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Antibacterial Activity of Kalimantan Native Seed Extracts of *Shorea macrophylla* and *Litsea garciae* Vidal Against *Helicobacter pylori*

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ABSTRACT

Introduction*: Infection with Helicobacter pylori remains a major cause of gastritis, and increasing antibiotic resistance has limited the effectiveness of conventional therapies. Natural products are therefore being explored as alternative antibacterial agents.* ***Aims****: This study aimed to evaluate the antibacterial potential of a combined ethanol extract of tengkawang tungkul seeds (Shorea macrophylla) and kalangkala seeds (Litsea garciae Vidal) against H. pylori.* ***Methods****: Extracts were prepared using Ultrasonic Assisted Extraction with 70% ethanol. Phytochemical screening, Fourier Transform Infrared (FTIR) analysis, and quantitative determination of total phenolic and flavonoid contents were performed. Antibacterial activity was assessed in vitro using inhibition zone assays with chloramphenicol (30 µg) as the positive control, and in vivo using a mouse gastric infection model with ciprofloxacin as the reference drug.* ***Result****: Both extracts contained alkaloids, flavonoids, terpenoids, and tannins, while saponins were detected only in L. garciae. FTIR spectra confirmed functional groups associated with phenolic and flavonoid compounds. L. garciae showed higher total phenolic content (9.56 mg GAE/g), whereas S. macrophylla exhibited higher flavonoid content (7.77 mg QE/g). In vitro testing demonstrated moderate antibacterial activity, with the 75%:25% extract ratio producing the largest inhibition zone (7.95 ± 0.41 mm), equivalent to 46.88% of the inhibition zone of chloramphenicol. In vivo, the same ratio significantly reduced H. pylori colony counts (p=0.000), with antibacterial effectiveness approaching that of ciprofloxacin.* ***Conclusion****: The combined extracts of S. macrophylla and L. garciae exhibit promising antibacterial activity against H. pylori in vitro and in vivo. These findings suggest their potential as a natural source for developing alternative therapeutic agents for H. pylori–associated gastritis, although further studies are required to confirm interaction mechanisms and optimize dosage.*

KEYWORDS*: Gastritis, Helicobacter pylori, tengkawang tungkul, kalangkala, antibacterial.*

INTRODUCTION

Gastritis is inflammation of the gastric epithelium caused by infection, poor diet, stress, or prolonged drug use, with *Helicobacter pylori* as its principal infectious driver and a major global disease burden (Haddad and Abdulrahman 2020). More than half of the world's population is

estimated to carry *H. pylori*, though most infections are asymptomatic, and Indonesian prevalence varies regionally (Kasi et al. 2019). *H. pylori* causes about 44.3% of gastritis cases, ahead of NSAID use (10–25%) and smoking (13.5%) (Zamani et al. 2018; Pusmarani et al. 2023), and untreated infection can progress to peptic ulcer, gastric cancer, and gastric lymphoma (Ali and Alhussaini 2024).

Standard triple therapy, a proton pump inhibitor with amoxicillin and clarithromycin, is losing effectiveness as eradication rates fall to around 60% amid resistance that can emerge after one treatment failure (Tshibangu-kabamba and Yamaoka 2021; Losurdo et al. 2024). WHO has prioritized clarithromycin-resistant *H. pylori* for new antimicrobial development (Sanda et al. 2024; Vu et al. 2023); despite alternatives such as bismuth-based quadruple therapy and vonoprazan, Indonesian resistance remains high: 46.7% for metronidazole, 31.2% for levofloxacin, 9.1% for clarithromycin, 5.2% for amoxicillin, and 2.6% for tetracycline (Miftahussurur et al. 2016).

