

Original Article

Inhibitory Effect of an Ethanol Extract of Sembung Leaves (*Blumea balsamifera*) on the Growth of *Helicobacter pylori*: In Vitro Approach

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ABSTRACT

Background: *Helicobacter pylori* infection is a global health problem with a high prevalence in developing countries and plays a role in the pathogenesis of various gastrointestinal disorders. Antibiotic resistance is a major challenge in the eradication of *H. pylori*, prompting the exploration of alternative antibacterial agents derived from traditional medicinal plants. **Objective:** To analyze the antibacterial activity of an ethanol extract of sembung leaves (*Blumea balsamifera*) against *Helicobacter pylori* growth in vitro. **Methods:** This experimental study used a posttest control group design with five concentrations of the ethanol extract of sembung leaves (10%, 20%, 40%, 60%, and 80%). The extraction was performed via the maceration method with 96% ethanol. Antibacterial activity was tested via the Kirby–Bauer disk diffusion method on *Mueller–Hinton agar* medium. The inhibition zone diameter data were analyzed via the Kruskal–Wallis and Mann–Whitney tests with a 95% confidence level. **Results:** None of the concentrations of the Sembung leaf ethanol extract produced an inhibition zone against *H. pylori*, with a diameter of 0 ± 0 mm in all the treatment groups. The positive control exhibited an inhibition zone of 23.15 ± 0.28 mm. The Kruskal–Wallis test revealed a significant difference ($p = 0.000$). However, the Mann–Whitney U test confirmed that there was no significant difference between the negative control and all extract concentrations ($p=1.000$). **Conclusion:** Ethanol extracts of *Blumea balsamifera* leaves at concentrations ranging from 10–80% did not exhibit antibacterial activity against *H. pylori* in vitro, as determined via the disk diffusion method.

KEYWORDS

Antibacterial, *Blumea balsamifera*, disk diffusion, *Helicobacter pylori*, maceration, antibiotic resistance

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INTRODUCTION

Helicobacter pylori infection affects more than 44% of the global population, with a higher prevalence in developing countries (93.6%) than in developed countries (15.5%) [1,2]. These spiral-shaped gram-negative bacteria colonize the gastric mucosa and trigger local inflammation that can lead to atrophic gastritis, intestinal metaplasia, and peptic ulcers [3]. Local studies have shown that the prevalence rates of *H. pylori* infection in Denpasar and Tabanan are 41.2% and 43%, respectively [4].

The standard management of *H. pylori* infection uses a combination of two to three antibiotics (amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin) with acid-suppressing agents [5,6]. However, antibiotic resistance is the main cause of eradication failure, with resistance rates of 22.2% for clarithromycin, 69.2% for metronidazole, and 1.2% for amoxicillin in the United States and Europe [7]. A recent study from China reported dual resistance to clarithromycin and metronidazole in 75.1% of isolates [8]. This multidrug resistance reduces the effectiveness of standard therapy and increases treatment costs, therapy duration, and the risk of complications [9,10].

Exploring natural antibacterial agents from medicinal plants is an alternative strategy to address the antibiotic resistance crisis [11,12]. Sembung leaves (*Blumea balsamifera*) from the Asteraceae family have been traditionally used in Southeast Asia to treat infections, diarrhea, and digestive disorders [13,14]. Phytochemical analysis has shown that sembung leaves contain flavonoids (quercetin 6.9 mg/g dry weight), alkaloids, tannins, saponins, terpenoids, and essential oils with antibacterial activity [15].

Previous studies have reported the antibacterial activity of Sembung leaves against *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) of 0.5 mg/mL [15]; *Pseudomonas aeruginosa*, with an inhibition zone of 7.8–10.5 mm [16]; and *methicillin-resistant Staphylococcus aureus* (MRSA) [17]. Proteomic studies have shown that *B. balsamifera* essential oil kills *S. aureus* through four simultaneous mechanisms: destruction of cell membrane permeability, inhibition of nucleic acid and protein synthesis, and disruption of amino acid metabolism [18,19]. However, data on the antibacterial activity of sembung leaves against *H. pylori* are currently unavailable.

Given the unique characteristics of *H. pylori* as a microaerophilic gram-negative bacterium with a complex lipopolysaccharide outer membrane, evaluating the antibacterial activity of sembung leaves against this pathogen is important. Therefore, this study aimed to analyze the antibacterial activity of sembung leaf ethanol extracts against the growth of *H. pylori* *in vitro*, providing scientific data on the potential of sembung leaves as alternative antibacterial agents against *H. pylori* antibiotic resistance.

MATERIALS AND METHODS

Study design and setting

This experimental study used a posttest-only control group design and was conducted at the Laboratory of the Faculty of Medicine and Health Sciences, Warmadewa University, from August to September 2025. Sembung leaves were sampled from Jalan Dewi Uma, Banjar Pemogan Kelod, Pemogan Village, South Denpasar District, Denpasar City, Bali Province.

