic, flavonoid, triterpenoid, and saponin compounds were found in all extracts. The *n*-hexane fraction exhibited all secondary metabolites of phenolic, flavonoid, alkaloid, steroid, triterpenoid, and saponin. The analysis data for secondary metabolites of Sarang Semut (*M. pendans*), as shown in Table 1, indicated that all fractions contained different constituents. This preliminary data supported flavonoids being an essential antimicrobial compound in a prior study [31].

3.2. Antibacterial Activity

3.2.1. Antibacterial Activity of the Extracts

In order to evaluate the antibacterial potential from the natural source plant, Sarang Semut (*M. pendans*), the extracts were tested against *E. faecalis*, *S. mutans*, and *S. sanguinis*. The sensitivity of Sarang Semut extract against oral bacteria

was observed from their sample inhibition zones on bacteria growth by the Kirby-Bauer method. The samples were analyzed at concentrations of 1% with 2% chlorhexidine as a positive control, methanol, and water as the negative control. As shown in Table 2, this study revealed that *n*-hexane extract inhibited *E. faecalis* and *S. mutans* growth with different inhibition zone values for each bacteria. The *n*-hexane extract showed the highest activity against *S. mutans* with inhibition zone values of 10.5 mm at a concentration of 1%. The ethyl acetate extract was active against *E. faecalis* with an inhibition zone value of 9.8 ppm, while methanol and water extract was found in no inhibition zone.

3.2.2. Antibacterial Activity of the Combination Extracts

In order to confirm the effects of active constituents in the single and combination extracts, mixtures of extracts were prepared. Their antibacterial property was re-evaluated against *E. faecalis* ATCC 29212 bacteria, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556, respectively.

According to Table 3, antibacterial activity was shown by all of the combined extracts that could inhibit the growth of two bacteria, *E. faecalis* and *S. sanguinis*, with different inhibition zone values, while *S. mutans* did not get the effects.

Table 2. Antibacterial activity of Sarang Semut extracts against oral pathogenic bacteria *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10566.

Extract	Inhibition Zones (mm) at Concentration of 1%		
	<i>E. faecalis</i>	<i>S. mutans</i>	<i>S. sanguinis</i>
Methanol	0	0	0
<i>n</i> -hexane	7.15	10.45	0
Ethyl acetate	9.45	0	0
Water	0	0	0
2% Chlorhexidine	14.2	17.65	10.6

Table 3. Antibacterial activity of the combined extracts of Sarang Semut (*M. pendans*) at a concentration of 1% against oral pathogenic bacteria *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10566.

No	Samples	Inhibition Zones (mm) at Concentration of 1%		
		<i>E. faecalis</i>	<i>S. mutans</i>	<i>S. sanguinis</i>
1	M+Hex	7.85	0	7.65
2	M+EtOAc	6.75	0	6.9
3	M+H ₂ O	6.8	0	6.95
4	<i>n</i> -Hex+EtOAc	6.8	0	7.3
5	<i>n</i> -Hex+H ₂ O	6.8	0	0
6	EtOAc+H ₂ O	7.6	0	7.15
7	Chx	12.95	26.5	15.75

Note: M: methanol, *n*-Hex: *n*-hexane, EtOAc: ethyl acetate, H₂O: water, Chx: chlorhexidine.

Table 4. Antibacterial activity of the combinational extracts of Sarang Semut (*M. pendans*) and reference compounds at a concentration of 1% against oral pathogenic bacteria *E. faecalis* ATCC 29212, *S. mutans* ATCC 25172, and *S. sanguinis* ATCC 10566.

Samples	Inhibition Zones (mm) at Concentration of 1%		
	<i>E. faecalis</i>	<i>S. mutans</i>	<i>S. sanguinis</i>
M+Chx	6.9	0	6.9
M+F	19.1	20.25	9.15
M+Q	0	0	7.4
Hex+Chx	0	0	6.9
Hex+F	6.85	24.95	10.45
Hex+Q	17.35	17.35	0
EtOAc+Chx	6.75	0	0
EtOAc+F	17.6	20.95	12.2
EtOAc+Q	6.9	16	7.6
H ₂ O+Chx	6.85	14.1	0
H ₂ O+F	20	28.5	9.35
H ₂ O+Q	6.8	0	7.55

Note: M: methanol, Hex: *n*-hexane, EtOAc: ethyl acetate, H₂O: water, Chx: chlorhexidine, F: fosfomycin, Q: quercetin.

