Trop J Nat Prod Res, January 2026; 10(1): 6968 - 6974 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Comprehensive LC–HRMS Profiling, Antioxidant and Cytotoxic Evaluation of the Acetone and Methanolic Extracts of *Wendlandia paniculata* Leaves

Yulia D. Putri[1], Purwantiningsih Sugita[2]*, Auliya Ilmiawati[2], Prasetyawan Yunianto[3], Setyanto T. Wahyudi[4], Yuli Widyastuti[3]

[1]*Postgraduate Student of Chemistry Program, Pascasarjana School of IPB University, West Java 16680, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, West Java, Indonesia*
[3]*Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN) Republic of Indonesia, Tangerang Selatan 15314, Indonesia*
[4]*Department of Physics , Faculty of Mathematics and Natural Sciences, IPB University, West Java 16880, Indonesia*

ARTICLE INFO

Article history:
Received 04 January 2026
Revised 19 January 2026
Accepted 24 January 2026
Published online 01 February 2026

Copyright: © 2026 Putri *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

NCDs remain the leading cause of global mortality, urging the search for safer, natural therapeutic agents in line with sustainable chemistry. *Wendlandia paniculata* (lontrok), traditionally used as a remedy for chest pain and a labour stimulant, is an underexplored species with potential bioactive properties. The goal of this study is to explore the metabolite profile of *W. paniculata* leaves from Indonesia and to provide an initial assessment of their biological activities. Methanol and acetone extracts were prepared and examined by LC-HRMS, yielding tentative identification of 21 secondary metabolites, mainly phenolics, flavonoids, and terpenoids. These compound groupings were validated by qualitative phytochemical screening. Quantitative analysis showed that the acetone extract contained higher TPC (169.7 ± 3.2 mg GAE/g) and TFC (55.5 ± 1.7 mg QE/g) than the methanol extract. Antioxidant assays showed greater activity in the acetone extract than in the methanol extract, with values of 79.6 ± 8.0 µg TE/mL (DPPH), 11.6 ± 0.4 µg TE/mL (FRAP), and 69.0 ± 4.8 µg TE/mL (CUPRAC). BSLT showed measurable toxicity for both extracts, with LC_{50} values of 145.8 ± 12.2 µg/mL (acetone) and 209.8 ± 8.4 µg/mL (methanol), indicating greater potency of the acetone extract. A preliminary toxicity test using the BSLT showed moderate cytotoxicity for both extracts, with the acetone extract exhibiting lower LC_{50} values, 145.8 ± 12.2 µg/mL (acetone) and 209.8 ± 8.4 µg/mL (methanol). These results provide preliminary evidence of the bioactive potential of *W. paniculata* leaves, warranting further studies to isolate active constituents and substantiate their biological effects.

Keywords: Antioxidant Activity, Metabolite Profiling, Toxicity, *Wendlandia paniculata*.

Introduction

Non-communicable diseases (NCDs) like diabetes, cancer, and heart disorders are the leading cause of death worldwide, making up about 70% of all deaths. Their rates are also rising in Indonesia.[1] In parallel with this epidemiological burden, there is a growing scientific interest in identifying safer and more accessible therapeutic alternatives. Synthetic drugs frequently exhibit limitations such as resistance and adverse effects, prompting the search for safer and more sustainable therapeutic alternatives derived from natural products.[2]
Wendlandia paniculata (Roxb.) DC. is a member of the Rubiaceae family and is found all over South and Southeast Asia.[3] Previous studies indicate that several *Wendlandia* species possess diverse secondary metabolites and exhibit various biological activities, including antioxidant,[4] antimicrobial,[5] antidiabetic,[6] hepatoprotective,[7] and anti-inflammatory effects.[8] However, there is little scientific research on *W. paniculata*, despite its use in traditional medicine in Indonesia.

*Corresponding author. Email: purwantiningsih@apps.ipb.ac.id
Tel: +62-818-686-194

Citation: Yulia D. Putri, Purwantiningsih Sugita, Auliya Ilmiawati, Prasetyawan Yunianto, Setyanto T. Wahyudi, Yuli Widyastuti. Comprehensive LC–HRMS Profiling, Antioxidant and Cytotoxic Evaluation of the Acetone and Methanolic Extracts of *Wendlandia paniculata* Leaves. Trop J Nat Prod Res. 2026; 10(1): 6968 – 6974 https://doi.org/10.26538/tjnpr/v10i1.71

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

From an ethnopharmacological perspective, traditional medicinal practices employ *W. paniculata* leaves to treat chest pain,[3] suggesting their anti-inflammatory and analgesic properties. Additionally, traditional use reports describe the plant as a labour stimulant, consistent with chemotaxonomic evidence from other Rubiaceae species that contain similar classes of secondary metabolites and exhibit related pharmacological activities.[9] These traditional uses suggest that *W. paniculata* may contain compounds with potential biological effects. So far, only a few studies have examined its pain-relieving [10] and antidiarrheal [11] effects, and no comprehensive studies integrating metabolite profiling with biological activity evaluation have been reported, particularly for Indonesian plant materials.
Therefore, this study will explore the metabolite profile of *W. paniculata* leaves from Indonesia using liquid chromatography with high-resolution mass spectrometry (LC-HRMS) and assess their biological activities to determine their bioactive potential. The results should offer early scientific evidence to support further targeted isolation and pharmacological investigations of this underexplored species.