These limitations have driven interest in plant-derived antibacterials, though safety profiles are often poorly characterized (Ziegenhagen et al. 2021). Native Kalimantan plants offer promising candidates: tengkawang tungkul (*Shorea macrophylla*) seeds contain the antibacterial compound resveratrol (Rammohan, Vijaya, and Camilo 2020; Musa et al. 2023), and kalangkala (*Litsea garciae* Vidal) seed extract is active against *Propionibacterium acnes* (Ramadhan, Arsyad, and Sayakti 2020). Combining extracts can enhance efficacy through synergy, as shown for *Acorus calamus* and *Shorea robusta* (Kale et al. n.d.), supporting the rationale for combining the two species: flavonoids and polyphenols can inhibit *H. pylori* urease and adhesion (Tan, Scortecci, and Boylan 2025), while *L. garciae* phenolics and saponins may destabilize the bacterial membrane (Chen et al. 2025).

This study characterized the phytochemical profile, quantified total phenolic and flavonoid content, and evaluated the in vitro and in vivo antibacterial efficacy of combined *S. macrophylla* and *L. garciae* Vidal seed extracts against *H. pylori* across several extract ratios.

METHODS

Materials

The main ingredients used include tengkawang tungkul seeds (*Shorea macrophylla*) obtained from the Forest Products Technology Research and Development Institute (INTAN), Southeast Pontianak District, Pontianak, West Kalimantan, and kalangkala seeds (*Litsea garciae Vidal*) obtained in Sampit, Central

Kalimantan. Other materials included Nutrient Agar (Himedia), Ethanol 70% (Brataco), Acetic Anhydride (≥ 99%, Merck), HCl (37%, Merck), Amyl Alcohol (≥ 98%, Merck), Distilled Water (Brataco), Chloroform (≥ 99%, Merck), Magnesium Powder (analytical grade), H_2SO_4 (95–98%, Merck), NaCl 0.9% solution, Swiss Webster mice feed, Rice-Husk bedding, *Helicobacter pylori* strain (Oxoid), *Helicobacter pylori* ATCC strain (Oxoid), Male Swiss Webster mice, Columbia Agar (Himedia), Chloramphenicol antibiotic discs (30 µg, Oxoid), and Ciprofloxacin tablets. Major instruments used included a Laminar Air Flow (LAF) cabinet, Rotary Evaporator (Buchi R-100 II), UV–Vis spectrophotometer (Shimadzu UV-1800), Fourier Transform Infrared Spectrophotometer (Shimadzu IR Prestige), analytical balance (Ohaus), autoclave (GEA), Incubator (Memmert), Oven (Memmert), and additional standard Glassware (Iwaki) such as beakers, flasks, test tubes, and measuring cylinders.

This study was conducted at the Laboratory of Pharmacognosy, Pharmacology, and Microbiology, Faculty of Pharmacy, University of Muhammadiyah Banjarmasin.

Extraction

Powdered seeds (50 g) of each species were extracted with 70% ethanol (1:5 w/v) in an amber vessel by ultrasonic-assisted extraction (40 kHz, 30 min). The filtrate (Whatman No. 1) was concentrated under reduced pressure at 70 °C using a rotary evaporator to yield a semi-solid ethanolic extract (Kristina, Yusasrini, and Ni Made 2022).

Phytochemical Screening

Flavonoids were detected by heating 1 g of extract in 10 mL water, filtering, and treating an aliquot with magnesium powder, concentrated HCl, and amyl alcohol; a yellow amyl-alcohol layer indicated a positive result (Fitriyanti, Syamratul, and Putri 2020). Alkaloids were assessed by dissolving the extract in 2 N HCl, heating, filtering, and testing the filtrate with Mayer's, Wagner's, and Dragendorff's reagents, with precipitate formation in at least two tests taken as positive (Fitriyanti et al. 2020). Terpenoids were evaluated using chloroform, acetic anhydride, and concentrated H_2SO_4, a red-to-purple interface color indicating a positive reaction (Fitriyanti et al. 2020). Tannins were identified with 1% $FeCl_3$ (dark green/blue-black color) (Ali et al. 2022), and saponins by a froth test in which stable foam persisting for at least 10 minutes after HCl addition was considered positive (Singh, Rizvi, and Sara 2021).

FTIR Analysis

Spectra were acquired by the ATR method without additional sample prepar-

ration, using 1–2 mg of dried extract placed directly on the ATR crystal, scanned from 4000–500 cm^{-1} at 4 cm^{-1} resolution with 32 scans, followed by background and baseline correction (Purwanto et al. 2025; Purwanto et al. 2024).

