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Research Article

Metabolite Profiling and Preliminary Biological Assessment of *Sandoricum koetjape* (Burm. f.) Merr. Bark Extracts from Pekanbaru, Indonesia

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Abstract

Sandoricum koetjape (Burm. f.) Merr. is a tropical medicinal plant traditionally used in Southeast Asia; however, information on the metabolite composition and biological properties of its bark remains limited. This study aimed to characterize the metabolite profile of *S. koetjape* bark extracts obtained using solvents of different polarity and to conduct an initial evaluation of their biological activities. Bark samples collected from Pekanbaru, Indonesia, were extracted using *n*-hexane, ethyl acetate, and ethanol, and then followed by LC-MS/MS analysis. A total of 56 metabolites were identified, with triterpenoid-type compounds predominating in the ethyl acetate and ethanolic extracts, while sesquiterpenoid constituents were more characteristic of the *n*-hexane extract. Antioxidant activity was assessed using the DPPH radical scavenging assay, revealing that the ethanolic extract exhibited the strongest activity ($IC_{50} = 32.53\ \mu g\ mL^{-1}$), followed by the ethyl acetate extract ($IC_{50} = 103.73\ \mu g\ mL^{-1}$), while the *n*-hexane extract showed negligible activity within the tested range. Initial toxicity screening using the brine shrimp lethality test indicated that the ethanolic extract demonstrated higher toxicity ($LC_{50} = 94.93\ \mu g\ mL^{-1}$) compared with the other extracts. Overall, this study provides baseline phytochemical and preliminary biological data on *S. koetjape* bark extracts, highlighting solvent-dependent metabolite profiles and initial bioactivity patterns. These findings support further targeted pharmacological and mechanistic investigations of bioactive constituents from this plant.

Keywords: antioxidant, brine shrimp lethality test (BSLT), LC-MS/MS, metabolite profiling, *Sandoricum koetjape*

1. INTRODUCTION

Medicinal plants remain an important source of bioactive compounds, particularly in tropical regions where ecological diversity drives extensive chemical variation [1]. The chemical diversity of medicinal plants is strongly influenced by environmental conditions, genetic variation, and plant organ type [2][3]. Unique environmental pressures across Indonesia, including in Sumatra regions, which play a crucial role in shaping the metabolite profiles of plant species, leading to chemical diversity and potential differences in bioactivity and medicinal value [4]. Therefore, investigating the phytochemical characteristics of locally collected specimens is essential not only for supporting their potential pharmacological applications but also for contributing to the documentation of Indonesia's biodiversity.

Among medicinal plants, *Sandoricum koetjape* (Burm. f.) Merr., locally known as "sentul" or "kecapi", has long been used in traditional medicine in Indonesia and other regions to manage metabolic and inflammatory disorders [5]–[7]. In Bali, its use is recorded in the *Taru Pramana* manuscripts, and the Baduy community also employs this plant to treat diarrhea [8][9]. In Pekanbaru, Riau, the fruit peel of sentul is used as an herbal tea, a daily beverage believed to offer various health benefits [10]. Previous studies have reported that sentul contains diverse secondary metabolites, including triterpenoids, limonoids, flavonoids, tannins, polyalcohols, phenolics, and alkaloids, which associated with various pharmacological activities [7][11].

Most phytochemical investigations on this species have examined the bark, focusing on both isolated compounds and their biological properties [7]. Studies of bark extracts from West Sumatra reported variations in metabolite classes, including the absence of triterpenoids, steroids, alkaloids, coumarins, and phenolics, along with demonstrated antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [12]. Other studies from the different location but same region similarly noted

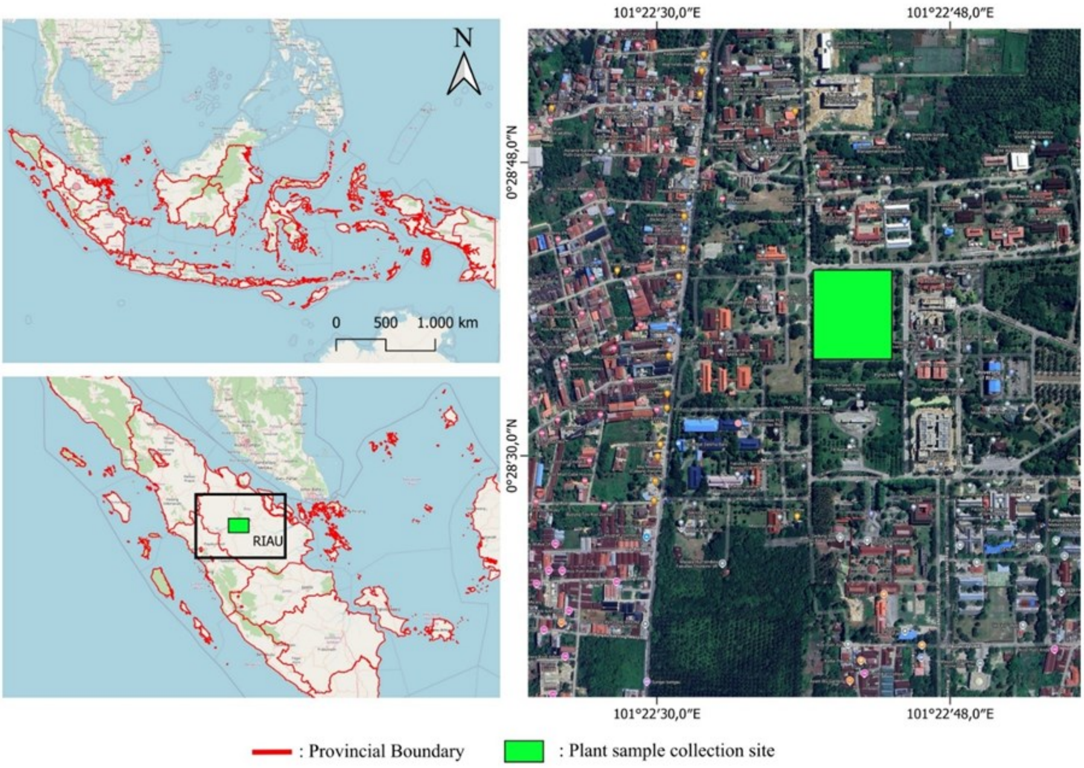

Figure 1. Location of plant sample collection at Simpang Baru, Panam, Pekanbaru, Riau Province, Indonesia. The map features provincial boundaries, a scale bar and a north arrow, indicating the precise position where bark samples of sentul. Base map sourced from open map and Esri satelite, generated using QGIS.

the absence of flavonoids, phenolics, triterpenoids, and coumarins [13]. Research on samples from Malaysia showed that *n*-hexane extracts of the stem bark exhibit cytotoxic and apoptotic effects against breast cancer cell lines (MCF-7, MDA-MB-231, T47D, and MCF-10A) [14]. In addition, aqueous acetone extracts yielded koetjapic acid and 3-oxo-olean-12-en-29-oic acid, both demonstrating ichthyotoxic and anticarcinogenic potential [15]. Despite these findings, detailed information on the metabolite composition of sentul bark remains limited, particularly regarding solvent-dependent metabolite profiles. Bark tissues may contain metabolites distinct from other plant parts due to tissue-specific biosynthetic pathways, yet these differences have not been comprehensively explored. To date, no study has applied multi-solvent extraction combined with LC–MS/MS analysis to characterize the bark of sentul collected from Pekanbaru, Riau. This gap highlights the need for a further investigation of the metabolite diversity and solvent-specific chemical distribution in this plant part.

Given the limited information on the metabolite profile of sentul bark from Pekanbaru, Riau, further evaluation of its biological potential is also essential. Early-stage biological assays provide a practical means to explore these functional characteristics. The DPPH radical scavenging assay is a widely used method for evaluating antioxidant activity, typically associated with phenolic and flavonoid constituents [16][17]. Meanwhile, the brine shrimp lethality test (BSLT) offers an initial indication of general cytotoxicity and can help reveal the presence of biologically active or potentially toxic compounds [18]. In combination with metabolite profiling, these assays support a more detailed characterization of extract bioactivity. Thus, assessing local samples is essential to identify potential differences in functional properties across regions.