Materials and Methods

Chemicals and Reagents

The materials used in this study consisted of *W. paniculata* leaves (Centre for Traditional Medicinal Plants, Tawangmangu, Central Java, Indonesia), and organic solvents such as methanol, acetone, ethanol, n-hexane, and ethyl acetate were distilled before use. Distilled water, silica gel 60 F_{254} TLC plates (0.25 mm), as well as silica gel 60 (70–230 and 230–400 mesh), were obtained from Merck (Germany), reagent $FeCl_3$ 5% (Merck, Germany), H_2SO_4 (Merck, Germany), Dragendorff reagent (Merck, Germany), magnesium powder (Merck, Germany), HCl (Merck, Germany), CH_3COOH (Merck, Germany), α-naphthol (Merck, Germany), $Ce(SO_4)_2{\cdot}4H_2O$ (Merck, Germany), $AlCl_3$ 10% (Merck, Germany), CH_3COONa (Merck, Germany), Folin–Ciocalteu reagent (Merck, Germany), Na_2CO_3 7.5% (Merck, Germany), quercetin

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

gallic acid (Sigma-Aldrich, USA), 1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich, USA), acetate buffer 300 mM pH 3.6) composed of sodium acetate and acetic acid (Merck, Germany), 2,4,6-tripyridyl-*s*-triazine (Sigma-Aldrich, USA), $FeCl_3 \cdot 6H_2O$; Merck, Germany), $CuCl_2 \cdot 6H_2O$ (Merck, Germany), neocuproine (Sigma-Aldrich, USA), and ammonium acetate buffer (Merck, Germany), Trolox (Sigma-Aldrich, USA), *Artemia salina* Leach eggs and NaCl were obtained from Sigma-Aldrich USA.

Plant Material

Wendlandia paniculata's leaves (lontrok) were collected from the Research Centre for Medicinal Raw Materials and Traditional Medicine (PR BBOOT), National Research and Innovation Agency (BRIN), located in Tawamangu, Karanganyar, Central Java, Indonesia (geographical coordinates: 7°41′12″ S, 111°6′15″ E; altitude 910 m above sea level). The plant specimen was taxonomically identified and verified under ELSA ID 227109 with customer sample code 6449-227109-1 at the BRIN Drug Raw Material Laboratory. The official Test Report (LHU No. 227109), dated August 7, 2025, contained the identifying results. Taxonomic authentication was ensured through verification conducted via the E-Layanan Sains (ELSA) BRIN platform.

2.2 Extraction of W. paniculata Leaves

The procedure involved washing *W. paniculata* leaves with running water, oven-drying them at 45 °C to constant weight, and grinding and sieving the material to obtain a 40-mesh powder. The study used 2,000 g of powdered simplicia for methanolic extraction and 44.4 g for acetone extraction. Extraction was performed by maceration at a solid-to-solvent ratio of 1:5 (w/v) for three consecutive 24-h cycles. After maceration, the mixture was filtered under reduced pressure using a Büchner funnel (porcelain, Duran®, 90 mm diameter). The filtrate was concentrated under reduced pressure using a rotary evaporator (Büchi R-200, Switzerland) at temperatures below 40 °C to obtain a viscous extract.[12]

Qualitative Phytochemical Screening

Major classes of phytochemicals, such as alkaloids (Dragendorff reagent), flavonoids (Shinoda test), terpenoids, and steroids (Liebermann–Burchard reagent), were qualitatively examined in the extracts.[13]

Determination of Total Flavonoid Contents

The total flavonoid content was determined using a spectrophotometric colourimetric method.[14] The extracts were first dissolved in methanol and acetone. Subsequently, the procedure mixed 10 µL of the extract solution (1.5 mg/mL) with 30 µL of 70% ethanol, 60 µL of $AlCl_3$, 60 µL of $NaCH_3COO$, and 60 µL of distilled water, and measured the absorbance at 415 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan). The study used quercetin as the calibration standard at concentrations ranging from 15.625 to 500 µg/mL,[15] the results were expressed as milligrams of quercetin equivalents per gram of extract (mg QAE/g), and the experiment was performed all measurements in triplicate.

Determination of Total Phenolic Contents

The total phenolic content was determined using the Folin–Ciocalteu colorimetric method.[14] The procedure combined 20 µL of the extract solution (1.5 mg/mL) dissolved in acetone or methanol, with 100 µL of 10 % Folin–Ciocalteu reagent, and the resulting mixture incubated for 5 min. Subsequently, 80 µL of 7.5% Na_2CO_3 was added and incubated for an additional 30 min. The analysis measured absorbance at 750 nm using a UV–Vis spectrophotometer. Gallic acid was employed as the calibration standard in the investigation, with concentrations ranging from 15.625 to 250 µg/mL.[16] The analysis expressed the data as milligrams of gallic acid equivalents per gram of extract (mg GAE/g), and the measurement was carried out in triplicate.