Population and sample

The study population consisted of all sembung leaves (*B. balsamifera*) growing at the study site. Sembung leaf samples were selected on the basis of the following inclusion criteria: young to mature leaves, healthy without physical damage, and not exposed to pesticides. The exclusion criteria were leaves that were too young, wilted, or affected by pests or diseases. The test bacteria were *H. pylori* clinical isolates obtained from laboratory samples.

Research procedures

Preparation of the crude drug and extraction

Freshly eaten leaves were washed thoroughly with running water, wet-sorted, drained, cut into small pieces, and weighed. Drying was carried out in an air-conditioned room without direct sunlight exposure to prevent the degradation of bioactive compounds [20]. After drying, dry sorting was performed, and the mixture was ground using a blender and sieved to obtain a homogeneous powder. Extraction was performed via the maceration method, following the procedures described by Ameliana et al. [21] and Romadhina et al. [22], with modifications. A total of 100 g of powdered simple was macerated with 96% ethanol until completely submerged, left for 72 h protected from light, and stirred via a *magnetic stirrer* every 24 h. The filtrate was filtered, and the residue was remacerated via the same solvent. All the filtrates were combined and evaporated via a *rotary evaporator* at 40–60°C until a thick extract was obtained. The total mass of the extract was determined via an analytical balance.

Preparation of various concentrations

The thick extract was diluted with 96% ethanol to produce five concentrations (10%, 20%, 40%, 60%, and 80% w/v) in 5 mL volumetric flasks according to the procedure described by Sanda et al. [23]. The extract mass calculation was adjusted via the weight-per-volume percentage formula to obtain a homogeneous solution.

Preparation of media and test bacteria

Mueller–Hinton agar (MHA) medium was prepared by dissolving MHA powder in distilled water and heating it while stirring until homogeneous. The pH of the medium was measured via a *pH stick* to ensure a pH of 7.3 ± 0 . *H. pylori* colonies from pure cultures were revived on *nutrient agar* medium and incubated at 37°C for 24 hours. Colonies aged 1×24 h were suspended in sterile 0.9% physiological NaCl until they reached a turbidity equivalent to the 0.5 McFarland standard (1.5×10^8 CFU/mL) measured via a McFarland densitometer [11,24].

Antibacterial activity test

The test was performed via the Kirby–Bauer disk diffusion method, following the procedures described by Putri, Hafida, and Megawati [23] and American Society for Microbiology [25]. Sterile 6 mm diameter disks were saturated with 20 μ L of each concentration of the extract. The negative control used 96% ethanol, and the positive control used a 30 μ g chloramphenicol antibiotic disc. A standard 0.5 McFarland suspension of *H. pylori* was inoculated onto the surface of the MHA medium via sterile cotton swabs until it was evenly distributed. The medium was left for 15 min to allow the suspension to soak. The saturated discs were placed on the surface of the medium at a distance of ± 15 mm between the discs. The Petri dishes were incubated at 37°C for 24 h [26,27].

Measurement and data analysis

The diameter of the inhibition zone was measured via a caliper with an accuracy of 0.1 mm. The inhibition zone diameter data were analyzed via the Kolmogorov–Smirnov normality test. Because the data were not normally distributed, the analysis was continued with the Kruskal–Wallis nonparametric test to determine the differences between groups with a 95% confidence level ($\alpha=0.05$). The Mann–Whitney U test was used to identify pairs of groups that were significantly different. Data processing was performed via SPSS software (version 25) [28,29].

RESULTS

Antibacterial activity

Testing the antibacterial activity of ethanol extracts of Sembung leaves at five concentrations (10%, 20%, 40%, 60%, and 80%) against *H. pylori* yielded negative results. Table 1 presents the measured inhibition zone diameters for each treatment group. The negative control (96% ethanol) did not produce an inhibition zone in any of the replicates, with an average value of 0 ± 0 mm, confirming that the solvent had no antibacterial activity against *H. pylori*. The positive control

(30 µg chloramphenicol) had a consistent inhibition zone in all the replicates, with diameters ranging from 22.8–23.5 mm and averages of 23.5 mm and 23.35 mm. The average diameter of the inhibition zone in the positive control was 23.15 ± 0.28 . Inhibition zones ≥ 21 mm were classified as very strong, confirming that the test bacteria were still sensitive to standard antibiotics and that the validity of the testing system was fulfilled. None of the concentrations of the Sembung leaf ethanol extract (10%, 20%, 40%, 60%, or 80%) produced inhibition zones in any of the replicates, with an average value of 0 ± 0 mm. The absence of an inhibition zone indicates that the ethanol extract of Sembung leaves at these concentrations could not inhibit the growth of *H. pylori in vitro*, as determined via the disk diffusion method.