3.2.3. Antibacterial Activity of the Combination Extracts and References Compounds

The activity effects of active constituents in the single and reference compounds were analyzed further. Chlorhexidine, fosfomycin, and quercetin were used in this assay, the mixtures extracts were prepared, and then their activities were evaluated against oral bacteria of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556, respectively.

The antibacterial activity of the extract combinations that were added to the reference compounds is shown in Table 4. All reference compounds give different effects depending on the type of bacteria. This data revealed that some different extract combinations inactively inhibited the growth of bacteria. There are five extract combinations of M+F, Hex+F, EtOAc+F, EtOAc+Q, and H₂O+F that were found to actively inhibit the growth of all bacteria, including *E. faecalis*, *S. mutans*, and *S. sanguinis* with different inhibition zone values. The antibacterial activity was represented by inhibition zone values, which were in the range of 6.8 to 28.5 mm.

4. DISCUSSION

The antibacterial drug that is commonly used in oral disease treatment is resistant, as the use of antibiotics has increased over this decade. Exploration and discovery of new antibacterial agents have been the focus for treatment and prevention of the growth of pathogenic oral bacteria. Dental caries is mainly caused by infection of certain bacteria, such as *E. faecalis*, *S. mutans*, and *S. sanguinis*. Chlorhexidine is mostly used in oral treatment and is considered the gold standard [32, 33]. However, it may cause discoloration of teeth and drug resistance [34, 35]. Hence, the new compounds should be more selective, effective, and efficient, with fewer negative side effects.

Currently, research focuses on developing antibacterial drugs or chemicals using natural compounds. The investigation of active compounds sourced from medicinal plants is motivated by their affordability and the belief that they are more effective in healing and minimizing side effects [36]. This study provides the preliminary antibacterial activity data of edible plants selected based on this plant consumed daily by humans [37].

M. pendans was locally named Sarang Semut, distributed, and was a native plant in Papua, Eastern Indonesia [38, 39]. This plant was used as herbal medicine by native people to heal various kinds of diseases, showing minimal side effects [23, 24, 40]. Sarang Semut is rich in phytochemicals, including phenolics, flavonoids, triterpenoids, steroids, saponins, and alkaloids, distributed into several extracts according to their chemical polarity, as described in Table 1. Pharmacological studies reveal that phytoconstituents of Sarang Semut have shown good antioxidant activities [16, 21, 41], anti-inflammatory [42], antitumor [19], anticancer [43 - 45], antimicrobial [41, 46], and antibacterial [47 - 51] properties. According to these facts, this study revealed a correlation between the chemical constituents contained in the extract with the pharmacological activity of this plant. Sarang Semut has potential and can be used as a promising plant source to obtain new antibacterial compounds.

The extracts of Sarang Semut have been evaluated for their susceptibility to inhibit oral bacteria growth of *E. faecalis*, *S. mutans*, and *S. sanguinis*. The inhibition zone was determined by the Kirby-Bauer method. Table 2 shows that each extract has a different sensitivity to variant bacteria, suggesting that their mechanisms of inhibiting bacterial growth are also different. Most extracts actively inhibit *E. faecalis* growth, which is assumed from the evaluating data. Two extracts, *n*-hexane and ethyl acetate, were active at a concentration of 1% with inhibition zone values of 7.15 and 9.45 mm, respectively.

Further data analysis showed that only *n*-hexane extract could inhibit two oral bacteria, *E. faecalis* and *S. mutans*, with inhibition zone values of 7.15 and 10.45 ppm, respectively. This value was lower than the standard, *i.e.*, 2% chlorhexidine against those bacteria with inhibition zone values of 14.2 and 17.65 mm, respectively. This data corresponds to a previous report, according to which each *n*-hexane and ethyl acetate extract was active against *E. faecalis* [50].

