Pharmaciana
Vol.15, No.1, March 2025, Page. 146-153
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v15i1.30099

The phytochemical and antibacterial activity of ethanolic extract of *Laportea decumana* and *Laportea aestuans*

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Submitted: 19-09-2024 ***Reviewed:23-01-2025*** ***Accepted: 15-03-2025***

ABSTRACT

Plants have been an important medicinal resource for centuries, offering a rich source of bioactive compounds used in traditional and modern medicine. This study aims to analyze the comparison between *Laportea decumana* and *Laportea aestuans*, focusing on the two main aspects, phytochemical screening and antibacterial activity. The extraction of the maceration process uses ethanol 96% as the solvent at room temperature for 72 hours. The qualitative phytochemical analysis uses HCl and $FeCl_3$ 0.1% reagents to determine flavonoids and phenolic compounds, respectively. The quantitative analysis of total flavonoid content is measured using colorimetric $AlCl_3$ method, while the determination of phenolic compounds is performed by the Folin-Ciocalteu. The diffusion method is used to determine the antibacterial activity. The 96% ethanol extract of *L. decumana* leaves has higher flavonoids (69.4 mg GAE/g extract) than phenolics (55.1 mg QE/g extract). However, the *L. aestuans* has higher phenolic content (55.3 mg QE/g extract) than flavonoids (35.3 mg GAE/g extract). The antibacterial test using agar diffusion method shows that both extracts inhibited the growth of pure strains of *E. coli* ATCC and *S. aureus* ATCC, with different potencies. The *L. decumana* extract showed 1.5 times stronger activity than the *L. aestuans* extract on the growth inhibitory activity against *E. coli* bacteria. The results show that the relationship between the antibacterial activity and the phytochemical profile against *E. coli* is strong but weak against *S. aureus*.

Keywords: Phytochemistry, antibacterial, *Laportea decumana*, *Laportea aestuans*

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INTRODUCTION

Plants have been important medicinal resources for centuries, offering rich sources of bioactive compounds used in traditional and modern medicine. Genus *Laportea* belongs to the family of *Urticaceae* includes several species known to have therapeutic potential (Simaremare et al., 2015). *Laportea decumana* and *Laportea aestuans*, localy known as Itchy leaf have always been interesting for their diverse phytochemical contents and reported medicinal properties (Thalib et al., 2021; Kiat et al., 2019).

L. decumana and *L. aestuans* are widespread in tropical and subtropical regions, often used in traditional medicine for their antioxidant activity (Okereke & Elekwa, 2020), analgesic used (Thalib et al., 2021), and antimicrobial properties (Simaremare et al., 2017; 2020). Despite their widespread usage, much scientific literature comparing the phytochemical profiles and antimicrobial activities is limited. Understanding these aspects is crucial to validate the traditional uses and explore potential new applications in the pharmaceutical and therapeutic fields.

The evaluation of antimicrobial activity is essential to identify the potential natural alternatives for synthetic antibiotics, especially to overcome the increasing antimicrobial resistance (Hidayati et al., 2023). Phytochemical testing, which identifies the presence of bioactive compounds, such as alkaloids, flavonoids, phenolics, and saponins, contributes to the usage of the medicinal properties of plants (Liu et al., 2018). The 90% ethanol, which is extracted from the *Laportea* genus leaves by a maceration process, has shown the antibacterial activity to destroy or inhibit the growth of Gram-negative and Gram-positive bacteria, for the various contents of antimicrobial metabolite compounds such as tannins, flavonoids, and saponins (Uddin et al., 2016)

This study aims to compare the analysis of phytochemical screening and antibacterial activity against L. *documana* and L. *aestuans*. Identifying and quantifying phytochemical contents and evaluating antibacterial activity in this study will provide a comprehensive understanding of the medicinal potential of these species. These findings are expected to expand the knowledge of medicinal plants and support the development of new therapeutic agents from *Laportea* species.