Total Phenolic Content

Determined using the Folin–Ciocalteu method. A 100 mg extract stock solution was diluted, and 0.2 mL of the working solution was reacted with Folin–Ciocalteu reagent and water for 8 minutes before adding sodium carbonate to create alkaline conditions. After 2 hours at room temperature, absorbance was read at 765 nm, and results were expressed as mg gallic acid equivalents (GAE) per gram of extract using a gallic acid calibration curve (Ginting et al. 2024).

Total Flavonoid Content

Measured by aluminum chloride complexation. A 100 mg extract was dissolved overnight in 10 mL water; 2 mL of this solution was reacted with $AlCl_3$ and sodium acetate for 30 minutes, and absorbance was read at 430 nm against a quercetin standard curve, with results expressed as mg quercetin equivalents (QE) per gram of extract (Syakri et al. 2024).

Extract Combinations

The two ethanolic extracts were combined by vortex mixing at 25:75, 50:50, and 75:25 (w/w) ratios of *S. macrophylla* to *L. garciae*, each prepared at a concentration of 1 g extract per 10 mL.

In Vitro Antibacterial Assay

H. pylori was cultured on Nutrient Agar under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2; Oxoid CampyGen™) at 37 °C for 48–72 hours and adjusted to a 0.5 McFarland standard (≈ 1×10^8 CFU/mL). Because culture-medium composition and incubation atmosphere strongly influence *H. pylori* recovery and viability (Hortelano et al. 2020; AlRubaye and Al-Thwaini 2024), Nutrient Agar was chosen as it supplies adequate carbon, nitrogen, and mineral sources under controlled microaerophilic conditions, consistent with reports that non-enriched media can still support *H. pylori* growth when incubation conditions are appropriate (Hortelano et al. 2020). Antibacterial activity was assessed by disk diffusion (Sundar et al. 2021): a 24-hour *H. pylori* suspension in 0.9% NaCl was spread on Nutrient Agar, and discs impregnated with the extract combinations were applied, with distilled water as the negative control and 30 µg chloramphenicol discs as the positive control. Plates were incubated microaerophilically at 37 °C for 48 hours (Sanda et al. 2024).

In Vivo Antibacterial Assay

Male Swiss Webster mice were acclimatized for 7 days, fasted for 12 hours, and then orally inoculated with *H. pylori*

suspension (10^8 CFU/mL) four times at 2-day intervals over 21 days. Five groups were used: a negative control, three extract-treatment groups (25:75, 50:50, 75:25) receiving combined extract at 10 mL/kg body weight orally once daily for 7 days, and a positive-control group given ciprofloxacin (500 mg/kg, a dose based on prior preclinical studies to account for the faster metabolism and drug elimination of mice) (Mabeku et al. 2017). After treatment, gastric tissue was homogenized in phosphate-buffered saline, serially diluted, cultured on Columbia Agar, and incubated microaerophilically for bacterial colony counting (Mabeku et al. 2017). All animal procedures were approved by the Institutional Animal Ethics Committee, Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin (Approval No. 495), in accordance with national guidelines for laboratory animal care.

Statistical Analysis

In vitro assays were performed in triplicate, and in vivo experiments used n = X animals per group; data are presented as mean ± standard deviation. Analyses were performed in SPSS version 25 (IBM Corp.), with normality assessed by the Shapiro–Wilk test and variance homogeneity by Levene's test. Between-group differences were analyzed by one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Extraction Yield

Ultrasonic-assisted extraction of 500 g tengkawang tungkul powder (from 552 g total simplicia, 7% drying loss) yielded 10.3 g of viscous extract (2.06%), while 500 g kalangkala powder (from 521.44 g total simplicia, 8.5% drying loss) yielded 13.4 g of extract (2.68%). Moisture content of both simplicia was below the 10% storage limit, meeting quality requirements and providing adequate extract yield for subsequent antibacterial testing (RI 2017).