Table 1. Inhibition zone diameter of the Sembung leaf ethanol extract against *H. pylori*

Treatment	Inhibition zone diameter (mm)					Mean $\pm$ SD
	I	II	III	IV	V	
K- (96% ethanol)	0	0	0	0	0	± 0 e 0
K+ (Chloramphenicol 30 µg)	22.8	23	23.1	23.5	23.35	23.15 ± 0.28
10% extract	0	0	0	0	0	$\pm$
20% extract	0	0	0	0	0	$\pm$
40% extract	0	0	0	0	0	$\pm$
60% extract	0	0	0	0	0	$\pm$
80% extract	0	0	0	0	0	$\pm$

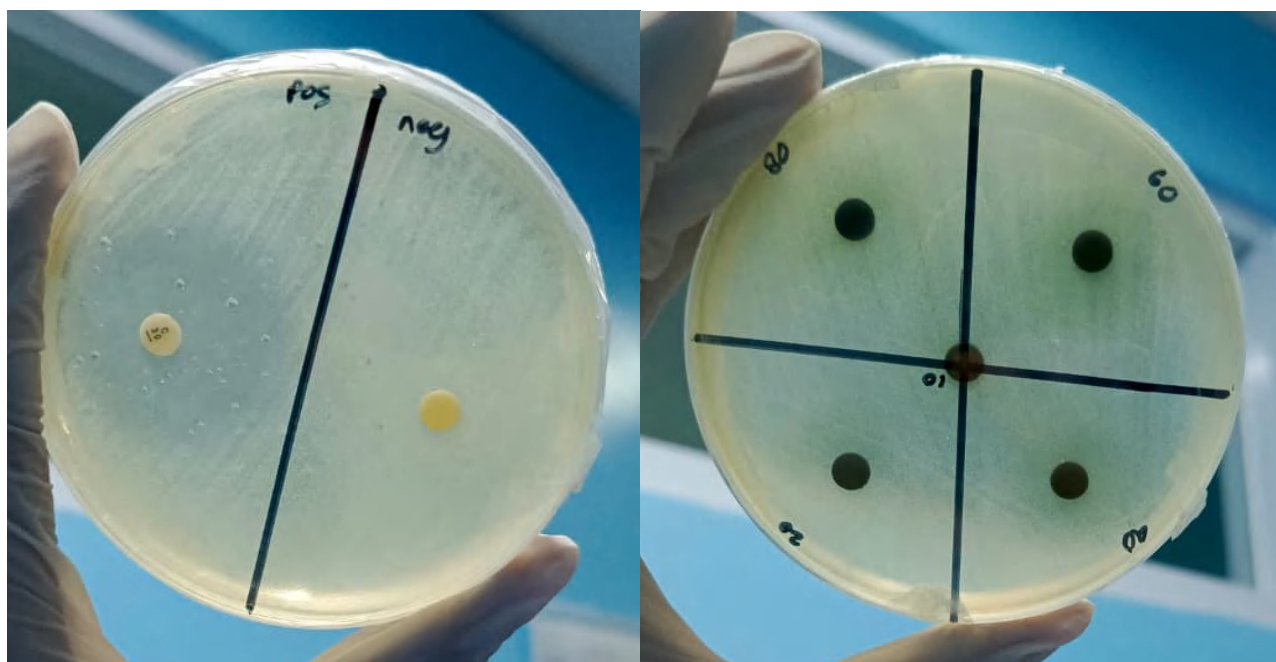


Figure 1. Inhibitory effect of the ethanol extract of Sembung leaves on the growth of *H. pylori*. Description: (a) Inhibitory effects of the positive and negative controls on the growth of *H. pylori* bacteria and (b) examination of various variations in the Sembung leaf extract

Statistical analysis of differences in antibacterial activity between groups

The Kolmogorov-Smirnov test for normality of the inhibition zone diameter data revealed that all treatment groups and the negative control had *p* values < 0.05 , indicating that the data were not normally distributed. The positive control had a *p* value > 0.05 , indicating a normal distribution. As most of the data were not normally distributed, the analysis was continued via the nonparametric kruskal-wallis test (Table 2).

The kruskal-wallis test was performed to determine the differences between the treatment groups, with a confidence level of 95% ($\alpha = 0.05$). The test results showed a significant value of $p = 0.000$ ($p < 0.05$), indicating a significant difference in the diameter of the inhibition zone among the treatment groups (Table 3).