Metabolite Profiling by LC-HRMS

The study conducted metabolite profiling using LC–HRMS (Thermo Scientific Q Exactive™ Orbitrap). Each extract (5 mg) was dissolved in 1 mL of LC–HRMS–grade methanol, filtered through a 0.2 µm membrane, and 2.0 µL of the filtrate was injected into the system. Chromatographic separation was achieved on an Accucore™ C18 column using 0.1 % formic acid in water (A) and acetonitrile (B) with gradient elution at a flow rate of 0.2 mL/min. Mass spectrometric analysis was performed in positive electrospray ionisation (ESI^+) mode over an m/z range of 100–1500,[17] applying collision energies of 18, 35, and 53 eV. The operating conditions included a capillary temperature of 320 °C, a spray voltage of 3.8 kV, and a resolution of 70,000 FWHM. Data processing was carried out using MZmine version 4.7.8.[18]

Antioxidant Activity

This study evaluated antioxidant activity using three assays i.e. DPPH (radical scavenging activity), FRAP (ferric-reducing antioxidant power), and CUPRAC (cupric-reducing antioxidant capacity).[19] The study performed each assay in triplicate and expressed antioxidant activity as Trolox equivalents at a fixed sample concentration.

DPPH Assay

Herein DPPH was dissolved in methanol, and 180 µL of the resulting solution was mixed with 20 µL of the extract sample. Then the solution was incubated in the dark for 30 min, using Trolox as the calibration standard at concentrations ranging from 1.625 to 100 µg/mL.[19] Absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 517 nm. Data were obtained by comparing the responses of the samples and the standard using a linear regression equation (equation 1) and were expressed as antioxidant capacity.

$$y = a + bx \qquad \text{equation 1}$$

FRAP Assay

FRAP reagent were mixed with acetate buffer (pH 3.6), $FeCl_3$ (20 mM), and TPTZ (10 mM in 40 mM HCl) at a ratio of 10:1:1 (v/v/v). Then 180 µL of resulting solution was mixed with 20 µL of the extract solution, the resulting mixture was then incubated in the dark for 30 min, and absorbance was measured at 593 nm. Trolox (2.343–150 µg/mL) was used as the standard,[19] and antioxidant capacity was determined using linear regression i.e. equation 1.

CUPRAC Assay

The extracts were dissolved in the appropriate solvent and 50 µL of the extract solution was mixed with 50 µL of $CuCl_2 \cdot 6H_2O$, 50 µL of neocuproine, and 50 µL of ammonium acetate buffer (pH 7) and incubated in the dark for 30 min. Trolox was employed as the calibration standard at concentrations ranging from 2.343 to 150 µg/mL.[19] The analysis measured absorbance at 593 nm using a UV–Vis spectrophotometer and calculated antioxidant capacity by comparing sample and standard responses using linear regression (equation 1).

Toxicity assay using the Brine Shrimp Lethality Test (BSLT)

In artificial seawater with 3.8% (w/v) NaCl, *Artemia salina* eggs were cultivated for a full day until they reached the nauplius stage. In the meantime, the extract samples were dissolved in 1% DMSO in 10 mL vials at different concentrations (10–1000 µg/mL). After that, ten nauplii were put into each vial and diluted to a final volume of five millilitres using artificial seawater. The study prepared extract samples in 1% DMSO at concentrations of 10 to 1000 µg/mL in 10 mL vials. Probit analysis was used to assess the extracts' harmful effects after counting larval mortality.[20]

Statistical Analysis

We conducted all experiments in triplicate and reported results as mean ± standard deviation (SD). The analysis employed standard calibration curves to quantify total phenolic content (TPC) and total flavonoid content (TFC), and applied linear regression analysis to evaluate antioxidant assays (DPPH, FRAP, and CUPRAC). The coefficients of determination (R^2) were used to assess linearity. For antioxidant assays, the study calculated sample antioxidant capacity from Trolox standard curves using regression equations and expressed the results as Trolox equivalents.[19] The analysis determined the median lethal concentration (LC_{50}) in the brine shrimp lethality test from concentration–mortality

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

data using probit analysis. Given the exploratory nature of the study and the limited sample size, the analysis applied descriptive statistics only, without inferential comparisons between extracts. All data processing and statistical calculations were performed using Microsoft Excel (version 2019, Microsoft Corporation, Redmond, WA, USA).

Results and Discussion

Simplicia's Quality Assessment

Wendlandia paniculata simplicia met the stated quality standards for dried plant materials since its moisture level was less than 10% (**Error! Reference source not found.**). These findings indicate that the simplicia met acceptable quality criteria and were suitable for subsequent extraction and bioactivity analyses.[21]

Table 1: Moisture content of *Wendlandia paniculata* leaf simplicia determined by the oven-drying method

Sample	Weight (g)	Moisture Content (%)	Mean±SD
WP I	0.9241	8.50	
WP II	0.9249	8.48	8.46 ± 0.04
WP III	0.9259	8.42	

Description: WP I, WP II, and WP III represent replicates 1, 2, and 3 of *Wendlandia paniculata* leaf simplicia, respectively.