MATERIALS AND METHODS

Materials

The research method usedsa laboratory experimental design method. The plant samples are *Laportea decumana* and *Laportea aestuans*, collected from Salatiga, Central Java, by purposive sampling. The plant used is leaf parts, 96% ethanol leaves, HCl, $FeCl_3$, *Folin-ciocalteu*, gallic acid, Na_2CO_3, $AlCl_3$, CH_3COOK, pure strains *Escherichia coli* ATCC and *Staphylococcus aureus* ATCC obtained from Microbiology Laboratory, Faculty of Biology, UKSW, Nutrient agar (NA), $BaCl_2$, H_2SO_4. The main tools include oven (memmert OV 24), blender (Philip HR1538), vacuum-assisted rotary evaporator R114 Buchi (Eyela A-1000S), UV-Visible spectrophotometer (Shimadzu UV mini1240), autoclave (hirayama HG50).

Methods

Sample preparation and extraction

Research samples (Figure 1) are taken in fresh condition, sorted, cleaned, then dried using an oven at 40°C for 24 hours. Furthermore, the dried leaves are mashed using a blender to obtain dry powdered simplicia. The 96% ethanol extract is used as a solvent to make the extract. The maceration method is repeated 3 times each for 72 hours. After the maceration process, the extract is filtered and macerated again. The filtrate obtained is dried at 40°C using a rotavapor R114 Buchi for 30 minutes and then aerated until the extract is obtained (Kristiani et al., 2024). Research samples (Figure 1) are taken in fresh condition, sorted, cleaned, then dried using an oven at 40°C for 24 hours.

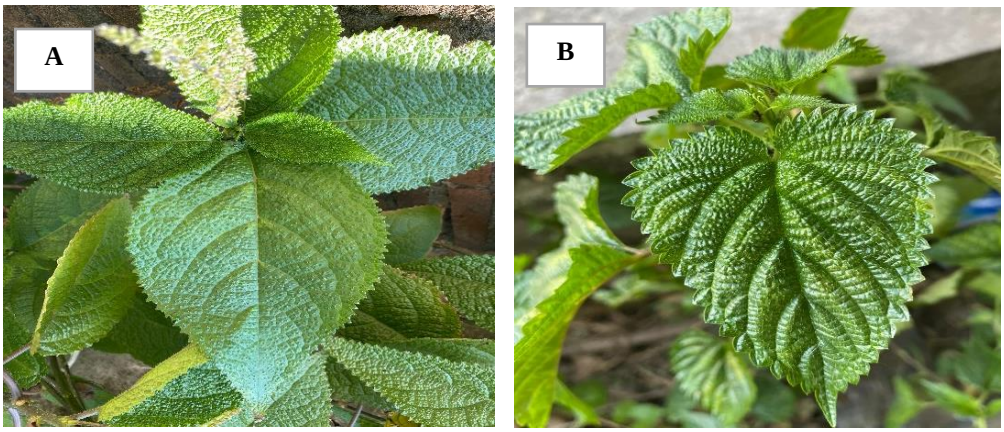

Figure 1. Research samples. (A) *Laportea decumana* leaf, (B) *Laportea aestuans* leaf

The phytochemical analysis

Qualitative test of flavonoid compounds

Put 0.5 grams of sample extract in a test tube dissolved in 10 mL of 96% ethanol and filter using Whatman filter paper. Furthermore, 4-5 drops of concentrated HCl are added and then heated in a water bath at 40°C for 10 minutes. The color change to red or yellow indicated the positive result contained flavonoids (flavones and aurones) (Samejo et al., 2013).

The qualitative test of phenolic compounds

Put 0.5 grams of sample extract in a test tube, dissolved in 10 mL of distilled water, and filtered using *Whatman* filter paper. Furthermore, 3-4 drops of $FeCl_3$ 0.1% are added and then heated in a water bath at 40°C for 10 minutes. The formation of the black blue color indicates the positive result of the phenol (Samejo et al., 2013).

The quantitative determination of total flavonoid content

The flavonoid content is determined using the AlCl3 reagent according to Jhon et al. (2019), with slight modifications. The measurement of sample absorbance is performed by UV-Visible spectrophotometer (Shimadzu UV mini1240) at 415 nm. The quercetin solution is used with a concentration range of 20, 40, 60, 80, 100 µg/mL. The test solution uses a mixture between 0.2 mL of the sample and 0.6 mL of 95% ethanol, then shaken. Then add 0.04 mL of 10% aluminum chloride and 0.04 ml of CH3COOK 1M distilled water until it reaches a volume of 2 mL, then incubated at 25 °C for 30 minutes. The determination of total flavonoid concentration in the sample is calculated based on the linear equation of quercetin: QE = c (V/m). QE = quercetin equivalent, c = total flavonoid concentration of quercetin standard curve (mg/l), V = extract volume (mL), m = extract weight (g).