The lack of detailed metabolite data and biological evaluation of sentul bark from Pekanbaru, Riau, a comprehensive analysis is required. This study therefore aimed to characterize the metabolite profile of bark extracts obtained using three solvents of differing polarity (*n*-hexane, ethyl acetate, and ethanol) through LC-MS/MS analysis; assess their antioxidant properties using the DPPH assay; and determine their preliminary toxicity through the BSLT bioassay. The resulting data are expected to broaden the phytochemical information on sentul, clarify the effects of solvent polarity on metabolite composition, and serve as baseline knowledge for subsequent pharmacological studies and biodiversity-related research.

2. MATERIALS AND METHODS

2.1. Plant Collection and Extraction

Bark material of sentul including portions of both the outer and inner bark, was collected in Pekanbaru, Riau, Indonesia (Figure 1). The species identification was confirmed by the Directorate for Scientific Collection Management, BRIN Cibinong, Bogor (Certificate No. B-3142/II.6.2/IR.01.02/9/2024). A total of 50 g of dried and powdered bark was subjected to maceration in three solvents of differing polarity: *n*-hexane, ethyl acetate, and ethanol, using a 1:10 (w/v) ratio. Each sample was macerated for 6 h with continuous stirring and the process was repeated twice. The combined extracts were filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain the crude extracts [19]. The extract yield percentage was calculated using Equation (1):

$$\text{Total extract yield (\%)} = \frac{\text{weight of crude extract}}{\text{weight of powdered plant material}} \times 100\% \qquad (1)$$

2.2. Compound Identification by LC-MS/MS

Metabolite profiling of the bark extracts was performed using an UHPLC Vanquish Tandem system coupled to a Q Exactive Plus Orbitrap HRMS (Thermo Scientific) followed the standard protocol at Advanced Laboratory of IPB University. A total of 5 mg of each extract was dissolved in 1 mL of methanol and filtered through a 0.2 µm nylon membrane prior to analysis. Chromatographic separation was carried out on an Accucore C18 column (100 × 2.1 mm, 1.5 µm; Thermo Scientific) with a flow rate of 0.2 mL/min. The mobile phases consisted of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The gradient program was set as follows: 0–1 min (5% B), 1–25 min (5–95% B), 25–28 min (95% B), and 28–33 min (5% B) for re-equilibration. The column oven was maintained at 30 °C, and the injection volume was 2 µL. Mass spectrometric detection was performed in both adduct types $[M+H]^+$ and $[M-H]^-$ using a heated electrospray ionization (HESI) source. Full-scan MS data were acquired over an m/z range of 100–1500 at a resolution of 70,000. Data-dependent MS/MS (dd-MS^2) acquisition was carried out at a resolution of 17,500 with stepped normalized collision energies (NCE) of 18, 35, and 52. Source conditions were as follows: sheath gas flow 15, auxiliary gas flow 3, sweep gas flow 0, spray voltage 3.80 kV, capillary temperature 320 °C, S-lens RF level 50, and auxiliary gas heater temperature 0 °C. Tentative compound identification was conducted by matching accurate mass, isotopic patterns, and MS/MS fragmentation spectra against online databases including ChemSpider and mzCloud. Compound identification was added by MS-DIAL ver. 3.82 followed the method by Umar, et al. [20]. Raw LC–MS/MS files were converted to .abf format using Abf Converter 4.0.0 and MS FileReader 2.2.62, and the resulting data were examined for retention time (RT), peak detection, filtering, and alignment. Retention times were recorded independently for

Table 1. LC_{50} value categories.

Clarkson's toxicity index		**McLaughlin's toxicity index**	
LC_{50} (µg mL^{-1})	**Category**	**LC_{50} (µg mL^{-1})**	**Category**
$LC_{50} > 1000$	Non toxic	$LC_{50} > 1000$	Non toxic
LC_{50} 500–1000	Low toxic	$LC_{50} < 1000$	Toxic
LC_{50} 100–500	Medium toxic	LC_{50} 30–200	Potential antimicrobial
$LC_{50} < 100$	Highly toxic	$LC_{50} < 30$	Potential anticancer

Table 2. Percentage yield of S. koetjape bark extracts obtained using three different solvent.

Type of extraction solvent	Yield (%)	Total compounds
n-Hexane	6.96	29
Ethyl acetate	7.56	32
Ethanol	14.98	36

compounds detected in different solvent extracts, as minor RT shifts may occur depending on extract matrix composition.

2.3. Antioxidant Activity (DPPH Assay)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was conducted following the standard protocol of the Tropical Biopharmaca Research Center (TropBRC), IPB University. The analysis was performed in a 96-well microplate and measured using a microplate reader. A DPPH stock solution (50 ppm) was prepared by dissolving 2.50 mg of DPPH in ethanol PA to a final volume of 50 mL. Ascorbic acid was used as the positive control, prepared at 10,000 ppm by dissolving 10 mg in 1 mL of DMSO and subsequently diluted to 20, 10, 5, 2.5, and 1.25 µg mL^{-1}. Each bark extract (*n*-hexane, ethyl acetate, and ethanol) was prepared at 10,000 ppm by dissolving 10 mg of extract in 1 mL of DMSO, then diluted with ethanol PA to obtain concentrations of 125, 100, 75, 50, and 25 µg mL^{-1}. All samples were analyzed in triplicate. For each well, 200 µL of sample, standard, or control solution was added, mixed, and incubated in the dark for 30 min. Absorbance was then measured at 517 nm.

2.4. Toxicity Activity

The BSLT was followed and modified from Hamidi, et al. [21] and Pasaribu, et al. [22]. Artificial seawater with a salinity of 25–35 ppm was used for hatching and maintaining *Artemia salina* larvae. This salinity range corresponds to the optimal conditions for *A. salina* survival, while the species is known to tolerate a wider salinity range up to approximately 300 ppm and reproduce optimally only at much higher salinities (>1500 ppm) [23]. The hatching tank was divided into dark and illuminated compartments; 0.5 g of *A. salina* eggs was placed in the dark section, while the illuminated side served as the light source. Aeration was provided continuously, and nauplii were collected after 48 h. A 10,000 ppm stock solution was prepared by dissolving 40 mg of extract in 50 µL DMSO and 4 mL of distilled water, and serial concentrations of 1000, 100, 10, and 0 µg mL^{-1} were prepared by mixing 1 mL of each solution with 5 mL artificial seawater. Ten *A. salina* nauplii were transferred into each flask, and all treatments were performed in triplicate. Mortality was recorded after 24 h, and larval death (%) was calculated using Equation (2):

$$\text{Percentation of larval death (\%)} = \frac{\text{number of dead larvae}}{\text{total alrvae}} \times 100\% \qquad \textbf{(2)}$$

LC_{50} values were determined using linear regression of probit mortality against log concentration ($y = ax + b$) in Microsoft Excel. Positive control was not included in this experiment because the objective of BSLT was to determine the intrinsic toxicity of the extract and compare LC50 values among treatments, consistent with Clarkson et al. [24] and McLaughlin et al. [25]. The interpretation of LC_{50} values followed the toxicity categories as summarized in Table 1.

2.5. Data Analysis

All experimental data were analyzed using descriptive and inferential statistical approaches. Percentage inhibition values from the DPPH antioxidant assay and percentage mortality from the BSLT assay were expressed as mean ± standard deviation (SD) from three independent replicates. IC_{50} (for DPPH) and LC_{50} (for BSLT) values were determined using linear regression analysis between the concentration series (log-transformed) and their respective responses (percent inhibition or probit mortality). Probit analysis performed in Microsoft Excel by transforming mortality proportions into probit values using the NORM.S.INV function (probit = NORM.S.INV(p) + 5), followed by linear regression analysis. Prior to further statistical testing performed in RStudio, the data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When both assumptions were met, two-way analysis of variance (ANOVA) was employed to evaluate differences among treatment groups, followed by Tukey's HSD post-hoc test to determine significant pairwise comparisons. Data analysis, including scatter and regression plots for IC_{50}/LC_{50} determination, was performed using Microsoft Excel and RStudio. The significance level for all statistical tests was set at $p < 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Sample Extraction and LC–MS/MS Profiling of Bioactive Compounds

Extraction using three solvents of different polarity levels, including non-polar, semi-polar, and polar, which are produced paste-type crude extracts with varying yields and number of detected metabolites (Table 2). Among them, the polar solvent (ethanol) produced the highest extract weight, yield percentage, and number of compounds while the non-polar solvent (*n*-hexane) resulted in the lowest yield and lowest number of compounds, consistent with the broader solubility range of seondary metabolites in more polar solvent. This finding is consistent with previous work, in which the use of a more polar solvent in the extraction of *Scenedesmus obliquus* resulted in a higher extractive value and facilitated the recovery of a wider range of compounds, particularly phenolics and flavonoids [26].