Effects of Different Solvents on Extraction Yield.

Methanol and acetone were used as extraction solvents because both efficiently recover phenolic and flavonoid compounds that are critical to bioactivity-related research.[22] In this study, methanol produced a higher extraction yield (20.6%) than acetone (14.7%) (Figure 1), indicating that solvent polarity influences extraction efficiency. However, the study did not evaluate biological potential based solely on extraction yield. Solvent polarity also determines the classes of compounds extracted; therefore, a higher yield does not necessarily correspond to a higher content of bioactive metabolites. [23] Based on this consideration and the primary objectives of the study, the study retained both extracts for subsequent metabolite profiling and bioactivity evaluation.

Qualitative Phytochemical Analysis

The results of the qualitative phytochemical analysis presented in Table **2** revealed the presence of flavonoids, steroids, terpenoids, saponins, and phenolics in both methanol and acetone extracts, whereas alkaloids were not detected.

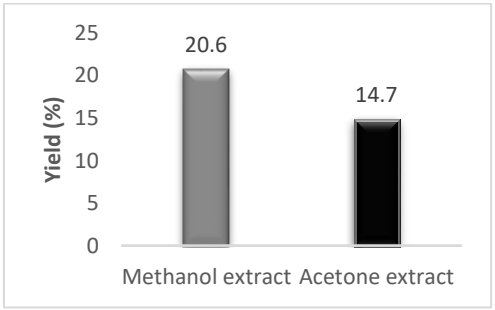

Figure 1: Effect of solvent type on the extraction yield of *Wendlandia paniculata* leaf extracts

Table 2: Qualitative phytochemical screening of methanolic and acetone extracts of *Wendlandia paniculata* leaves

Test	Extract	
	Methanol	Acetone
Alkaloid	-	-
Flavonoid	+	+
Steroid	+	+
Terpenoid	+	+
Saponin	+	+
Phenolic	+	+

Description: (+) indicates the presence of the corresponding phytochemical constituent, while (–) indicates its absence.

Determination of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

Evaluation of total flavonoids and phenolics from both extracts showed that the acetone extract had a higher total amount of phenolics and flavonoids, amounting to 169.7191 mg GAE/g dry weight and 55.5606 mg QE/g dry weight, respectively (**Error! Reference source not found.**). These results indicate that acetone acts as a more effective solvent than methanol for extracting phenolic and flavonoid compounds from *W. paniculata* leaves. Owing to its intermediate polarity, acetone efficiently solubilises both polar and moderately non-polar constituents.[24] In contrast, the higher polarity of methanol limits its ability to extract less polar compounds. Consequently, acetone facilitates the extraction of phenolic and flavonoid compounds, particularly their aglycone forms. This observation aligns with the review by Lee *et al.*,[23] which reported that semi-polar solvents generally recover higher amounts of terpenoids, complex phenolics, and flavonoid aglycones than highly polar or non-polar solvents.

Table 3: Total phenolic and flavonoid contents of methanolic and acetone extracts of *Wendlandia paniculata* leaves

Sample	Total Phenolic Content (mg GAE/g dry weight)	Total Flavonoid Content (mg QE/g dry weight)
Methanol extract	134.8 ± 7.9	43.1 ± 2.7
Acetone extract	169.7 ± 3.2	55.5 ± 1.7

Metabolite Profiling Analysis using LC-HRMS

Metabolite profiling of *W. paniculata* leaves led to the identification of 21 secondary metabolites in methanol and acetone extracts (**Error! Reference source not found.**, Figure 2, Figure 3). Most of these compounds were phenolics, flavonoids, or terpenoids, and their distribution varied depending on the solvent used.

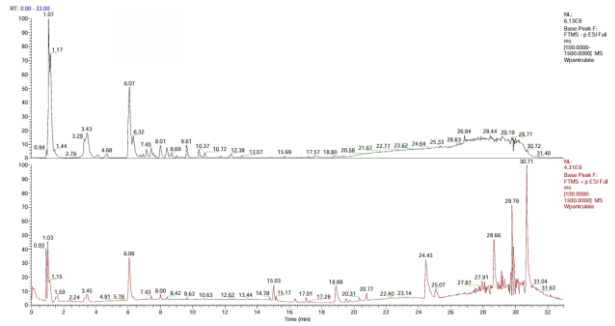

Figure 2: LC–HRMS chromatogram of the methanolic extract of *Wendlandia paniculata* leaves

Both extracts contained several phenolic and flavonoid compounds, such as ferulic acid, chlorogenic acid, rutin, catechin, and myricetin. The enhanced antioxidant activity observed in the DPPH, FRAP, and CUPRAC assays may be attributed to the presence of these compounds, which correlate with higher total phenolic and flavonoid contents, particularly in the acetone extract. These compounds contribute to antioxidant capacity through redox-related mechanisms, including hydrogen or electron donation and metal chelation, consistent with the observed *in vitro* antioxidant responses. The analysis also identified several terpenoid and triterpenoid compounds, including loliolide, stigmasterol, and ursolic acid. Previous studies on other plant species frequently associate these metabolites with general cytotoxic or bioactive effects. Although the BSLT provides only a preliminary

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

indication of cytotoxicity and does not reflect specific pharmacological activities, the presence of these compounds may contribute to the mild toxicity observed in this study. To clarify the roles of individual compounds and to better understand their specific biological effects, further studies involving targeted isolation and bioactivity evaluation are required.