Quantitative determination of total phenolic content

According to Jhon et al. (2019), the phenolic content was determined using the *Folin-ciocalteu* reagent with slight modifications. Gallic acid is used as the standard for phenolic compounds, with a concentration range of 100, 200, 300, 400, and 500 µg/mL. The absorbance of the extract is calculated using a UV-visible spectrophotometer (Shimadzu UV mini1240) at a wavelength of 550 nm. For the test solution, 0.2 ml of sample is used, then 1 mL of *Folin-Ciocalteu* 10% is added, shaken, and 0.8 ml of Na2CO3 75% is added, then incubated at 25^0C for 30 minutes. The total concentration in the sample is determined using the linear regression equation for gallic acid. The calculation of the concentration was done using the equation GAE = c (V/m). GAE = gallic acid equivalent, c = total phenolic concentration of the gallic acid standard curve (mg/l), V = volume of extract (l), m = weight of extract (g).

The antibacterial activity test

The nutrient agar (NA) is used as a media prepared by weighing 28 g of the nutrient agar (NA) powder into a glass bottle, and then distilled water is added up to 1 L and homogenized. The mixture was then sterilized using an autoclave at 121°C for 24 hours. The Agar media was removed and poured into a Petri dish, and left to solidify. In the process of rejuvenating bacteria and preparing bacterial suspensions, the tested bacteria are taken from the stock culture with an Ose needle, streaked in slant nutrient agar medium, and incubated at 37°C for 24 hours. The bacteria that have been inoculated with a needle are taken and suspended in 5 mL of 0.9% NaCl solution. Shake the solution until homogeneous and compare the turbidity of the suspension with McFarland solution. The McFarland 0.5 turbidity standard is prepared using 0.5 mL of 1% BaCl2 solution added by 9.5 mL of 1% H_2SO_4 solution. The antibacterial tests of ethanol extracts of *L. decumana* and *L. aestuans* are performed using the disc diffusion method. The test concentrations of *L. decumana* and *L. aestuans* ethanol extracts used were 2,000; 1,500; and 1,000 ppm. 1 mL of the prepared bacterial suspensions was applied to agar medium. A sterile paper disc is filled with 10 µL of the test solution and allowed to stand for a while to allow the solvent to evaporate. The paper disc is then placed on the agar surface and incubated at 37°C for 24 hours. Tetracycline is used as a positive control. The antibacterial activity is indicated by the formation of a clear zone around the paper disc at the end of the incubation period (Hidayati et al., 2023). The diameters of the inhibition zone are measured to evaluate the antibacterial activity.

Data Analysis

The data are statistically analyzed using the SAS analysis test to determine the difference in the value of the real difference between the extracts and the test parameters and continued with the Tukey test to determine the correlation between the concentration of test compounds and antibacterial ability.

RESULT AND DISCUSSION

Phytochemical analysis

The qualitative determination of bioactive compounds in plants can be done by the phytochemical screening method, which is a preliminary test performed to determine the presence of bioactive compounds, both secondary metabolites and in an extract (Gracelin et al., 2013). The two groups of bioactive phenolic and flavonoid compounds have been reported to have antibacterial properties. The types of compounds tested in this study are flavonoids and phenolics (Table 1). The extract of *L. decumana* and *L. aestuans* leaves contains flavonoids and phenolic compounds. The flavonoid test on *L. aestuans* shows a lighter color than the other. These results indicate that both extracts contain both flavonoids and phenolic compounds, possibly at different levels. Ethanol is a solvent that is similar to ethyl, so the extraction using ethanol can dissolve flavonoids and phenolic compounds generally in the same polarity. According to Ahwan et al. (2024), flavonoids are polyphenolic compounds, chemical phenolic compounds that can dissolve in bases. It is because they are a group of polar compounds that have an -OH group that can dissolve in polar solvents such as ethanol.