Phytochemical Screening

Combined qualitative screening (Table 1) showed that both extracts contained alkaloids, flavonoids, terpenoids, and tannins, while saponins were detected only in *L. garciae*; *S. macrophylla* was additionally Wagner-negative for alkaloids, whereas *L. garciae* was Wagner-positive. These metabolites act antibacterially through distinct mechanisms — alkaloids disrupting DNA synthesis, flavonoids damaging cell membranes, terpenoids altering wall permeability, and tannins precipitating bacterial proteins — so the saponin content unique to *L. garciae* may further enhance activity by compromising membrane integrity (Fitriyanti et al. 2020). The differing Wagner response likely reflects the fact that precipitation-based alkaloid reagents do not react uniformly

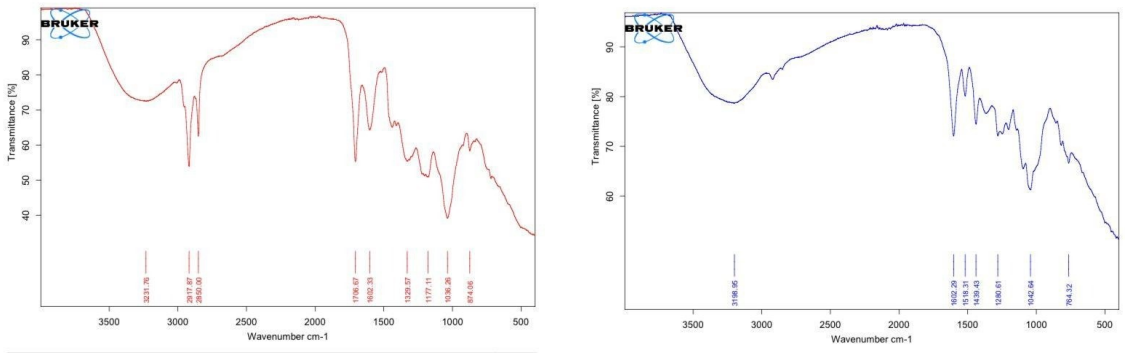
Table 1. Phytochemical Constituents of *S. macrophylla* and *L. garciae* Vidal Seed Extracts

Secondary Metabolite	Reagent/Test	*S. macrophylla*	*L. garciae*	Indicator
Alkaloid	Mayer	+	+	White precipitate
	Wagner	–	+	Brown precipitate (positive) / absent (negative)
	Dragendorff	+	+	Brick-red precipitate
Flavonoid	Mg + HCl + amyl alcohol	+	+	Yellow amyl-alcohol layer
Terpenoid	Acetic anhydride + H_2SO_4	+	+	Red-to-purple color at the interface
Tannin	$FeCl_3$ 1%	+	+	Dark green color
Saponin	Water (shaken) + HCl 2N	–	+	Stable foam ≥ 10 minutes

with all alkaloid structures and can be influenced by co-occurring constituents (Raal et al. 2020); since many antibacterial alkaloids act on bacterial DNA/topoisomerases or the cytoplasmic membrane (Sulaiman et al. 2022), the Wagner-positive alkaloids detected in *L. garciae* may add to the overall antibacterial contribution of the combined extract.

FTIR Analysis

FTIR spectra (Figure 1) showed functional groups typical of phenolic and flavonoid compounds in both extracts. The *S. macrophylla* extract exhibited a broad N–H (amide) band at 3231 cm^{-1} and a strong C=O (carbonyl) band at 1706 cm^{-1}, while the *L. garciae* extract showed bands at 3198 cm^{-1} (N–H, amide) and 1518 cm^{-1} (C=C, aromatic). These signals are consistent with phenolic and flavonoid functional groups, although FTIR alone cannot confirm specific compound identity (Made and Sanjiwani 2021).



(a) *(b)*

Figure 1. FTIR spectra of (a) *S. macrophylla* and (b) *L. garciae* Vidal seed extracts.