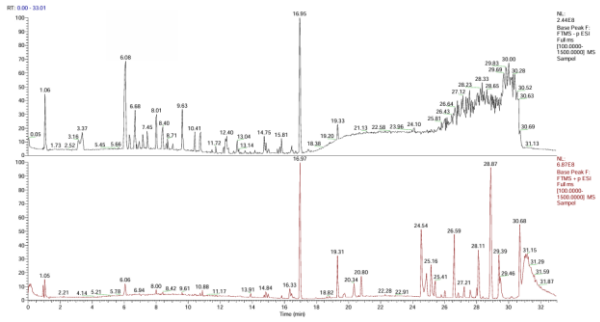

Figure 3: LC–HRMS chromatogram of the acetone extract of *Wendlandia paniculata* leaves

Table 4: Extended list of putatively identified metabolites in methanolic and acetone extracts of *Wendlandia paniculata* leaves*

No	Name	Molecular Formula	Molecular Mass	tR (min)	Annot. Delta mass	Ion Mode	MS/MS	Class	Solvent	
									RAT	RMET
1	L-(+)-Valine	$C_5H_{11}NO_2$	117.07898	1.0571	0.21	[M+H]+	118, 70, 59	Amino acid	√	√
2	Sorbitol	$C_6H_{14}O_6$	182.07904	1.0571	-0.64	$[M+H]^+$	184, 147,104,69	Sugar alcohol	–	√
3	Ferulic acid	$C_{10}H_{10}O_4$	194.05791	3.2761	-0.76	$[M+H]^+$	195, 177,149,121, 89	Phenolic	√	√
4	Scopoletin	$C_{10}H_8O_4$	192.04226	3.4833	-7.32	$[M-H]^-$	163, 145, 91	Phenolic	–	√
5	4-hydroxycoumarin	$C_9H_6O_3$	162.03169	6.0581	-1.31	$[M+H]^+$	163, 145, 135, 117, 107, 89, 79	Phenolic	√	√
6	5-O-caffeoylquinic acid (chlorogenic acid)	$C_{16}H_{18}O_9$	354.09508	6.0581	-1.62	$[M+H]^+$	163, 145, 89, 70	Phenolic	√	√
7	procyanidin B1	$C_{30}H_{26}O_{12}$	578.14243	6.7601	0.02	$[M+H]^+$	409, 291, 247, 127	Flavonoid	√	√
8	Catechin	$C_{15}H_{14}O_6$	290.07904	7.1471	-2.07	$[M+H]^+$	273, 207, 165, 139, 123	Flavonoid	√	√
9	Rutin	$C_{27}H_{30}O_{16}$	610.15338	8.422	-1.38	$[M+H]^+$	573, 479, 390, 303, 85	Flavonoid	√	√
10	Myricitin	$C_{21}H_{20}O_{12}$	464.09548	8.7044	-0.29	$[M+H]^+$	303, 229, 153, 127, 61	Flavonoid	√	√
11	loliolide	$C_{11}H_{16}O_3$	196.10994	8.9891	-1.15	$[M+H]^+$	179, 161,135, 107, 93, 73, 55	Terpenoid	√	√
12	clethric acid	$C_{30}H_{48}O_7$	505.34509	14.9908	-0.62	$[M+H]^+$	405, 279, 249, 159, 119	Lipid	√	√
13	Linolenic acid	$C_{18}H_{30}O_2$	278.22458	18.7782	-4.93	$[M-H]^-$	277, 235, 163, 135,121, 107,93	Lipid	√	√
14	3β-hydroxy-11-oxours-12-en-28-oic acid	$C_{30}H_{46}O_4$	470.33961	20.2748	-0,79	$[M-H]^+$	453, 407, 367, 201, 147, 105	Triterpenoid	√	√
15	Stigmasterol	$C_{29}H_{48}O$	412.37052	24.7585	-3.8	$[M-H]^-$	411, 341, 297, 231, 203, 163,95	Triterpenoid	√	√
16	Ursolic acid	$C_{30}H_{48}O_3$	456.36034	24.7585	-1.29	$[M+H]^+$	439, 411, 326, 231,191,95	Triterpenoid	√	√
17	Palmitamide	$C_{16}H_{33}NO$	255.25621	24.8835	-0.85	[M+H]+	256, 161, 144, 88, 57	Lipid	√	–