The quantitative test of total flavonoid and phenolic content of ethanol extracts of *L. decumana* and *L. aestuans* shows that all extracts have different flavonoid and phenolic content (Table 2). The ethanol extract of *L. decumana* leaves shows the levels of flavonoid compounds (69.4 mg GAE/g extract) are higher than phenolics (55.1 mg QE/g extract). On the contrary, the extract in *L. aestuans* is (35.3 mg GAE/g extract) of flavonoids and (55.3 mg QE/g extract) of phenolics. These results follow the color intensity in the qualitative test on flavonoids in *L. aestuan*, which is lighter than the color of *L. decumana*.

Table 1. The bioactive compounds content of the ethanol extract of *L. decumana* and *L. aestuans* leaves

Ethanol extract of plant leaves	Compounds assay			
	Flavonoid	Results	Phenolic	Results
L. decumana	++		++	
L. aestuans	+		++	

+: the positive sample contained a phytochemical assay

Table 2. The bioactive compounds content of the ethanol extract of *L. decumana* and *L. aestuans* leaves

Ethanol extract from plant leaves	Bioactive content	
	Flavonoids (GAE/g extract)	Phenolics (QE/g extract)
L. decumana	69.4 ± 1.55	55.1 ± 0.35
L. aestuans	35.3 ± 0.72	51.3 ± 1.02

GAE: gallic acids equivalent; QE: quercetin equivalent

The antibacterial activity

The agar diffusion method is used to test the antibacterial ability. The diffusion method is used to be quick, easy, and simple (Klančnik et al., 2010). The antibacterial activity of *L. aestuan* and *L. decumana* against *E. coli* and *S. aureus* is indicated by the zone of inhibition on nutrient agar media, as presented in Figure 2.

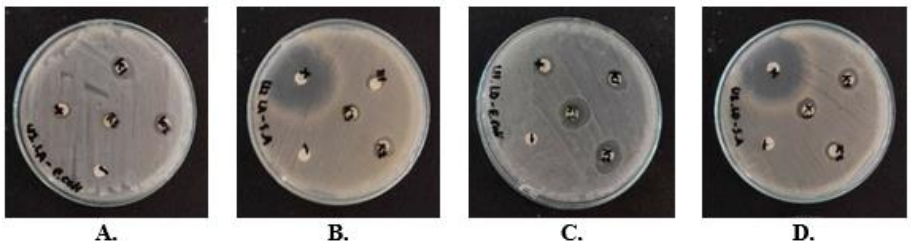

Figure 2. The antibacterial activity assay of *L. aestuan* and *L. decumana* against *E. coli* and *S. aureus*. A. *L. aestuan* against *E. coli*; B. *L. aestuan* against *S. aureus*; C. *L. decumana* against *E. coli*; and *D. L. decumana* against *S. aureus*. (-) negative control; (+) tetracycline as positive control; (K1, K2, K3) treatment concentration

The diameter of the inhibition zone formed in the agar diffusion test of *Laportea* leaf ethanol extract in inhibiting the growth of *S. aureus* and *E. coli* varied between the concentrations of extract (Table 3). According to Ołdak et al., (2017), the criteria for extract potency based on the diameter of the inhibition zone is weak if the inhibition zone is less than 5 mm, moderate if the inhibition zone is 5-10 mm, strong if the inhibition zone is 10-20 mm and very strong if the inhibition zone is 20 mm or more.

Table 3. The antibacterial activity of ethanol extract of *L. decumana* and *L. aestuans* leaves against *S. aureus* and *E. coli*

Species of *Laportea*	Concentration (µg/mL)	Diameter of inhibition zone (mm)	
		E. coli	***S. aureus***
L. decumana	Tetracycline	4.33 ± 0.26^{de}	33.0 ± 1.29^{a}
	1000	9.33 ± 2.88^{ab}	7.50 ± 1.95^{bc}
	1500	11.83 ± 0.68^{a}	7.26 ± 0.93^{bc}
	2000	11.76 ± 0.68^{a}	8.33 ± 1.57^{b}
L. aestuans	Tetracycline	3.00 ± 0.45^{e}	32.00 ± 0.45^{a}
	1000	6.26 ± 2.11^{cd}	5.50 ± 2.1^{c}
	1500	7.33 ± 1.13^{bc}	6.26 ± 1.13^{bc}
	2000	6.76 ± 1.03^{cd}	5.83 ± 1.03^{bc}

Letters next to different DIZ values in the same column indicate a significant difference