According to the phytochemical analysis, *n*-hexane extract containing phenolic, flavonoid, triterpenoid, steroid, saponin, and alkaloid could inhibit two oral bacteria, *E. faecalis* and *S. mutans*, and it is considered to have a synergistic effect resulting from the chemical compounds. However, further assay and specific study are necessary to support this assumption.

The inhibition zone value of single and combination extracts was determined against all bacteria. The evaluation used the same method: sample formulations were used to determine the synergistic effects of those extracts. Table 3 shows that most of the combined extracts were active as an antibacterial against *E. faecalis* and *S. sanguinis* with distinct inhibition zone values, while the combination of *n*-Hex+H₂O obtained no inhibition zone against *S. sanguinis*. It is suggested that the addition of *n*-hexane extract into the others might cause the synergistic effect on their activity against *S. sanguinis*. Each extract was inactive to inhibit this bacteria growth. In contrast, none of these combined extracts had antibacterial activity against *S. mutans*, suggesting that chemical constituents in each extract had antagonistic effects when combined and mixed. This finding is confirmed by a prior study that some extracts had synergistic and antagonistic

effects [52]. Preliminary data can be used as key information and a guided procedure to determine the most appropriate methods to obtain the pure active compounds from the plant extract.

As stated earlier, this research aims to obtain new antibacterial agents as drug candidates. The plant extracts were combined with the reference compounds for evaluating their effect against oral bacteria. Chlorhexidine, fosfomycin, and quercetin were used as a reference in this study. Chlorhexidine is a broad-spectrum antibacterial agent that causes disturbance of cell membranes. It is also used orally as antiseptic mouthwash to prevent bacterial and dental plaque accumulation [53, 54]. Fosfomycin is also an antibiotic and has been evaluated for its action against several bacteria by inhibiting the first step in cell wall synthesis [33]. Quercetin is one of the bioactive compounds found in plants, which is known for its pharmacological potential. These bioflavonoids exhibit good antibacterial effects against most strains of bacteria [55].

The antibacterial activity of extracts combined with reference compounds, as seen in Table 4, showed that different combinations generated increasing activity against oral bacteria. The highest inhibition zones were obtained in the combination of **M+F**, **H₂O+F**, and **EtOAc+F** against *E. faecalis*, *S. mutans*, and *S. sanguinis* with values of 19.1, 28.5, and 12.2 mm, respectively. It indicated that bioactive compounds in methanol, water, and ethyl acetate extract together with fosfomycin were merged, increasing their ability to inhibit bacteria growth and resulting in synergistic effects. Other important data revealed that the **M+F** and **Hex+F** had similarly high inhibition zones against *S. mutans* with 20.25 and 24.95 mm, respectively. Both extracts were less active against the other bacteria, indicating that this extract could inhibit *S. mutans* more than *E. faecalis* and *S. sanguinis*. Fosfomycin is one of the antibacterial agents known for its ability as an inhibitor of the MurA enzyme. All extracts combined with this antibiotic actively inhibited the formation of *E. faecalis* and *S. mutans* cell walls [56]. Despite the lack of comprehensive studies concerning principal mechanisms that contribute to the antibacterial activity of extracts, a previous report has suggested that each compound would attack different bacteria cell components [57].

According to this research data, Sarang Semut has potential activity as an antibacterial agent. Variation of activity values of single and combinational extracts together with reference drugs provided necessary biomarkers as antibacterial compounds based on the oral bacteria species. Determination of structure compound and activity as an antibacterial agent isolated from the active extract could be conducted by guiding certain phytochemical screenings and evaluation activity assays.

CONCLUSION

The epiphytic plant of Sarang Semut (*M. pendans*) exhibited antibacterial activity against oral pathogenic bacteria of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556 *in vitro*. This result could be an important database for further clinical studies to determine the

effectiveness and exact dosages in practical application. Toxicity studies should be conducted to determine the safety uses since this plant is consumed by humans. Isolation and structure characterization of the active compound as an antibacterial is required in future work. The finding of new antibacterial agents from the potential natural products may lead to obtaining antibacterial compounds with clinical efficacy against dental caries and other oral pathogenic bacteria.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the Repository of Universitas Padjadjaran at <http://repository.unpad.ac.id/frontdoor/index/index/docId/200578>, reference number 200578.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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