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

No	Name	Molecular Formula	Molecular Mass	tR (min)	Annot. Delta mass	Ion Mode	MS/MS	Class	Solvent	
									RAT	RMET
18	Oleic acid amide	$C_{18}H_{35}NO$	281.27186	25.4077	-1.12	[M+H]+	282, 265, 247, 135,69	Lipid	√	–
19	Phytol	$C_{20}H_{40}O$	296.30792	27.3465	-0.96	[M+H]+	298, 256, 226, 171, 57	Terpenoid	√	–
20	Stearic amide	$C_{18}H_{37}NO$	283.28751	27.9144	-1.47	$[M+H]^+$	284, 240, 116, 57	Lipid	√	√
21	Cis-11-Eicosenamide	$C_{20}H_{39}NO$	309.30316	28.3861	-1.54	[M+H]+	311, 275, 240, 161, 135, 69	Lipid	√	–

Description: tR: retention time (min); RAT: detected in the acetone extract; RMET: detected in the methanolic extract. Data were processed using MZmine 4.7.8 and adjusted according to the family- and genus-level databases of Wendlandia paniculata species.

Antioxidant Activity of W. paniculata Extract

Antioxidant activity evaluation revealed that the acetone extract exhibited higher antioxidant activity than the methanol extract in all tests, particularly in the FRAP and CUPRAC methods (Table 4). This result aligns with the total phenolic and flavonoid content data, which showed that the acetone extract contained higher levels of both constituents. The literature consistently indicates that higher phenolic and flavonoid contents correlate with increased antioxidant activity.[25] Other *Wendlandia* species have shown similar patterns. For instance, in *W. tinctoria*, the dichloromethane fraction had the highest total phenolic content (289.87 ± 0.47 mg GAE/g) and the maximum DPPH scavenging activity (IC_{50} = 18.83 ± 0.07 µg/mL).[4] In *W. exserta*, the aqueous fraction exhibited the highest total phenolic content (87.10 ± 1.10 mg GAE/g) and the most pronounced DPPH inhibitory activity (85.94 ± 0.35% at 1000 µg/mL).[26] Likewise, in *W. heynei*, the ethyl acetate fraction displayed the highest total phenolic content (263.6 ± 2.24 mg GAE/g), total flavonoid content (137.3 ± 1.92 mg RE/g), and the highest DPPH scavenging activity (IC_{50} = 23.12 ± 0.41 µg/mL).[27] Collectively, these findings support the association between higher total phenolic and flavonoid contents and enhanced DPPH radical scavenging activity, leading to greater DPPH scavenging activity.

Toxicity Assay using Brine Shrimp Lethality Test (BSLT)

Table 4: Antioxidant capacities of methanolic and acetone extracts of *Wendlandia paniculata* leaves determined by DPPH, FRAP, and CUPRAC assays

Sample	DPPH (µg/mL Trolox)	FRAP (µg/mL Trolox)	CUPRAC (µg/mL Trolox)
Methanol extract	73.9 ± 4.8	8.7 ± 0.7	57.0 ± 4.6
Acetone Extract	79.6 ± 8.0	11.6 ± 0.4	69.0 ± 4.8

Description: DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: ferric reducing antioxidant power, CUPRAC: cupric ion-reducing antioxidant capacity, All values are the mean ± SD (n = 3)

Based on the BSLT assay, the acetone extract of *W. paniculata* leaves exhibited higher toxicity than the methanol extract, with LC_{50} values of 145.80 µg/mL and 209.80 µg/mL, respectively. Both extracts fall into the poisonous category (LC_{50} < 1000 µg/mL).[28]

Table 5: Toxicity of methanolic and acetone extracts of *Wendlandia paniculata* leaves evaluated by the Brine Shrimp Lethality Test (BSLT)

Sample	LC_{50}
Methanol extract	209.8 ± 8.4
Acetone extract	145.8 ± 12.2

All values are the mean ± SD (n = 3)

Although the BSLT assay does not identify specific cell types or metabolic pathways, the metabolite composition of each extract may contribute to the observed differences in toxicity. LC–HRMS analysis (**Error! Reference source not found.**) showed that the acetone extract contained higher levels of sterols, fatty acid–related compounds, and triterpenoids. Previous studies have frequently associated these metabolite classes with cytotoxic effects in biological systems, particularly through interactions with cell membranes.[29] The greater lethality seen for the acetone extract may be partially explained by this association, even if it is still indirect and needs more research. Because the BSLT assay provides only preliminary toxicity information and cannot be directly predictive of mammalian cell systems, the study interprets these findings with caution. Further *in vitro* studies using mammalian cell lines are required to confirm the observed cytotoxic effects and to establish their pharmacological relevance.