In the assay of antibacterial activity against *E. coli*, both (1,000-2,000 µg/mL) strongly significantly inhibit the growth of *E. coli* compared to tetracycline as antibacterial standard. The activity against *S. aureus* is the opposite. Both extracts inhibit the growth of *S. aureus* more than tetracycline. Examining the findings presented in Table 2 and Table 3, it appears that there is a different influence between flavonoids and phenolics, the genus of the test plant, and the test bacteria. The activity against *E. coli*, a negative gram strain of bacteria, the ability of the extract from *L. decumana* was significantly 1.5 times stronger than the extract from *L. aestuans*. In this case, it appears that the antibacterial ability against *E. coli* by the presence of flavonoids is more important than by phenolic compounds. On the other hand, in the case of *S. aureus*, a positive gram strain of bacteria, the antibacterial ability of both extracts is similar at all concentrations, which shows that phenolic compounds have a greater role in inhibiting the growth of *S. aureus*.

According to Ramona et al. (2017), the differences in cell wall structure determine the penetration, bonding, and activity of bacterial compounds. The gram-positive bacteria have a cell wall structure with a thicker peptidoglycan layer and contain polysaccharides, while the gram-negative bacteria contain more lipids, a thinner peptidoglycan layer, and an outer membrane in the form of a bilayer consisting of non-polar phospholipids and lipopolysaccharides (Lopez-Romero et al., 2015). This layer is difficult for the antibacterial substances to enter the cell. The ability of secondary metabolite compounds, such as flavonoids and phenols in plant extracts, has a role in antibacterial inhibitory ability. According to Suriyaprom et al. (2022), the mechanism of action of flavonoids in inhibiting bacteria by denaturing proteins to stop the metabolic power of bacterial cells. In the protoplasm, the phenolic processes act as toxins that destroy the cell walls (Rempe et al., 2017).

The correlation of phenolic content, flavonoids, and antibacterial activity

The compounds in the extract affect the content of metabolites that have antibacterial properties (Alibi et al., 2021). In this study, the Tukey test is used to determine the correlation between antibacterial activity and flavonoid and phenolic bioactive compounds (Table 4). R count higher than R table indicate a significant relationship while R count lower than R table indicate no significant relationship. The significant level is r-table 5% 0.279 and 1% 0.361. Both phenol and flavonoid compounds show a reciprocal relationship that was significantly different. The test results show that

the correlation between the antibacterial activity of extracts against *S. aureus* with metabolite compounds produces an R-calculated lower than the R table value, 0.036 and 0.047 for phenolic and flavonoids, respectively. On the other hand, the activity against *E. coli* bacteria shows an R-calculated higher than the R table value, 0.479 and 0.538 for phenolic and flavonoids, respectively.

Table 4. The correlation of phenolic content, flavonoids, and antibacterial activity of ethanol extracts of *Laportea* genus against *S. aureus* and *E. coli* bacteria

	DDHSA	DDHEC	Phenolic	Flavonoid
DDHSA	0	-0.608	0.036	0.047
DDHEC		0	0.479	0.538
Phenolic			0	0.941
Flavonoid				0

DDHSA = diameter of inhibition zone of *S. aureus*, DDHEC = diameter of inhibition zone of *E. coli*

These results show that *E. coli* has a positive correlation with metabolite compounds of the genus *Laportea* compared to *S. aureus*, which has a lower correlation value. This is possible because of the different cell wall structures.

CONCLUSION

Both qualitative and quantitative phytochemical analysis show the presence of flavonoids and phenolic compounds in *L. decumana* and *L. aestuans* extracts. The total flavonoid content in *L. decumana* extract (69.4 mg GAE/g extract) and *L. aestuans* show higher phenolic content (55.3 mg QE/g extract). The antibacterial activity of *L. decumana* is stronger than *L. aestuans* extract against *E. coli*. The flavonoid compounds play a major role in antibacterial activity. Further research in vivo is recommended to evaluate the ability of *L. decumana* and *L. aestuans* plants as sources of antibacterial agents

ACKNOWLEDGEMENT

The author would like to thank the Faculty of Biology, Department of Master of Biology, Satya Wacana Christian University; All staff of the Molecular Biology Laboratory and Biochemistry Laboratory for methodological support and laboratory facilities used in the research, and those who assisted in the implementation of this research

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