Phytochemical screening and compound identification of the *S. koetjape* stem bark using LC–MS/MS generated chromatographic profiles for each extract and blanks in both positive and negative ionization modes (Figure 2). Based on the LC–MS/MS results, a total of 56 compounds were putatively characterized by database matching and literature comparison (Table 3). The majority belonged to terpenoids (tri-/sesquiterpene/ terpenoid) with 29 compounds, followed by phenolics (10 compounds), alkaloids (9 compounds), flavonoids including isoflavones (3 compounds), and one nitrogen-containing heterocyclic compound. Some compounds were unique to specific solvent extracts, while others were shared across two or more extracts. Compounds detected in multiple extracts exhibited slight RT variations, indicating matrix-dependent chromatographic behavior.

The heatmap provides a qualitative visualization

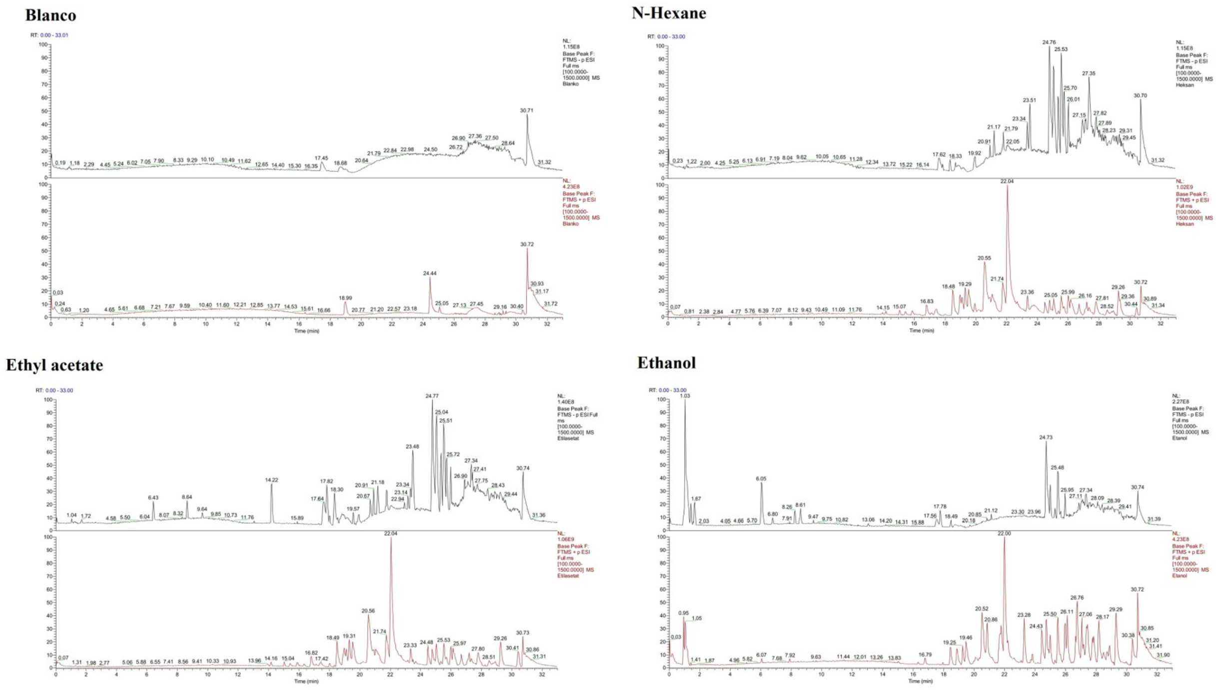

Figure 2. Chromatographic value of LC-MS/MS. The chromatogram with a black trace (upper panel) corresponds to the positive ionization mode, whereas the chromatogram with a red trace (lower panel) corresponds to the negative ionization mode.

Table 3. Tentatively identified compounds detected in *Sandoricum koetjape* bark extracts based on LC-MS/MS analysis.

No	Compound name	Formula	Molecular weight	Class	RT (min)	Extract presence		
						1	2	3
1	Curcumene	$C_{15}H_{22}$	202.1722	Sesquiterpene	21.76	+	-	-
					22.05	-	+	-
					21.99	-	-	+
2	Butylated hydroxytoluene	$C_{15}H_{24}O$	220.18227	Phenolic	22.14	+	-	-
					22.09	-	+	-
3	(E.e)-α-farnesene	$C_{15}H_{24}$	204.18743	Sesquiterpene	21.77	+	-	-
					22.17	-	+	-
					26.75	-	-	+
4	3,4-Dihydrocadalene	$C_{15}H_{20}$	200.15615	Sesquiterpene	19.02	+	+	+
5	(+)-Nootkatone	$C_{15}H_{22}O$	218.16655	Sesquiterpene	19.30	+	+	-
					19.49	-	-	+
6	Cryptophorine	$C_{17}H_{27}NO$	261.2093	Alkaloid	21.75	+	+	-
					21.69	-	-	+
7	Enoxolone	$C_{30}H_{46}O_4$	470.3396	Triterpenoid	25.54	+	+	+
8	Betulinicaldehyde	$C_{30}H_{48}O_2$	440.3654	Triterpenoid	26.15	+	-	-
					26.13	-	+	-
					26.11	-	-	+
9	3-Oxoglycyrrhetinic acid	$C_{30}H_{44}O_4$	468.324	Triterpenoid	23.15	+	-	-
					23.50	-	+	-
10	Oleanolic acid	$C_{30}H_{48}O_3$	456.3603	Triterpenoid	26.00	+	-	-
					25.98	-	+	+
11	Valerenic acid	$C_{15}H_{22}O_2$	234.162	Sesquiterpenoid	15.44	+	-	-
					14.16	-	+	-

Table 3. *Cont.*

No	Compound name	Formula	Molecular weight	Class	RT (min)	Extract presence		
						1	2	3
12	Alisol A	$C_{30}H_{50}O_5$	491.37	Triterpenoid	23.88	+	-	-
					8.16	-	-	+
13	Panaxadiol	$C_{30}H_{52}O_3$	461.39859	Triterpenoid	25.55	+	-	-
14	Dihydrochalcone	$C_{15}H_{14}O$	211.11729	Flavonoid	19.54	+	-	-
15	Benzoic acid	$C_7H_6O_2$	123.04043	Phenolic	0.99	+	-	-
16	6-Shogaol	$C_{17}H_{24}O_3$	277.17749	Phenolic	13.03	+	-	-
17	Anethole	$C_{10}H_{12}O$	149.09612	Phenolic	18.55	+	-	-
18	Thermopsine	$C_{15}H_{20}N_{2O}$	245.15088	Phenolic	16.39	+	-	-
19	Garcinoic acid	$C_{27}H_{38}O_4$	427.28369	Terpenoid	20.92	+	-	-
20	20(r)-protopanaxadiol	$C_{30}H_{52}O_3$	461.39877	Triterpenoid	22.77	+	-	-
21	Andrachcinidine	$C_{13}H_{25}NO_2$	228.19562	Alkaloid	26.83	+	-	-
					14.28	-	-	+
22	Kuwanon C	$C_{25}H_{26}O_6$	422.1729	Flavonoid	19.54	-	-	+
					19.56	-	+	-
23	Humulene (α)	$C_{15}H_{24}$	205.19461	Sesquiterpenoid	21.77	-	+	-
					26.78	-	-	+
24	α-Cyperone	$C_{15}H_{22}O$	219.1738	Sesquiterpenoid	20.66	-	+	-
25	3-Hydroxy-11-ursen-28,13-olide	$C_{30}H_{46}O_3$	455.35077	Sesquiterpenoid	28.89	-	+	-
26	Soyasapogenol A	$C_{30}H_{50}O_4$	475.37769	Triterpenoid	25.19	-	+	-
					22.52	-	-	+
27	Betulin	$C_{30}H_{50}O_2$	443.3891	Triterpenoid	22.77	-	+	+
28	Indole	C_8H_7N	118.06522	Alkaloid	22.08	-	+	-
29	4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	179.07001	Phenolic	25.21	-	+	-
30	Reserpine	$C_{33}H_{40}N_2O_9$	609.2702	Alkaloid	25.91	-	+	-