Table 2. Results of the kolmogorov-smirnov normality test

Treatment	Mean± SD	P Value
K- (96% Ethanol)	0± 0	0.000***
K+ (Chloramphenicol 30 µg)	23.15± 0.28	0.200**
10% extract	±0 0	0.000***
20% extract	0± 0	0.000***
40% extract	0± 0	0.000***
60% extract	0± 0	0.000***
80% extract	0± 0	0.000***

Remarks: * There is a significant difference, significance level 95% (P<0.05)

Table 3. Results of the kruskal-wallis test

Treatment	Mean± SD	P Value
K- (96% Ethanol)	0± 0	
K+ (Chloramphenicol 30 µg)	23.15± 0.28	
10% extract	0± 0	
20% extract	0± 0	0.000***
40% extract	0± 0	
60% extract	0± 0	
80% extract	0± 0	

The findings indicate that there are significant differences, mainly due to the contrast between the positive control and the other groups. To identify the group pairs that caused significant differences, a Mann-Whitney test was performed (Table 4). The Mann-Whitney test revealed a significant difference between the positive control, negative control, and all concentrations of the extract (p=0.005). However, there were no significant differences between the negative control and all concentrations of the Sembung leaf extract (p=1.000) or between the extract concentrations (p=1.000). These results confirmed that all extract concentrations had an effect equivalent to that of the negative control; that is, they were unable to inhibit the growth of *H. pylori*.

Table 4. Mann-Whitney test results

Treatment	P Value						
	K-	K+	10%	20%	40%	60%	80%
K- (96% Ethanol)		*0.005	1,000	1,000	1,000	1,000	1,000
K+ (Chloramphenicol 30 µg)	*0.005		*0.005	*0.005	*0.005	*0.005	*0.005
10% extract	1,000	*0.005		1,000	1,000	1,000	1,000
20% extract	1,000	*0.005	1,000		1,000	1,000	1,000
40% extract	1,000	*0.005	1,000	1,000		1,000	1,000
60% extract	1,000	*0.005	1,000	1,000	1,000		1,000
80% extract	1,000	*0.005	1,000	1,000	1,000	1,000	

Remarks: * There is a significant difference, significance level 95% (P<0.05).

DISCUSSIONS

This study aimed to analyze the antibacterial activity of an ethanol extract of Sembung leaves against *H. pylori* *in vitro*. The results showed that the ethanol extract of Sembung leaves at concentrations of 10–80% did not produce an inhibition zone against *H. pylori*, as indicated by an inhibition zone diameter of 0 ± 0 mm in all the treatment groups. This finding indicates that within the tested concentration range, the extract of sembung leaves does not exhibit antibacterial activity against *H. pylori* according to the disk diffusion method.

The negative control (96% ethanol) did not show an inhibition zone (0 ± 0 mm), indicating that the solvent did not contribute to antibacterial activity [30]. Ethanol (96%) was selected as the extraction solvent because of its ability to dissolve polar to semipolar secondary metabolites, such as flavonoids, phenolics, tannins, and alkaloids, which possess antibacterial properties [31,32]. Ethanol has high penetration power into plant cell walls, thereby increasing the extraction efficiency compared with that of lower-concentration solvents [33]. Jirakitticharoen et al. [15] reported that, compared with extraction with other solvents, extraction of *B. balsamifera* leaves with 50% ethanol produced 1.2 times greater total phenolic content and 1.1 times greater total flavonoid content.

The positive control (30 µg chloramphenicol) produced a consistent inhibition zone with an average diameter of 23.15 ± 0.28 mm, which was classified as very strong according to AlSheikh et al. [11]. These results prove that *H. pylori* is sensitive to standard antibiotics and optimal testing conditions [30]. Chloramphenicol inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit, producing a bacteriostatic or bactericidal effect depending on the concentration [1,34]. The sensitivity of *H. pylori* to chloramphenicol confirmed the validity of the testing system and demonstrated that the negative results of the sembung leaf extract were not due to a failure of the testing system but rather reflected the characteristics of the biological interaction between the extract and the test bacteria.

The absence of an inhibition zone at all concentrations of the sembung leaf ethanol extract can be explained by several biological and methodological mechanisms. First, *H. pylori* is a microaerophilic gram-negative bacterium with a complex outer membrane structure containing lipopolysaccharides that functions as a barrier to the diffusion of antibacterial compounds [35]. The outer membrane of gram-negative bacteria consists of lipopolysaccharides (LPS), phospholipids, and porin proteins that form a selective permeability barrier to hydrophilic and lipophilic molecules [36,37].

The phytochemical compounds in sembung leaves, particularly flavonoids and terpenoids, are lipophilic and have relatively large molecular weights [38,39]. Quercetin, the main flavonoid in *B. balsamifera* (6.9 mg/g dry weight), has a molecular weight of 302.24 g/mol, indicating moderate lipophilicity [15,40]. Lipophilic compounds can easily diffuse through the phospholipid membranes of gram-positive bacteria but are blocked by the outer LPS membrane of gram-negative bacteria [40,41].