Nevertheless, the study requires further investigation, including targeted biological assays and fractionation-guided isolation, to substantiate these observations. This work provides a basis for future research and contributes new baseline data on an understudied Indonesian plant species. However, findings such as this, should be viewed as preliminary screening results rather than evidence of confirmed pharmacological effects.[30]

Conclusion

This study presents the first metabolites profile and assessment of bioactive metabolites sourced from *Wendlandia paniculata* leaves native to Indonesia. Solvent polarity significantly influenced extraction yield, metabolite composition, and biological activity. More phenolic and flavonoid compounds, which are associated with improved antioxidant ability, were significantly present in the acetone extract. Preliminary BSLT results indicate that the acetone extract merits further investigation. To confirm the pharmacological significance, safety, and therapeutic potential of *W. paniculata* leaf extracts, further studies utilising mammalian cell models and mechanistic techniques are required.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Acknowledgements
The authors acknowledge financial support from the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through the Doctoral Dissertation Research Program (Contract No. 23144/IT3.D10/PT.01.03/P/B/2025).

References

1. Arifin H, Chou KR, Ibrahim K, Fitri SUR, Pradipta RO, Rias YA, Sitorus N, Wiratama BS, Setiawan A, Setyowati S, Kuswanto H, Mediarti D, Rosnani R, Sulistini R, Pahria T. Analysis of Modifiable, Non-Modifiable, and Physiological Risk Factors of Non-Communicable Diseases in Indonesia: Evidence from the 2018 Indonesian Basic Health Research. J Multidiscip Healthc. 2022;15(2):2203–2221.
2. Laksemi DAAS, Sukrama IDM, Suwanti LT, Sudarmaja IM, Damayanti PAA, Tunas IK, Wiryanthini IAD, Linawati NM. A Comprehensive Review on Medicinal Plants Potentially as Antimalarial. Trop J Nat Prod Res. 2022; 6:287–298.
3. Hossain MJ, Maliha F, Hawlader MB, Farzana M, Rashid MA. Ethnomedicinal uses, phytochemistry, pharmacology and toxicological aspects of genus *Wendlandia*: an overview. J Bangladesh Acad Sci. 2023;47(2):139–154.
4. Farzana M, Hossain J, El-shehawi AM, Sikder AA, Rahman MS, Al-mansur MA, Albogami S, Elseehy MM, Roy A, Uddin MA, Rashid MA. Phenolic Constituents from *Wendlandia tinctoria* var. grandis (Roxb.) DC. Stem Deciphering Pharmacological Potentials against Oxidation, Hyperglycemia, and Diarrhea: Phyto-Pharmacological and Computational Approaches. Molecules. 2022;27(18):5957.
5. Shahzadi T, Riaz T, Abbasi MA, Mazhar F, Shahid M, Ajaib M. *Wendlandia exserta*: A pertinent source of antioxidant and antimicrobial agent. Turk J Biochem. 2018;43(4):456–463.
6. Sheikh Y, Chanu MB, Mondal G, Manna P, Chattoraj A, Chandra Deka D, Chandra Talukdar N, Chandra Borah J. Procyanidin A2, an anti-diabetic condensed tannin extracted from: *Wendlandia glabrata*, reduces elevated G-6-Pase and mRNA levels in diabetic mice and increases glucose uptake in CC1 hepatocytes and C1C12 myoblast cells. RSC Adv. 2019;9(30):17211–17219.
7. Maryam S, Khan MR, Shah SA, Zahra Z, Majid M, Sajid M, Ali S. *In vitro* antioxidant efficacy and the therapeutic potential of *Wendlandia heynei* (Schult.) Santapau & Merchant against bisphenol A-induced hepatotoxicity in rats. Toxicol Res (Camb). 2018;7(6):1173–1190.
8. Tasdemir D, Dönmez AA, Çalis I, Rüedi P. Evaluation of biological activity of Turkish plants. Rapid screening for the antimicrobial, antioxidant, and acetylcholinesterase inhibitory potential by TLC bioautographic methods. Pharm Biol. 2004;42(4–5):374–383.
9. Gruber CW, O'Brien M. Uterotonic plants and their bioactive constituents. Vol. 77, Planta Med. 2011; 77(3):207–220.
10. Razu R. Analgesic & Antidiarrheal Activities Investigation on Methanol Extract of *Wendlandia Paniculata* : *In Vivo* Approach. Int J Innov Sci Res Technol. 2021;6(1):1188. https://www.researchgate.net/publication/348920092_Analgesic_antidiarrheal_activities_investigation_on_methanol_extract_of_*Wendlandia_paniculata*_In_Vivo_approach
11. Hasan MM, Hossain MS, Taher MA, Rahman T. Evaluation of analgesic, antidiarrheal and hypoglycemic activities of *Wendlandia paniculata* (Roxb.) DC leaves extract using mice model. Toxicol Int. 2021;28(2):155–163.