Table 3. *Cont.*

No	Compound name	Formula	Molecular weight	Class	RT (min)	Extract presence		
						1	2	3
31	Conessine	$C_{24}H_{40}N_2$	357.33627	Alkaloid	26.80	-	+	-
32	Hexadecyl ferulate	$C_{26}H_{42}O_4$	419.31558	Phenolic	28.73	-	+	-
33	9-Epiblumenol B	$C_{13}H_{22}O_3$	227.16396	Sesquiterpenoid	9.01	-	+	-
34	3-Methyl-2-butenyl caffeate	$C_{14}H_{16}O_4$	248.1049	Phenolic	7.93	-	-	+
35	Corydaline	$C_{22}H_{27}NO_4$	370.20114	Alkaloid	22.24	-	-	+
36	Niacinamide	$C_6H_6N_2O$	123.05511	Alkaloid	22.039	-	+	-
					29.101	-	-	+
37	Gentisyl alcohol	$C_7H_8O_3$	141.05109	Phenolic	0.99	-	-	+
38	18α-Glycyrrhetinic acid	$C_{30}H_{46}O_4$	471.34579	Triterpenoid	30.60	-	-	+
39	4-Methylcoumarin	$C_{10}H_8O_2$	161.05948	Phenolic	25.22	-	-	+
40	Corosolic acid	$C_{30}H_{48}O_4$	473.36169	Triterpenoid	23.21	-	-	+
41	Diosgenin	$C_{27}H_{42}O_3$	415.3197	Triterpenoid	24.05	-	-	+
42	Catharanthine	$C_{21}H_{24}N_2O_2$	337.18475	Alkaloid	24.77	-	-	+
43	Matrine	$C_{15}H_{24}N_2O$	249.18509	Alkaloid	20.07	-	-	+
44	Pterin	$C_6H_5N_5O$	164.06709	Heterosiklik nitrogen	0.987	-	-	+
45	20-Epikoetjapic acid	$C_{30}H_{46}O_4$	471.34589	Triterpene	25.526	+	-	-
					25.020	-	-	+
46	Sandorinic acid B	$C_{30}H_{46}O_5$	487.34036	Triterpenoid	21.075	-	+	-
					24.727	-	-	+
47	Sandorinic acid C	$C_{30}H_{46}O_4$	470.33316	Triterpenoid	23.142	+	-	-
					26.982	-	+	-
					23.491	-	-	+
48	Katononic acid	$C_{30}H_{46}O_3$	454.33890	Triterpene	23.618	+	-	-
					26.689	-	+	-
					24.784	-	-	+

Table 3. *Cont.*

No	Compound name	Formula	Molecular weight	Class	RT (min)	Extract presence		
						1	2	3
49	Oleanoic acid	$C_{30}H_{48}O_3$	457.36630	Triterpenoid	25.999	-	-	+
50	Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	Phenolic	6.42	-	+	-
					6.07	-	-	+
51	1,3,24-Trihydroxy-24-(hydroxymethyl)-9,19-cyclolanostan-28-oic acid	$C_{31}H_{52}O_6$	520.3764	Triterpenoid	26.01	+	-	-
					25.96	-	-	+
52	Asiatic acid	$C_{30}H_{48}O_5$	488.3502	Triterpenoid	25.73	+	-	-
					25.71	-	+	-
					25.30	-	-	+
53	Genistein	$C_{15}H_{10}O_5$	270.0528	Isoflavone	17.83	-	+	-
					17.79	-	-	+
54	Quillaic acid	$C_{30}H_{46}O_5$	486.3345	Triterpenoid	21.15	+	-	-
					21.18	-	+	+
55	Visnadine	$C_{21}H_{24}O_7$	388.1522	Phenolic	20.90	-	+	-
56	Phytolaccagenin	$C_{31}H_{48}O_7$	532.34	Triterpenoid	23.50	+	-	-
Total number of compound in each extract type						**29**	**32**	**35**

Notes: (+/-) indicates the detected/ not detectd of compounds in extract 1 (*n*-hexane extract), 2 (ethyl acetate extract), and/or 3 (ethanolic extract). Compounds were tentatively identified based on LC-MS/MS spectral data by comparison with mass spectral databases and published literature. Identification was not confirmed using authentic reference standards.

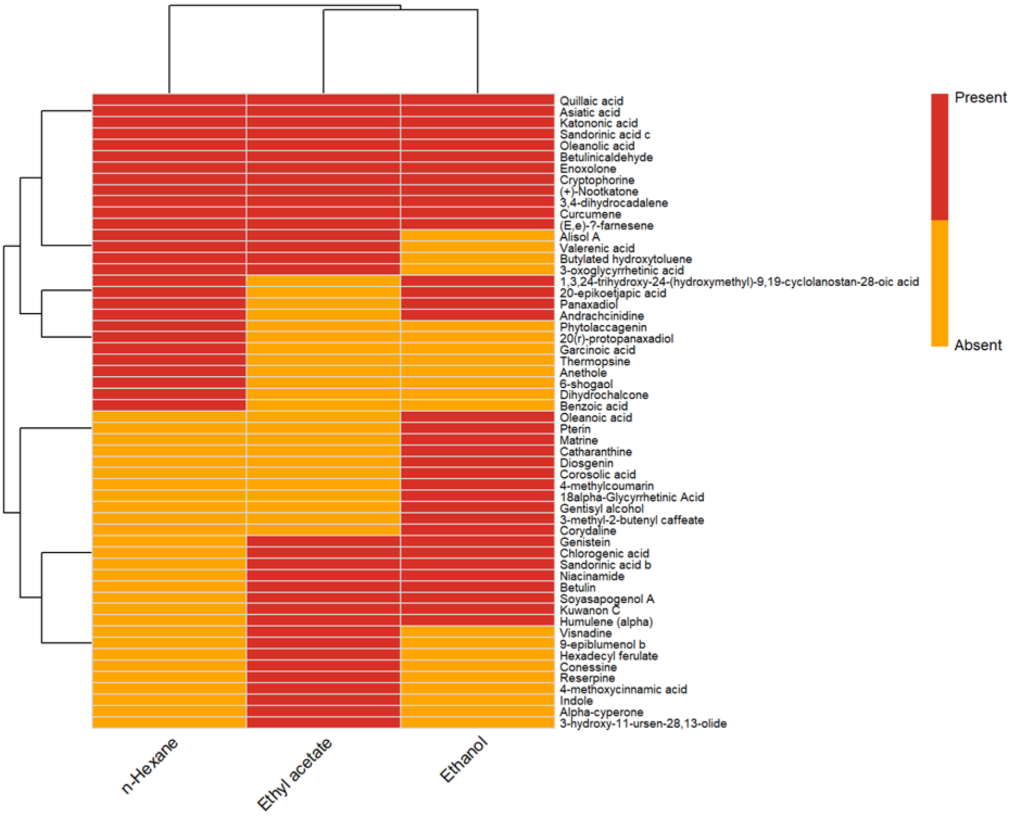

Figure 3. Heatmap of solvent-dependent metabolite distribution in *S. koetjape* bark extracts. Heatmap showing the presence or absence of tentatively identified metabolites across *n*-hexane, ethyl acetate, and ethanolic extracts based on LC–MS/MS analysis. The visualization highlights solvent-dependent metabolite distribution patterns, based on qualitative data.

of solvent-dependent metabolite distribution across *n*-hexane, ethyl acetate, and ethanolic extracts, revealing both shared and solvent-specific metabolite patterns (Figure 3). Several metabolites were consistently detected in all extracts, indicating a common core profile, while distinct clusters were selectively associated with solvents of different polarity. Non-polar metabolites were primarily linked to the *n*-hexane extract, whereas ethyl acetate and ethanolic extracts exhibited greater metabolite diversity. This clustering pattern highlights the influence of solvent polarity on extraction efficiency and complements the LC–MS/MS findings by emphasizing presence–absence relationships rather than quantitative abundance. The broader metabolite coverage observed in the ethanolic extract further supports its suitability for capturing chemically diverse secondary metabolites that may contribute to the higher biological activity observed in preliminary antioxidant screening.