Sakee et al. [42] reported that a *B. balsamifera* methanol extract produced an inhibition zone against three gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*) with a diameter of 7.8–10.5 mm. Jirakitticharoen et al. [15] reported that a 50% ethanol extract of immature *B. balsamifera* leaves had an MIC of 0.5 mg/mL against *S. aureus*. Yang et al. [18] used proteomic analysis to show that *B. balsamifera* essential oil kills *S. aureus* by destroying cell membrane integrity, inhibiting nucleic acid and protein synthesis, and disrupting amino acid metabolism. Wongarmart et al. [43] reported that *B. balsamifera* extract exhibits antibacterial activity against multidrug-resistant bacteria, including *Klebsiella pneumoniae* and *Acinetobacter baumannii*.

However, the effectiveness of *B. balsamifera* extract is highly dependent on the target bacterial species tested. Binh et al. [44] reported that essential oils from sembung leaves were effective against gram-positive bacteria but showed much lower activity against certain gram-negative bacteria. Second, the disk diffusion method has limitations in detecting the antibacterial activity of natural compounds, especially nonpolar or volatile compounds such as essential oils [30]. Bubonja-Šonje et al. [35] reported that most polyphenols are lipophilic compounds with poor diffusion through water-based agar and that the standard disc diffusion method is optimized for hydrophilic antibiotics.

Other studies testing natural materials against *H. pylori* have reported similar results. Pang et al. [41] reported that various plant extracts showed low or undetectable activity through the disk diffusion method but showed inhibitory activity via the liquid dilution method or MIC test. Pertiwi et al. [45] evaluated the antibacterial activity of mangrove leaf extract (*Rhizophora mucronata*) against *H. pylori* and reported no inhibition zone formation via a disk diffusion assay.

Hasan et al. [46] tested the effects of botto-botto leaf extract (*Chromolaena odorata*) against *H. pylori* with similar results.

Statistical analysis via the kruskal-wallis test revealed a significant difference between the treatment groups ($p = 0.000$). However, the Mann-Whitney test confirmed that this difference was due mainly to the positive control activity, which was significantly different from that of the other groups ($p=0.005$). There were no significant differences between the negative control and all extract concentrations ($p=1.000$) or between the extract concentrations ($p=1.000$). These results indicate that an increase in extract concentration was not accompanied by an increase in antibacterial effect; thus, there was no dose-dependent response to the test bacteria.

Alternative strategies for evaluating the antibacterial potential of sembung leaf extracts against *H. pylori* include (1) using the liquid dilution or broth microdilution method for more accurate MIC and MBC determination; (2) fractionating the extract to isolate specific bioactive compounds; (3) modifying the extract formulation by adding outer membrane permeabilizers such as EDTA; (4) testing the synergy between the sembung leaf extract and standard antibiotics; and (5) testing antibiofilm activity, given *H. pylori*'s ability to form biofilms as a defense mechanism against antimicrobials [30,47].

Clinical and public health implications

The results of this study have important implications for the development of natural antibacterial agents for *H. pylori* eradication. Although the ethanol extracts of Sembung leaves did not show antibacterial activity against *H. pylori* at the tested concentrations, these findings provide valuable information that antibacterial screening of natural materials requires a comprehensive methodological approach that is not limited to the disk diffusion method. Given the global rise in *H. pylori* antibiotic resistance, exploring alternative antibacterial agents from traditional medicinal plants is crucial. Clarithromycin resistance rates reached 22.2%, and metronidazole resistance rates reached 69.2% in the United States and Europe [48], with dual resistance in 75.1% of isolates in China [8], highlighting the urgency of developing new antibacterial agents with mechanisms of action different from those of conventional antibiotics. This study also provides information to healthcare practitioners and the public that the empirical use of Sembung leaves as a traditional therapy for stomach disorders cannot be assumed to be effective against *H. pylori* infection without adequate scientific evidence of its efficacy.

Limitations

This study had several limitations. First, the disk diffusion method has limitations in detecting the antibacterial activity of lipophilic and volatile compounds that may not diffuse optimally in agar media. Alternative methods, such as broth microdilution, can provide more accurate results for MIC and MBC determination. Second, this study tested only crude extracts without fractionation to isolate specific bioactive compounds. It is possible that there are active antibacterial compounds in the extract that are masked by other components or whose concentrations are too low in the crude extract to produce an antibacterial effect. Third, the variability in the phytochemical content of Sembung leaves depends on geographical factors, harvest season, and plant age, which were not strictly controlled in this study. Fourth, this study only used one clinical isolate of *H. pylori* without characterizing its antibiotic resistance profile. Fifth, this study was limited to *in vitro* evaluation and did not provide information on *the in vivo* antibacterial activity, pharmacokinetics, or safety of the extract.