12. Purwoko M, Indarto D, Purwanto B, Soetrisno S, Kariosentono H. Identification of Chemical Compounds in *Plumbago zeylanica* Linn Leaves from Indonesia. Trop J Nat Prod Res. 2022;6(9):1440–1442.
13. Binoodha Remina C, Vimal Priya S, Karthika K. Screening of phytochemical constituents and quantitative estimation of total flavonoids and phenolic compounds of leaf extracts of *Mitracarpus hirtus* (rubiaceae). Kongunadu Res J. 2022 ;9(1):47–52.
14. Abeysinghe DT, Kumara KAH, Kaushalya KAD, Chandrika UG, Alwis DDDH. Phytochemical screening, total polyphenol, flavonoid content, *in vitro* antioxidant and antibacterial activities of Sri Lankan varieties of *Murraya koenigii* and *Micromelum minutum* leaves. Heliyon. 2021;7(7):7449. https://doi.org/10.1016/j.heliyon.2021.e07449
15. Seno DSH, Sembiring DPDB, Kurniatin PA, Nurcholis W. Total Phenolic, Total Flavonoid Contents, and Radical Acavenging Activity of Different Concentrations of Ethanol Extract of *Pyrrosia piloselloides* (L) M. G. Price. Trop J Na`t Prod Res. 2022;6(4):484–487.
16. Utami YP, Yulianty R, Djabir YY, Alam G. Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of Etlingera elatior (Jack) R.M. Smith from North Luwu, Indonesia. Trop J Nat Prod Res. 2024;8(1):5955–5961.
17. Rafi M, Madya MM, Karomah AH, Septaningsih DA, Ridwan T, Rohaeti E, Aisyah S, Idroes R. LC-HRMS-Based Metabolomics for Profiling the Metabolites in Different Plant Parts of *Centella asiatica*. Hayati. 2024;31(6):1106–1115.
18. Osman N, Zahari A, Hazni H, Nazneem WN, Othman W, Rasol NE, Ismail NH, Champy P, Beniddir MA, Litaudon M, Awang K. Isolation and Characterization of Compounds from *Ochreinauclea maingayi* (Hook. f.) Ridsd. (Rubiaceae) with the Aid of LCMS/MS Molecular Networking. Separations. 2023;10(2):74. : https://doi.org/10.3390/separations10020074
19. Makkiyah FA, Pradana DLC, Putra RP, Nurcholis W. Optimization Microwave-Assisted Extraction of Moringa oleifera Leaves Using Response Surface Methodology Focused on Extracting Phenolic And Flavonoid With Antioxidant Activity. Trop J Nat Prod Res. 2024;8(6):7474–7482.
20. Ahamed HN, Y I, Navabshan I, S MZ, TS S, Jaleel I, LH TA. Investigating the toxicity of malachite green and copper sulfate in brine shrimp: *In-vivo* and computational study. Toxicol Rep. 2024;13(13):101811.
21. Manalu LP, Adinegoro H. Drying process conditions for producing standardized *Curcuma zedoaria* (temu putih) simplicia. J Standardization. 2018;18(1):63.
22. Truong DH, 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.
23. Lee JE, Jayakody JTM, Kim J Il, Jeong JW, Choi KM, Kim TS, Seo C, Azimi I, Hyun JM, Ryu BM. The Influence of Solvent Choice on the Extraction of Bioactive Compounds from Asteraceae: A Comparative Review. Foods. 2024; 13(19):3151
24. El Mannoubi I. Impact of different solvents on extraction yield, phenolic composition, *in vitro* antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit. J Umm Al-Qura Univ Appl Sci. 2023;9(2):176–184.
25. Rahim NA, Roslan MNF, Muhamad M, Seeni A. Antioxidant Activity, Total Phenolic and Flavonoid Content and LC–MS Profiling of Leaves Extracts of Alstonia angustiloba. Separations. 2022;9(9).
26. Shahzadi T, Riaz T, Abbasi MA, Mazhar F, Shahid M, Ajaib M. *Wendlandia exserta*: A pertinent source of antioxidant and antimicrobial agent. Turk J Biochem. 2018;43(4):456–463.
27. Maryam S, Khan MR, Shah SA, Zahra Z, Majid M, Sajid M, Ali S. *In vitro* antioxidant efficacy and the therapeutic potential of *Wendlandia heynei* (Schult.) Santapau & Merchant against bisphenol A-induced hepatotoxicity in rats. Toxicol Res (Camb). 2018;7(6):1173–1190.

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

28. Zakaria Z, Soekamto NH, Dali N, Amalia HAM, Syarifuddin SH. Toxicity Test Using The Brine Shrimp Lethality Test (BSLT) Method on Extracts of Stem Bark, Stem Wood, and Leaves on Bayur (Pterospermum diversifolium B. Rob.). Elkawnie. 2024;10(1):42.
29. Radman S, Čagalj M, Šimat V, Jerković I. Seasonal Monitoring of Volatiles and Antioxidant Activity of Brown Alga Cladostephus spongiosus. Mar Drugs. 2023;21(7).
30. Maslikah SI, Lestari SR, Siri S, Yunus M, El Baroroh AR, Miasih DS, Rifa'i M, Warsito, Djati MS. Phytochemical Screening and Antioxidant Potential of Ethanol Extract From Piper crocatum Leaves. Trop J Nat Prod Res. 2025;9(7):3114–3119.

© 2026 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License