Several compounds identified in the LC-MS/MS analysis have been previously reported in *S. koetjape* from other geographical origins. α-Humulene was detected in GC-MS results from methanolic stem bark extract of sentul collected in Tangerang [27], while coumarin derivatives, including 4-methylcoumarin and visnadine, were previously isolated from *n*-hexane and ethyl acetate extracts of stem bark from West Sumatra [28]. Chlorogenic acid was also identified from methanolic extracts of stems originating from Bogor [29]. Additionally, several triterpenoids such as 20-epikoetjapic acid, sandorinic acid B-C, katononic acid, and oleanoic acid were previously isolated from Thai samples [30]-[32].

Notably, several metabolites tentatively identified in the present study have not been reported in earlier investigations of *S. koetjape* bark from other geographical origins. These include curcumene, (E,E)-α-farnesene, 3,4-dihydrocadalene, (+)-nootkatone, cryptophorine, enoxolone, betulinicaldehyde, oleanolic acid, asiatic acid, and quillaic acid, which were detected in the Pekanbaru samples but have not been documented in previous studies conducted in Tangerang, Bogor, West Sumatra, or Thailand. This observation suggests a degree of geographical or ecological influence on the metabolite composition of *S. koetjape* collected from Pekanbaru, Riau. Conversely, several compounds reported in earlier studies were not detected in the present analysis, further supporting the presence of regional chemotaxonomic variation [7]. Collectively, these findings highlight the scientific merit of investigating *S. koetjape* from underexplored locations and emphasize the importance of geographical origin in shaping its metabolite profile. Nevertheless, structural confirmation through compound isolation and elucidation remains necessary to validate these tentative identifications.

Secondary metabolites were predominantly triterpenoids and sesquiterpenoids, consistently found across extract types, including curcumene, (E,E)-α-farnesene, 3,4-dihydrocadalene, (+)-nootkatone, cryptophorine, enoxolone, betulinicaldehyde, oleanolic acid, katononic acid, asiatic acid, and quillaic acid. Their detection in multiple extraction solvents suggests that these compounds act as major metabolites, indicating high abundance and chemical stability in sentul from Pekanbaru, Riau. Several of these metabolites

Table 4. Antioxidant activity of bark extracts at different concentrations.

Sample extract	Concentration (μg mL^{-1})	%Inhibition (Mean±SD)	IC$_{50}$ (μg mL^{-1})
n-Hexane	125	4.18 ± 0.16^{i}	Not Determined
	100	3.36 ± 0.16^{fi}	
	75	2.63 ± 0.42^{f}	
	50	1.45 ± 0.31^{b}	
	25	0.54 ± 0.27^{b}	
	0	0	
Ethyl acetate	125	56.04 ± 0.42^{j}	103.73
	100	49.05 ± 0.27^{g}	
	75	40.87 ± 0.00^{a}	
	50	29.97 ± 0.27^{c}	
	25	15.17 ± 0.16^{a}	
	0	0	
Ethanol	125	91.372 ± 0.16^{h}	32.53
	100	86.19 ± 0.16^{h}	
	75	75.93 ± 0.57^{e}	
	50	65.21 ± 0.57^{d}	
	25	40.78 ± 0.42^{k}	
	0	0	
Ascorbic acid	20	91.46±0.31	5.65
	10	67.94±0.57	
	5	48.86±0.16	
	2.5	26.16±0.55	
	1.25	9.81±0.27	
	0	0	

Note: Different lowercase letters indicate significant differences among treatments based on Tukey's HSD post-hoc test ($p < 0.05$). Identical letters denote groups that are not significantly different.

have documented pharmacological activities. Curcumene exhibits antimicrobial activity against bacteria and yeasts, including *Saccharomyces cerevisiae* (MIC/MFC = 0.8 mg/mL) [32]. (E,E)-α-Farnesene demonstrates anti-inflammatory, anticancer, antiviral, neuroprotective, and antidepressant activities [33]–[36]. The 3,4-dihydrocadalene demonstrates anti-breast cancer [37], nootkatone as antimicrobial, insecticidal, antioxidant, anti-inflammatory, anticancer, cardioprotective, neuroprotective, hepatoprotective, and nephroprotective [38], enoxolone as anti-inflammatory and anticancer [39], and betulinicaldehyde shows anti-lung cancer activity against A549 cells [40]. Previous studies have reported that oleanolic acid exhibits potential anticancer, anti-osteoporotic, and anti-inflammatory activities [41][42]. Katononic acid shows anti-inflammatory, cytotoxic (ED_{50} = 0.61 µg/mL), α-glucosidase inhibitory (IC_{50} = 15.7 ± 0.8 µM), ichthyotoxic, and anti-EBV activities [15][31][32][39][40][43]. Asiatic acid is well-known for neuroprotective, anti-inflammatory, antibacterial, antihypertensive, cardioprotective, and antitumor effects [44]–[46], while quillaic acid as anticancer [47], and immunoadjuvant [48]. The detection of triterpenoids and sesquiterpenoids in this study, may contribute to the observed biological responses, as similar compound classes have been reported to exhibit antioxidant and toxicity-related activities in previous studies [33]–[48].

3.2. Antioxidant Activity using the DPPH Assay

The two-way ANOVA revethyl acetate extractsled that solvent type, concentration, and their interaction significantly influenced DPPH radical scavenging activity ($p < 0.001$). Post-hoc analysis separated the treatments into 11 statistical groups (Table 4). The inhibition was observed in group A, consisting of ethanolic (25 µg mL^{-1}) and ethyl acetate extracts (75 µg mL^{-1}), indicating that low-concentration ethanolic and mid-level ethyl acetate extracts produced the strongest antioxidant response. High concentrations of ethanolic extracts (100 and 125 µg mL^{-1}) formed group H, confirming that ethanolic extracts consistently exhibited high antioxidant activity across concentrations. Ethyl acetate extracts at higher doses (100 and 125 µg mL^{-1}) clustered into groups G and J, reflecting a moderate-to-strong increase in activity with rising concentration. Treatments in groups C–F, including ethyl acetate (50 µg mL^{-1}), ethanolic (50 and 75 µg mL^{-1}), and *n*-hexane (75 µg mL^{-1}) extracts, demonstrated intermediate activity. Meanwhile, *n*-hexane at low doses (25 and 50 µg mL^{-1}) formed group B, showing minimal inhibitory effects, whereas *n*-hexane extracts (125 µg mL^{-1}; group I) exhibited very low activity. The lowest inhibition was recorded for ethyl acetate extracts (25 µg mL^{-1}; group K).

The ethanolic extract showed the highest DPPH radical scavenging activity among the tested extracts, indicating strong antioxidant capacity under the applied assay conditions, with an IC_{50} value of 32.53 µg mL^{-1}, followed by the ethyl acetate extract (103.73 µg mL^{-1}). The IC_{50} of the n-hexane extract could not be determined within the tested concentration range. Compared with the positive control, ascorbic acid (IC_{50} 5.65 µg mL^{-1}), ethanolic was the most effective extraction solvent. Ethyl acetate demonstrated a concentration-dependent increase in activity, whereas *n*-hexane contributed minimally. These patterns indicate strong solvent–concentration interaction effects on antioxidant activity. The differences in antioxidant activity among the extracts are likely related to solvent polarity, which affects the solubility of phenolic and flavonoid compounds as the main contributors to radical-scavenging activity [49][50]. Polar solvents such as ethanolic tend to extract higher amounts of phenolic compounds compared to semi-polar or non-polar solvents like ethyl acetate and *n*-hexane.