CONCLUSIONS

Ethanol extracts of sembung leaves (*B. balsamifera*) at concentrations of 10–80% did not show antibacterial activity against *H. pylori* *in vitro* according to the disk diffusion method, as indicated by the absence of an inhibition zone (diameter 0 ± 0 mm) in all the treatment groups. Increasing the extract concentration did not produce a dose-dependent effect on the growth of the test bacteria. Recommendations for further research include (1) evaluating antibacterial activity via the broth microdilution method for more accurate MIC and MBC determination; (2) performing

fractionation of the extract for the isolation and testing of specific bioactive compounds; (3) modifying the extract formulation with an outer membrane permeabilizer to increase the penetration of active compounds; (4) testing the synergy between the extract and standard antibiotics; (5) testing antibiofilm activity; (6) quantitatively characterizing the phytochemical content of the extract; and (7) evaluating antibacterial activity against multiple *H. pylori* isolates with different resistance profiles.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision: IA., AAKS., and IWW.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

REFERENCES

- [1] Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of *Helicobacter pylori* infection. *Helicobacter* 2014;19:1–5. <https://doi.org/10.1111/hel.12165>.
- [2] Zamani M, Ebrahimitabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, Derakhshan MH. Systematic review with meta-analysis: the worldwide prevalence of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 2018;47:868–76. <https://doi.org/10.1111/apt.14561>.
- [3] Zahra H, Haridas RB, Gholam GM, Setiawan AG. Aktivitas antiulseratif berbagai tanaman herbal dan prospek masa depan sebagai tanaman budidaya. *J Sains Kesehat* 2022;4:343–53. <https://doi.org/10.25026/jsk.v4i3.1046>.
- [4] Mariadi IK, Wibawa IDN, Wibawa IBN. Detection of *Helicobacter pylori* CagA gene and Its association with endoscopic appearance in Balinese dyspepsia patients. *Indones J Gastroenter, Hepat, Diges Endos* 2017;17:99–105. <https://doi.org/10.24871/172201699-105>.
- [5] Parreira P, Soares BIG, Freire CSR, Silvestre AJD, Reis CA, Martins MCL, Duarte MF. *Eucalyptus* spp. outer bark extracts inhibit *Helicobacter pylori* growth: in vitro studies. *Ind Crops Prod* 2017;105:207–14. <https://doi.org/10.1016/j.indcrop.2017.05.012>.