Table 2. Total Phenolic and Flavonoid Content of *S. macrophylla* and *L. garciae* Vidal Seed Extracts

Seed Extract	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)
Tengkawang Tungkul (*S. macrophylla*)	7.542 ± 1.322	7.767 ± 2.101
Kalangkala (*L. garciae* Vidal)	9.562 ± 2.713	0.855 ± 1.212

Table 3. In vitro antibacterial activity against *H. pylori* (disk diffusion)

Treatment	Inhibition Zone (mm)	Category	Relative Inhibition (%)
Negative control (distilled water)	0	Negative	0
25% *S. macrophylla* : 75% *L. garciae*	5.88 ± 0.26	Moderate	34.67
50% *S. macrophylla* : 50% *L. garciae*	6.98 ± 0.55	Moderate	41.16
75% *S. macrophylla* : 25% *L. garciae*	7.95 ± 0.41	Moderate	46.88
Positive control (chloramphenicol 30 μg)	16.96 ± 1.51	Strong	100

Note: One-way ANOVA — F = 198.892; p < 0.001 (significant difference among treatments).

Total Phenolic and Flavonoid Content

L. garciae had a higher total phenolic content (9.56 mg GAE/g) than *S. macrophylla* (7.54 mg GAE/g) (Table 2), consistent with the role of phenolics in inhibiting microbial growth through membrane damage, protein disruption, and enzyme inhibition (Mabeku et al. 2017; Rammohan et al. 2020), which points to phenolics as a likely contributor from *L. garciae* to the combined extract's activity. Conversely, *S. macrophylla* had a markedly higher flavonoid content (7.77 mg QE/g) than *L. garciae* (0.86 mg QE/g) (Table 2), suggesting flavonoids — known to disrupt cytoplasmic membranes, suppress DNA replication, and inhibit key metabolic enzymes (Mabeku et al. 2017) — as the primary antibacterial contributor from *S. macrophylla*.

In Vitro Antibacterial Assay

Extract combinations produced moderate inhibition zones (5.88–7.95 mm) against *H. pylori*, compared with 16.96 ± 1.51 mm for the chloramphenicol positive control (Table 3), with a significant treatment effect by one-way ANOVA ($F = 198.892$, $p < 0.001$); no inhibition was observed for the negative control. Inhibition increased with the proportion of *S. macrophylla* in the mixture: 5.88 ± 0.26 mm (25:75), 6.98 ± 0.55 mm (50:50), and 7.95 ± 0.41 mm (75:25) — all within the moderate range (5–10 mm) (Mulyani, Artauli, and Turnip 2020) — corresponding to 34.67–46.88% of the positive control's activity, in line with reports that flavonoids and polyphenols are key contributors to anti-*H. pylori* activity (Majid et al. 2023). Tukey's test confirmed that the 75:25 ratio

Table 4. *Helicobacter pylori* colony counts in gastric tissue of swiss webster mice

Group	Treatment	*H. pylori* Colony Count ($\times 10^8$ CFU/mL)
1	Negative control	5.77 – 6.79
2	25% S. macrophylla : 75% L. garciae	3.61 – 4.45
3	50% S. macrophylla : 50% L. garciae	3.17 – 3.73
4	75% S. macrophylla : 25% L. garciae	2.13 – 2.95
5	Positive control (ciprofloxacin 500 mg/kg)	1.16 – 1.98

Note: One-way ANOVA — F = 44.670; p < 0.001 (significant difference among treatments).

produced the greatest inhibition, reflecting a proportional response in which a higher *S. macrophylla* fraction enhanced antibacterial performance (Seko, Sabuna, and Ngginak 2021).

The modest zone sizes observed are consistent with earlier diffusion-assay studies of plant extracts against *H. pylori*, since its slow growth rate, microaerophilic requirements, and limited phytochemical diffusion in solid media typically produce small inhibition zones despite genuine susceptibility, so the measurable zones seen here indicate biologically meaningful activity (Nostro et al. 2005). The stronger effect of *S. macrophylla*-dominant ratios likely reflects its higher flavonoid content, as flavonoids are widely reported to inhibit urease — an enzyme essential for *H. pylori* survival under acidic gastric conditions — thereby disrupting local pH regulation and reducing viability. Although urease inhibition and anti-adhesive activity were not directly measured in this study, they represent plausible, literature-supported mechanisms warranting further validation (Nostro et al. 2005). The phenolic content of *L. garciae* may additionally contribute via membrane destabilization and enzyme inactivation, so the combined flavonoid–phenolic profile likely explains the enhanced activity of the extract mixtures relative to either compound class alone (Nostro et al. 2005).