The 3-oxoglycyrrhetinic acid, valerenic acid, and phytolaccagenin, terpenoid types compound, were predominantly detected in the non-polar-polar extracts (*n*-hexane and ethyl acetate), which is consistent with their lipophilic chemical characteristics. These compounds are known to exhibit biological activities such as antiviral, antitumor and neuroprotector, making them relevant candidates for further investigation [51]–[53]. In contrast, phenolic and flavonoid compounds were more abundant in semi-polarpolar extracts (ethyl acetate and ethanol), including kuwanon C, 3-methyl-2-butenyl caffeate, gentisyl alcohol, 4-methylcoumarin, chlorogenic acid, and genistein. These compounds are well recognized for their

Table 5. Percentage of larva mortality, LC_{50} values of sentul bark extract and toxicity classification according to Clarkson's and Mc Laughlin.

Sample extract	Concentration (µg mL-1)	%Mortality (Mean±SD)	LC50 (µg mL-1)	Toxicity categories*
n-Hexane	1000	26.67 ± 1.52^{a}	>1000	Non toxic
	100	13.33 ± 1.52^{a}		
	10	6.67 ± 0.57^{a}		
	0	0		
Ethyl acetate	1000	13.33 ± 0.57^{a}	>1000	Non toxic
	100	6.67 ± 0.57^{a}		
	10	6.67 ± 0.57^{a}		
	0	0		
Ethanol	1000	96.67 ± 0.57^{b}	94.93	Highly toxic/ potential antimicrobial
	100	26.67 ± 1.15^{a}		
	10	13.33 ± 0.57^{a}		
	0	0		

Note: Means followed by different letters differ significantly ($p < 0.05$; two-way ANOVA followed by post-hoc test). %Mortality values are mean±SD ($n = 3$). *According to Clarkson's and McLaughlin's toxicity index for BSLT.

strong antioxidant properties and their role in protecting cells against oxidative stress, supporting the use of the ethanolic extract as a potential source of natural antioxidants [54]–[58]. The predominance of these compounds in the ethanolic extract may partly explain its higher DPPH radical scavenging activity, as phenolic constituents are widely recognized for their antioxidant properties. Additionally, compounds detected exclusively in a single extract, such as 20(r)-protopanaxodiol, alpha-cyperone, 3-hydroxy-11-ursen-28,13-olide, 9-epiblumenol B, and oleanoic acid, add value for targeted bioactivity exploration, particularly in relation to anticancer potential that has been widely reported for ursane- and oleanane-type triterpenoid derivatives [59]–[62].

There are still limited data on the antioxidant activity of sentul stem-bark extracts, although several previous studies have evaluated the antioxidant potential of its leaves and fruits. The crude methanolic leaf extract from samples collected in Bali, Indonesia showed a DPPH IC_{50} value of 8.14 µg/mL [63]. Meanwhile, the methanolic crude fruit extract from samples grown in Thailand exhibited IC_{50} values of 30.00 µg/mL and 48.00 µg/mL in the DPPH and ABTS assays, respectively. In contrast, the crude fruit flesh extract showed bigger IC_{50} values of 124.00 µg/mL (DPPH) and 217.00 µg/mL (ABTS) [64]. Compared with the findings of the present study, the crude stem-bark extract may not demonstrate stronger antioxidant activity than the leaf extract, but it shows comparable or better activity than the fruit extract. These differences may be influenced by the plant's geographical origin. Nevertheless, the present findings provide baseline data that may support further pharmacological and mechanistic investigations of *S. koetjape*.

3.3 Toxicity Evaluation using BSLT

The two-way ANOVA revealed that fraction type, concentration, and their interaction had a significant effect on larval mortality ($p < 0.05$). The post-hoc analysis showed that the ethanol fraction exhibited the highest toxicity, particularly at 1000 µg mL^{-1}, which was the only group that differed significantly (letter "b"). All other groups fell within a single statistical group ("a"), indicating no significant differences among fractions or concentrations in the 10–100 µg mL^{-1} range (Table 5). These results suggest that a consistent and pronounced increase in toxicity occurred only in the high-dose ethanol fraction, whereas the n-hexane and ethyl acetate fractions exhibited relatively low toxicity across all concentrations.

The ethanolic extract showed higher toxicity in the Brine Shrimp Lethality Test compared with other extracts, with an LC_{50} value of 94.93 µg mL^{-1}, categories as highly toxic and suggesting an initial indication of general toxicity. In contrast, the *n*-hexane and ethyl acetate extracts showed low toxicity, each with LC_{50} values >1000 µg mL^{-1}, classified as non-toxic. A higher LC_{50} value indicates low toxicity and general safety for long-term use at higher doses [65], whereas a lower LC_{50} value indicates that only a small dose is required to produce a biological effect, related to an advantageous pharmacological activities. Therefore, the toxicity observed in the BSLT represents a preliminary indication of general, nonspecific extract-level toxicity and does not differentiate between poisonous effects and selective cytotoxicity toward pathological cells, nor does it provide evidence of anticancer activity [66]. Furthermore, the observed toxicity does not imply that the traditional use of *S. koetjape* poses inherent risks to human health, as traditional applications typically involve different preparation methods, dosages, and exposure routes that are not addressed by the BSLT model. The toxicity data obtained from BSLT should be regarded as preliminary safety-related information rather than evidence of cytotoxic or therapeutic potential.

A previous study on the stem bark of *Sandoricum indicum* (synonym for *Sandoricum koetjape* (Burm. f.) Merr.) from Phitsanulok, Thailand, reported varying levels of toxicity across different extracts, namely methanol crude extract, *n* -hexane fraction, 90% methanol fraction, and water fraction (LC_{50} = 234.08, 48.89, 56.03, and 796.99 µg mL^{-1}, respectively) [67]. That study also showed that fractionation using n-hexane produced higher toxicity than the crude extract, indicating the presence of lipophilic metabolites, including terpenoids or limonoids, which commonly found in the Meliaceae family. Compared with the findings of Pisutthanan et al. [67], the ethanolic extract of sentul bark from Pekanbaru, Riau demonstrated a stronger toxic response, highlighting its potential as a source of pharmacologically active compounds. These findings also emphasize the importance of regional biodiversity in shaping metabolite composition.

This study is limited by the use of extract-level and screening-based assays, including a single antioxidant test and the BSLT, which do not provide mechanistic or target-specific insights. Although total phenolic content was not quantified, LC-MS/MS profiling indicated the presence of multiple phenolic and flavonoid compounds predominantly in the ethanolic extract. Given the qualitative nature of the metabolite profiling and the screening-level DPPH assay, no direct quantitative relationship between phenolic content and antioxidant activity can be established. Accordingly, the lack of total phenolic content analysis is acknowledged as a limitation and should be addressed in future studies using quantitative approaches. Further investigations involving compound isolation, cell-based assays, and mechanistic evaluations are necessary to substantiate and extend the present findings.

4. CONCLUSIONS

The present study demonstrates that the bark of *S. koetjape* contains a diverse range of secondary metabolites, as revealed by LC-MS/MS-based profiling, with triterpenoids and sesquiterpenoids as the predominant compound classes. Extraction using solvents of different polarity resulted in distinct metabolite compositions, which were reflected in their biological responses. The ethanolic extract exhibited the highest antioxidant activity in the DPPH assay and showed higher toxicity in the BSLT, whereas the *n*-hexane and ethyl acetate extracts displayed lower biological effects. This may be attributed to the presence of several dominant phenolic compounds in the ethanolic extract, such as kuwanon C, 3-methyl-2-butenyl caffeate, gentisyl alcohol, 4-methylcoumarin, chlorogenic acid, and genistein. Importantly, the biological assays employed in this study represent preliminary screening approaches and do not provide mechanistic or target-specific information. Nevertheless, the integration of metabolite profiling with initial bioactivity assessment offers valuable baseline data on *S. koetjape* bark extracts. These findings can serve as a foundation for future studies involving compound isolation, advanced bioassays, and mechanistic pharmacological evaluation.

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Conflicts of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

Not applicable.

REFERENCES

[1] L. Salvi, C. Brunetti, E. Cataldo, P. Storchi, and G. B. Mattii. (2020). "Eco-Physiological Traits and Phenylpropanoid Profiling on Potted Vitis vinifera L. cv Pinot Noir Subjected to Ascophyllum nodosum Treatments Under Post-Veraison Low Water Availability". *Applied Sciences.* **10** : 1-18. 10.3390/app10134473.

[2] I. Paul, M. Poddar, S. Pratap, and B. Singh. (2022). "Floral Secondary Metabolites in Context of Biotic and Abiotic Stress Factors". *Chemoecology.* **32** (2): 49-68. 10.1007/s00049-021-00366-0.