- [6] Syam AF, Simadibrata M, Makmun D, Abdullah M, Fauzi A, Renaldi K, Maulahela H, Utari AP. National consensus on management of dyspepsia and helicobacter pylori infection. *Acta Med Indones* 2017;49:279–87. <https://pubmed.ncbi.nlm.nih.gov/29093241/>
- [7] Graham DY, Canaan Y, Maher J, Wiener G, Hulten KG, Kalfus IN. Rifabutin-based triple therapy (RHB-105) for *Helicobacter pylori* Eradication. *Ann Intern Med* 2020;172:795–802. <https://doi.org/10.7326/M19-3734>.
- [8] Zhou Y, Zhong Z, Hu S, Wang J, Deng Y, Li X, Chen X, Li X, Tang Y, Li X, Hao Q, Liu J, Sang T, Bo Y, Bai F. A Survey of *Helicobacter pylori* antibiotic-resistant genotypes and strain lineages by whole-genome sequencing in China. *Antimicrob Agents Chemother* 2022;66:e02188-21. <https://doi.org/10.1128/aac.02188-21>.
- [9] Boyanova L, Hadzhiyski P, Gergova R, Markovska R. Evolution of *Helicobacter pylori* resistance to antibiotics: A topic of increasing concern. *Antibiotics* 2023;12:332. <https://doi.org/10.3390/antibiotics12020332>.
- [10] World Health Organization. Antimicrobial resistance: global report on surveillance. Sustainable Development Goals Series 2023;Part F2781:143–7. https://doi.org/10.1007/978-3-031-33851-9_22.
- [11] AlSheikh HM Al, Sultan I, Kumar V, Rather IA, Al-Sheikh H, Tasleem Jan A, Haq QMR. Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics* 2020;9:480. <https://doi.org/10.3390/antibiotics9080480>.
- [12] Angelini P. Plant-Derived Antimicrobials and Their crucial role in combating antimicrobial resistance. *Antibiotics* 2024;13:746. <https://doi.org/10.3390/antibiotics13080746>.
- [13] Kusumawati IGAW, Yogeswara IBA. Antioxidant and antibacterial capacity of Loloh Sembung (*Blumea Balsamifera*) based on extraction method. *Trad Med J* 2016;21:143–8.
- [14] Rahmi A, Afriani T, Hevira L, Widiawati W. Uji aktivitas antioksidan dan fenolik total fraksi etil asetat daun Sembung (*Blumea balsamifera* (L.) DC). *J Riset Kimia* 2021;12:383. <https://doi.org/10.25077/jrk.v12i2.383>.
- [15] Jirakitticharoen S, Wisuitiprot W, Jitareerat P, Wongs-Aree C. Phenolics, antioxidant and antibacterial activities of immature and mature *Blumea balsamifera* leaf extracts eluted with different solvents. *J Trop Med* 2022;2022:1–12. <https://doi.org/10.1155/2022/7794227>.
- [16] Satria D, Harahap U, Dalimunthe A, Septama AW, Hertiani T, Nasri N. Synergistic Antibacterial effect of ethyl acetate fraction of *Vernonia amygdalina* delile leaves with tetracycline against clinical isolate methicillin-resistant staphylococcus aureus (MRSA) and *Pseudomonas aeruginosa*. *Adv Pharmacol Pharm Sci* 2023;2023:1–11. <https://doi.org/10.1155/2023/2259534>.
- [17] Masyudi M, Noviyanti A, Ridhwan M, Nurman S, Jailani J, Armi A, Rafsanjani TM, Usman S, Hanafiah M, Marlina M. Antibacterial activity of *Blumea balsamifera* leaf extracts from Aceh, Indonesia. *Biodiversitas* 2023;24:d240862. <https://doi.org/10.13057/biodiv/d240862>.
- [18] Yang H, Gao Y, Long L, Cai Y, Liao J, Peng J, Wang L. Antibacterial effect of *Blumea balsamifera* (L.) DC. essential oil against *Staphylococcus aureus*. *Arch Microbiol* 2021;203:3981–8. <https://doi.org/10.1007/s00203-021-02384-6>.
- [19] Wolde R, Mahamed SA. Antibacterial activity of apis mellifera bees honey, garlic extracts and their combinations against *Salmonella* in Wolayta Sodo, Southern Ethiopia. *Svāsthya Trends Gen Med Public Health* 2024;1:e25. <https://doi.org/10.70347/svsthya.v1i2.25>.
- [20] Sudaryati NLG, Utomo B, Adnyana IMDM, Fauziyah S, Sucipto TH, Cahyaningrum PL, Samala Dewi IGAG, Kautsar R, Agustini DGD. Phytochemical profiling, dengue antiviral properties, and cytotoxicity of novel Baper tea polyherbal infusion: Insights from in silico and in vitro studies. *Salud, Ciencia y Tecnología* 2025;5:1791. <https://doi.org/10.56294/saludcyt20251791>.
- [21] Ameliana M, Sari NM, Zarta AR, Hernandi MF, Aryani F, Paurru P. Potential utilization of sembung leaves (*Blumea balsamifera*) with analysis of phytochemical content, antioxidant activity, and antibacterial activity. *J Trop Forest* 2022;6:188–96.
- [22] Romadhina R, Budi S, Rohama R. Formulation and antibacterial testing of liquid soap preparations from sembung leaf extract (*Blumea balsamifera*) against *Staphylococcus aureus* bacteria. *J Pharm Care Sci* 2023;4:129–39. <https://doi.org/10.33859/jpcs.v4i1.400>.