In Vivo Antibacterial Assay

In Swiss Webster mice, *H. pylori* colony counts were highest in the negative control (5.77–6.79 × 10^8 CFU/mL) and lowest in the positive control (1.16–1.98 × 10^8 CFU/mL) (Table 4). Among treated groups, colony counts decreased as the *S. macrophylla* proportion increased: 3.61–4.45 × 10^8 CFU/mL (25:75), 3.17–3.73 × 10^8 CFU/mL (50:50), and 2.13–2.95 × 10^8 CFU/mL (75:25), the ratio closest to the positive control, though still less effective overall. One-way ANOVA confirmed significant between-group differences ($F = 44.670$, $p < 0.001$), consistent with a clear ratio-dependent reduction in *H. pylori* colonization (Ali and Alhussaini 2024).

This reduction indicates that the extract combinations remained active under physiological gastric conditions, consistent with reports that plant-derived compounds can suppress *H. pylori* colonization both directly and by interfering with bacterial persistence mechanisms (Mabeku et al. 2017). One likely mechanism is inhibition of bacterial adhesion to gastric epithelial cells, since phenolic and flavonoid compounds have been shown to block *H. pylori* attachment and thereby limit stable colonization (Mabeku et al. 2017); flavonoids have also been reported to downregulate virulence-factor expression linked to mucosal damage and inflammation, weakening bacterial survival and facilitating clearance (Mabeku et al. 2017). Together, these observations suggest that the reduction in bacterial load reflects combined antibacterial, anti-adhesive, and anti-virulence effects.

Overall, the in vitro and in vivo findings were consistent: the 75:25 ratio produced both the largest inhibition zone (7.95 ± 0.41 mm) and the greatest reduction in bacterial load ($p < 0.001$), approaching the efficacy of the positive control. This concordance suggests that antibacterial activity observed under controlled culture conditions can translate into more complex biological systems, a pattern also reported in other studies combining plant extracts and bioactive agents against *H. pylori* in vitro and in vivo (Hasna et al. 2023; Tan et al. 2025). Broader systematic reviews likewise show that plant-derived extracts evaluated through combined in vitro/in vivo designs can suppress *H. pylori* colonization while reducing gastric mucosal damage, typically through flavonoids, phenolics, and terpenoids acting via urease inhibition, anti-adhesive effects, and immune modulation (Chen et al. 2025), reinforcing the promise of bioactive plant compounds as natural anti-*H. pylori* agents.

Study Limitations

This study has several limitations. Antibacterial testing relied on the disk diffusion method without determining minimum inhibitory or minimum bactericidal concentrations (MIC/MBC), and no formal synergy analysis (e.g., checkerboard or FICI) was performed to confirm interactions between the two extracts. Phytochemical characterization was limited to qualitative screening and FTIR analysis, without compound isolation or chromatographic profiling. The in vivo evaluation did not include gastric histopathology or toxicity assessment. Further studies addressing these gaps are needed to validate the pharmacological mechanisms and safety profile of the combined extract.

CONCLUSION

The combined ethanolic extract of tengkawang tungkul and kalangkala seeds showed antibacterial activity against *H. pylori* both in vitro and in vivo. In the disk diffusion assay, inhibition-zone diameter increased with the proportion of *S. macrophylla* in the mixture, with the 75:25 (*S. macrophylla*:*L. garciae*) ratio producing the largest zone (7.95 mm); the same ratio produced the greatest reduction in bacterial colony counts in vivo, approaching the effectiveness of ciprofloxacin. Flavonoids, phenolics, tannins, alkaloids, and saponins are plausible contributors to this activity, potentially acting through membrane disruption, enzyme inhibition, and interference with bacterial metabolism, although these mechanisms were not directly tested in this study. The combined extract therefore represents a promising candidate for developing standardized anti-*H. pylori* formulations, with potential translational value pending further pharmacological and safety evaluation.

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