[3] G. A. De Queiroz, E. F. Guimar, Y. J. Ramos, and D. D. L. Moreira. (2024). "Spatio-Temporal Variations of Volatile Metabolites as an Eco-Physiological Response of a Native Species in the Tropical Forest". *Plants.* **13** : 1-21. 10.3390/plants13182599.

[4] D. Mayasari, G. Faroliu, V. K. Utama, and Y. B. Murti. (2025). "Effect of Geographical Locations on the Antioxidant Activity of Melastoma malabathricum L. Leaves Extracts From Riau and Riau Island Provinces, Indonesia". *Tropical Journal of Natural Product Research.* **9** : 1385-1390. 10.26538/tjnpr/v9i4.4.

[5] T. H. R. Belgica, M. D. Suba, and G. J. D. Alejandro. (2021). "Quantitative Ethnobotanical Study of Medicinal Flora Used by Local Inhabitants in Selected Barangay of Malinao, Albay, Philippines".

Biodiversitas. **22** (7): 2711-2721. 10.13057/biodiv/d220720.

[6] H. H. Lwin and A. A. Aung. (2021). "Ethnomedicinal Study of Some Edible Plants in Kalwin Village, Myeik Township". *Journal of the Myanmar Academy of Arts and Science.* **19** (4): 425-433.

[7] M. D. Wijaya. (2022). "Ethnomedicinal, Phytochemicals, and Pharmacological Aspects of Sentul (Sandoricum koetjape)". *Biology, Medicine, and Natural Product Chemistry.* **11** (1): 65-73. 10.14421/biomedich.2022.111.65-73.

[8] P. E. S. Adnyana. (2020). "Lontar Taru Pramana: Pelestarian Budaya Pengobatan Tradisional Bali". *Jurnal Yoga dan Kesehatan.* **2** (2): 178. 10.25078/jyk.v2i2.1572.

[9] R. O. Igiastini, I. Wahyuni, and I. Saraswat. (2021). "Ethnobotanical Study of Digestive Systems Disorders in Baduy Ethnic Indonesia". *Biotropia.* **28** (1): 54-63. 10.11598/btb.0.0.0.1055.

[10] N. Izazah and N. Anisa. (2021). "Potensi Kecapi (Sandoricum koetjape): Pohon Larat Nan Kaya Manfaat Asal Riau". Taman Karya (IKAPI), Pekanbaru.

[11] C. Bailly. (2022). "The Health Benefits of Santol Fruits and Bioactive Products Isolated From Sandoricum koetjape Merr.: A Scoping Review". *Journal of Food Biochemistry.* **46** (7). 10.1111/jfbc.14152.

[12] S. P. Sari. (2018). "Isolasi Senyawa Metabolit Sekunder Dari Ekstrak Etil Asetat Kulit Kayu Kecapi (Sandoricum koetjape Merr.) dan Uji Aktivitas Antibakteri". Universitas Andalas, Padang.

[13] M. Famaidi. (2013). "Isolasi dan Karakterisasi Senyawa Metabolit Sekunder Dari Fraksi Aktif Terhadap Brine Shrimp Lethality Bioassay Dari Kulit Batang Kecapi (Sandoricum koetjape)," Universitas Andalas, Padang.

[14] A. F. A. Aisha, S. A. Alrokayan, and K. M. Abu-Salah. (2009). "In Vitro Cytotoxic and Apoptotic Properties of the Stem Bark Extract of Sandoricum koetjape on Breast Cancer Cells". *International Journal of Cancer Research.* **5** (3): 123-129. 10.3923/ijcr.2009.123.129.

[15] I. S. Ismail. (2003). "Ichthyotoxic and Anticarcinogenic Effects of Triterpenoids From Sandoricum koetjape Bark". *Biological and Pharmaceutical Bulletin.* **26** (9): 1351-1353. 10.1248/bpb.26.1351.

[16] S. Aryal, M. K. Baniya, K. Danekhu, P. Kunwar, R. Gurung, and N. Koirala. (2019). "Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables From Western Nepal". *Plants.* **8** (4): 1-12. 10.3390/plants8040096.

[17] M. Yamauchi, Y. Kitamura, H. Nagano, J. Kawatsu, and H. Gotoh. (2024). "DPPH Measurements and Structure–Activity Relationship Studies on the Antioxidant Capacity of Phenols". *Antioxidants.* **13** (3): 309. 10.3390/antiox13030309.

[18] H. Niksic, F. Becic, E. Koric, I. Gusic, E. Omeragic, and S. Muratovic. (2021). "Cytotoxicity Screening of Thymus vulgaris L. Essential Oil in Brine Shrimp Nauplii and Cancer Cell Lines". *Scientific Reports.* **11** : 13178-1. 10.1038/s41598-021-92679-x.

[19] N. Bennour, H. Mighri, H. Eljani, T. Zammouri, and A. Akrout. (2020). "Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of Moringa oleifera cultivated in Southern Tunisia". *South African Journal of Botany.* **129** : 181-190. 10.1016/j.sajb.2019.05.005.

[20] A. H. Umar. (2025). "Unveiling the Pharmacological Mechanism of Cosmos caudatus Compounds as Lung Cancer Drug Candidates: Pharmacology Networking, Molecular Docking, and Experimental Validation". *Journal of Pharmaceutical Innovation.* **20** (3): 1-19. 10.1007/s12247-025-09989-0.

[21] M. R. Hamidi, B. Jovanova, and T. K. Panovska. (2014). "Toxicological Evaluation of the Plant Products Using Brine Shrimp (Artemia salina L.) Model". *Macedonian Pharmaceutical Bulletin.* **60** (1): 9-18. 10.33320/maced.pharm.bull.2014.60.01.002.

[22] G. Pasaribu, E. Budianto, and A. H. Cahyana. (2021). "Toxicity and Total Phenolic Content of Saurauia vulcani Extracts From Cultivation". *IOP Conference Series:*

Materials Science and Engineering. **1011** : 012068. 10.1088/1757-899X/1011/1/012068.

[23] H. Hamdani and S. Astuti. (2001). "The Effect of Salinity on the Intrinsic Growth Rate of Artemia sp". *Jurnal Bionatura.* **3** (1): 18-26.

[24] C. Clarkson. (2004). "In Vitro Antiplasmodial Activity of Medicinal Plants Native to or Naturalised in South Africa". *Journal of Ethnopharmacology.* **92** (2-3): 177 -191. 10.1016/j.jep.2004.02.011.

[25] J. L. McLaughlin, J. E. Anderson, and C. M. Goetz. (1991). "A Blind Comparison of Simple Bench-Top Bioassays and Human Tumour Cell Cytotoxicities as Antitumor Prescreens". *Phytochemical Analysis.* **2** (3): 107-111. 10.1002/pca.2800020303.

[26] C. Mukherjee, P. G. Suryawanshi, M. Chandra, K. Dhanapati, and D. Donato. (2022). "Polarity-Wise Successive Solvent Extraction of Scenedesmus obliquus Biomass and Characterization of the Crude Extracts for Broad-Spectrum Antibacterial Activity". *Biomass Conversion and Biorefinery.* 10.1007/s13399-022-02432-1.

[27] Warsinah, E. Kusumawati, and S. Sunarto. (2015). "Identification of Antifungal Compounds of Sandoricum koetjape Stem and Activity Against Candida albicans". *Majalah Obat Tradisional.* **16** (3): 170-178. 10.22146/TRADMEDJ.8055.

[28] F. E. Susanti, M. Efdi, and Afrizal. (2016). "Isolasi Senyawa Kumarin Dari Kulit Batang Kecapi (Sandoricum koetjape) Sebagai Antibakteri". *Jurnal Katalisator.* **1** (1): 10.22216/jk.v1i1.994.

[29] S. M. Tulandi, S. Saadah, and R. A. Rohman. (2023). "Seven Types of Phenolic Acid and Inhibitory Activity for Protein Tyrosine Phosphatase 1B (PTP1B) From the Stem of Sandoricum koetjape". *IOP Conference Series: Earth and Environmental Science.* **1255** (1): 012070. 10.1088/1755-1315/1255/1/012070.