- [23] Putri AVAA, Widyastuti NH, Megawati V. The effect of the antibacterial activity of stevia leaf extract (*Stevia rebaudiana Bertonii*) at concentrations of 5%, 10%, 20%, 40% and 80% on *S. mutans* (in vitro). J Dental Sci 2017;1:9–14. <https://doi.org/10.23917/jikg.v1i1.4147>.
- [24] James S, Amy JM, April MB, Alexandra LB, Shelley C, Sharon KC. CLSI M100 performance standards for antimicrobial susceptibility testing. Wayne Pa Clin Lab Stand Inst 2025.
- [25] American Society for Microbiology. Kirby-Bauer Disk Diffusion Susceptibility. American Society for Microbiology 2016;3:6–13.
- [26] Ismail NA, Matawali A, Kanak FA, Lee P-C, How S-E, Goh LPW, Gansau JA. Antimicrobial activities and phytochemical properties of *Blumea balsamifera* against pathogenic microorganisms. J Med Life 2022;15:951–4. <https://doi.org/10.25122/jml-2021-0296>.
- [27] Mégraud F, Lehours P. *Helicobacter pylori* Detection and Antimicrobial Susceptibility Testing. Clin Microbiol Rev 2007;20:280–322. <https://doi.org/10.1128/CMR.00033-06>.
- [28] Adnyana IMDM. Desain Penelitian Kuantitatif. In: Akbar H, editor. Metodologi Penelitian. 1st ed., Bandung: CV. Media Sains Indonesia; 2024, p. 45–68.
- [29] Sukmawati AS, Hermawan IMA, Saputra EK, Adnyana IMDM, Aldyza N, Slamet NS, Hidayat B, Pandawa RM, Hamdani R, Maisura, Dara W, Sembodo A. Metodologi Penelitian. 1st ed. Bandung: CV. Media Sains Indonesia; 2024.
- [30] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71–9. <https://doi.org/10.1016/j.jpha.2015.11.005>.
- [31] Azwanida. A Review on the Extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants 2015;04:1000196. <https://doi.org/10.4172/2167-0412.1000196>.
- [32] Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y-H. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014;22:296–302. <https://doi.org/10.1016/j.jfda.2013.11.001>.
- [33] Truong D-H, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of *Severinia buxifolia*. J Food Qual 2019;2019:1–9. <https://doi.org/10.1155/2019/8178294>.
- [34] Parikh NS, Ahlawat R. *Helicobacter Pylori*. 1st ed. New York: StatPearls Publishing LLC; 2025. <https://doi.org/30480966>.
- [35] Bubonja-Šonje M, Knežević S, Abram M. Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds. Archives of Industrial Hygiene and Toxicology 2020;71:300–11. <https://doi.org/10.2478/aiht-2020-71-3396>.
- [36] Silhavy TJ, Kahne D, Walker S. The Bacterial Cell Envelope. Cold Spring Harb Perspect Biol 2010;2:a000414–a000414. <https://doi.org/10.1101/cshperspect.a000414>.
- [37] DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: a pathophysiologic approach 2014.
- [38] Aryasa IWT, Artini NPR. Green Synthesis Silver Nanoparticles Using Sembung (*Blumea balsamifera*) Leaf Extract as an Antibacterial and Antioxidant. Jurnal Penelitian Pendidikan IPA 2023;9:11877–86. <https://doi.org/10.29303/jppipa.v9i12.6609>.
- [39] Agung Yogeswara IB, Ayu Wita Kusumawati IG, Nursini NW. Antibacterial activity and cytotoxicity of sequentially extracted medicinal plant *Blumea balsamifera* Lin. (DC). Biocatal Agric Biotechnol 2022;43:102395. <https://doi.org/10.1016/j.bcab.2022.102395>.
- [40] Yuan G, Guan Y, Yi H, Lai S, Sun Y, Cao S. Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. Sci Rep 2021;11:10471. <https://doi.org/10.1038/s41598-021-90035-7>.
- [41] Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X, Wang K, Yuan L. *Blumea balsamifera*—A Phytochemical and Pharmacological Review. Molecules 2014;19:9453–77. <https://doi.org/10.3390/molecules19079453>.
- [42] Sakee U, Maneerat S, Cushnie TPT, De-eknamkul W. Antimicrobial activity of *Blumea balsamifera* (Lin.) DC. extracts and essential oil. Nat Prod Res 2011;25:1849–56. <https://doi.org/10.1080/14786419.2010.485573>.

- [43] Wongarmart U, Reungbun K, Tanampai B, Nateesirik P, Parathang R, Chaichamno E, Boongapim R, Rattanasuk S. Antibacterial activity of leaf extracts from *Blumea balsamifera*, *Morus alba* and *Limnocharis flava* against skin pathogenic bacteria. Pakistan J Biol Sci 2025;28:376–82. <https://doi.org/10.3923/pjbs.2025.376.382>.
- [44] Bình HT, Trí NM, Quân NH, Ngọc NV. Antibacterial activities and chemical composition of the essential oil of *Blumea balsamifera* (L.) DC. Dalat University Journal of Science 2020:3–13. [https://doi.org/10.37569/DalatUniversity.10.2.597\(2020\)](https://doi.org/10.37569/DalatUniversity.10.2.597(2020)).
- [45] Pertiwi R, S SY, Wibowo RH, Notriawan D, Nasution RP, Azhar AW. Aktivitas antibakteri ekstrak daun mangrove (*Rhizophora mucronata*) pada bakteri *helicobacter pylori* penyebab tukak lambung. Bioscientist 2024;12:202. <https://doi.org/10.33394/bioscientist.v12i1.9957>.
- [46] Hasan S, Jahan U, Saeed S, Kaur M, Gombra V, Rai A, Mansoori S. Prevalence of nutrient canals in mandibular anterior intra-oral periapical radiographs (IOPARS) in patients with chronic systemic diseases—a cross-sectional study. J Med Life 2022;15:661–8. <https://doi.org/10.25122/jml-2022-0009>.
- [47] Widhiantara IG, Jawi IM. Phytochemical composition and health properties of Sembung plant (*Blumea balsamifera*): A review. Vet World 2021;14:1185–96. <https://doi.org/10.14202/vetworld.2021.1185-1196>.
- [48] Zhang P-P, Li L, Qu H-Y, Chen G-Y, Xie M-Z, Chen Y-K. Traditional Chinese medicine in the treatment of *Helicobacter pylori* -related gastritis: The mechanisms of signalling pathway regulations. World J Gastroenterol 2025;31:96582. <https://doi.org/10.3748/wjg.v31.i3.96582>.