[30] N. Kaneda, J. M. Pezzuto, D. A. Kinghorn, and N. R. Farnsworth. (1992). "Plant Anticancer Agents, I. Cytotoxic Triterpenes From Sandoricum koetjape Stems". *Journal Of Natural Products.* **55** (5): 654-659. 10.1021/np50083a016.

[31] T. Tanaka. (2001). "New Multiflorane-Type Triterpenoid Acids From Sandoricum indicum". *Journal Of Natural Products.* **64** (9): 1243-1245. 10.1021/np010196w.

[32] G. Narjara. (2015). "Antimicrobial Evaluation of Sesquiterpene Alpha-Curcumene and Its Synergism With Imipenem". *Journal of Microbiology, Biotechnology and Food Science.* **4** (5): 434-436. 10.15414/jmbfs.2015.4.5.434-436.

[33] I. A. Schepetkin. (2022). "Neutrophil Immunomodulatory Activity of Nerolidol, a Major Component of Essential Oils From Populus balsamifera Buds and Propolis". *Plants.* **11** (23): 3399. 10.3390/plants11233399.

[34] O. T. Ayodele. (2023). "Biochemical Constituents and Anticancer Activities of the Essential Oils of Lannea egregia Engl. & K. Krause". *Journal of Complementary and Alternative Medical Research.* **21** (4): 33-42. 10.9734/jocamr/2023/v21i4446.

[35] A. Habibah, G. Zahira, M. Febri Angga, S. Nishfatul, and D. A. Rokhim. (2023). "Schematic Literature Review: Content of Alpha-Farnesene Compounds as an Anti-Virus". *Manganite: Journal of Chemistry and Education.* **2** (1): 24-29. 10.58330/manganite.v2i1.315.

[36] K. N. Tran, N. P. K. Nguyen, L. T. H. Nguyen, H. M. Shin, and I. J. Yang. (2023). "Screening for Neuroprotective and Rapid Antidepressant-Like Effects of 20 Essential Oils". *Biomedicines.* **11** (5): 1248. 10.3390/biomedicines11051248.

[37] A. Mendoza-Fuentes. (2023). "The Cytotoxicity Effect of 7-Hydroxy-3,4-Dihydrocadalene From Heterotheca inuloides and Semisynthetic Cadalenes Derivates Towards Breast Cancer Cells: Involvement of Oxidative Stress-Mediated Apoptosis". *PeerJ.* **11** : e15586. 10.7717/peerj.15586.

[38] L. L. Zhang, Y. Chen, Z. J. Li, G. Fan, and X. Li. (2023). "Production, Function, and Applications of the Sesquiterpenes Valencene and Nootkatone: A Comprehensive Review". *Journal of Agricultural and Food Chemistry.* **71** (1): 121

-142. 10.1021/acs.jafc.2c07543.

[39] G. Hong. (2024). "Enoxolone Suppresses Apoptosis in Chondrocytes and Progression of Osteoarthritis via Modulating the ERK1/2 Signaling Pathway". *Archives of Medical Science.* **3** : 93211. 10.5114/aoms.2020.93211.

[40] P. Huang, X. Duan, Z. Tang, Z. Zou, and W. Song. (2023). "Betulinaldehyde Exhibits Effective Anti-Tumor Effects in A549 Cells by Regulating Intracellular Autophagy". *Scientific Reports.* **13** : 743-1. 10.1038/s41598-023-27580-w.

[41] C. Lin, X. Wen, and H. Sun. (2016). "Oleanolic Acid Derivatives for Pharmaceutical Use: A Patent Review". *Expert Opinion On Therapeutic Patents.* **26** (6): 643-655. 10.1080/13543776.2016.1182988.

[42] M. A. Rasadah, S. Khozirah, A. A. Aznie, and M. M. Nik. (2004). "Anti-Inflammatory Agents From Sandoricum koetjape Merr". *Phytomedicine.* **11** (2-3): 261-263. 10.1078/0944-7113-00339.

[43] T. X. H. Hang, S. Jarupinthusophon, R. Hairani, V. K. Nguyen, and W. Chavasiri. (2024). "Cycloartane-Type Triterpenoids From the Leaves of Sandoricum koetjape and Their Efficacy on Alpha-Glucosidase Inhibition Activity". *Journal of Natural Medicines.* **78** (3): 655-663. 10.1007/s11418-023-01778-8.

[44] C. Hao. (2017). "Asiatic Acid Inhibits LPS-Induced Inflammatory Response in Human Gingival Fibroblasts". *International Immunopharmacology.* **50** : 313-318. 10.1016/j.intimp.2017.07.005.

[45] M. Fizur, N. Meeran, S. N. Goyal, K. Suchal, and C. Sharma. (2018). "Pharmacological Properties, Molecular Mechanisms, and Pharmaceutical Development of Asiatic Acid: A Pentacyclic Triterpenoid of Therapeutic Promise". *Frontiers in Pharmacology.* **9** : 892-1. 10.3389/fphar.2018.00892.

[46] L. Ding, T. Liu, and J. Ma. (2023). "Neuroprotective Mechanisms of Asiatic Acid". *Heliyon.* **9** (5): e15853. 10.1016/j.heliyon.2023.e15853.

[47] X. Huang. (2022). "Synthesis and Evaluation of Anticancer Activity of Quillaic Acid Derivatives: A Cell Cycle Arrest and Apoptosis Inducer Through NF-\kappa B and MAPK Pathways". *Frontiers in Chemistry.* **10** : 951713-1. 10.3389/fchem.2022.951713.

[48] I. Arslan. (2020). "Quillaic Acid-Containing Saponin-Based Immunoadjuvants Trigger Early Immune Responses". *Revista Brasileira de Farmacognosia.* **30** (4): 467-473. 10.1007/s43450-020-00080-y.

[49] H. U. Chowdhury, S. B. Uddin, and M. O. Faruque. (2025). "Untangling the Biological Efficacy and Chemical Composition of Different Solvent Extractives From Brownlowia elata Roxb. Leaves". *Food Chemistry Advances.* **9** : 101160. 10.1016/j.focha.2025.101160.

[50] M. Dikamu, D. Ezez, H. Birhanu, A. Mamo, and M. Tefera. (2025). "Analysis of Phytochemical Constituents Using GC–MS, Evaluation of Antioxidants and Antibacterial Activities of Maerua oblongifolia Root Bark Extracts". *Discover Applied Sciences.* **7** : 417. 10.1007/s42452-025-06939-w.

[51] B. Xu. (2017). "An Overview of Structurally Modified Glycyrrhetinic Acid Derivatives as Antitumor Agents". *Molecules.* **22** (6): 924. 10.3390/molecules22060924.

[52] Q. Lu, Y. Ding, Y. Li, and Q. Lu. (2020). "5-HT Receptor Agonist Valerenic Acid Enhances the Innate Immunity Signal and Suppresses Glioblastoma Cell Growth and Invasion". *International Journal of Biological Sciences.* **16** : 1427-1442. 10.7150/ijbs.44906.

[53] Z. Kokanova-Nedialkova, M. Kondeva-Burdina, and P. Nedialkov. (2021). "Saponins From the Roots of Chenopodium bonus-henricus L. With Neuroprotective and Anti-\alpha-Glucosidase Activities". *Pharmacia.* **68** : 387-392. 10.3897/pharmacia.68.e64425.

[54] W. Ko. (2020). "Neuroprotective and Anti-Inflammatory Effects of Kuwanon C From Cudrania tricuspidata Are Mediated by Heme Oxygenase-1". *International Journal of Molecular Sciences.* **21** (14): 4839-1. 10.3390/ijms21144839.

[55] S. N. Ugariogu, I. A. Duru, F. C. Onwumere, and J. O. Igoli. (2021). "Physicochemical Assessment and Drug Potential of Some Phenylpropanoid and Flavonoid Compounds of Ethyl Acetate Eluate From Umudike Propolis". *Tropical Journal of Natural Product Research.* **4** (12): 1208-1214. 10.26538/tjnpr/v4i12.30.

[56] J. Ham. (2019). "Gentisyl Alcohol Inhibits Proliferation and Induces Apoptosis via Mitochondrial Dysfunction and Regulation of MAPK and PI3K/AKT Pathways in Epithelial Ovarian Cancer Cells". *Marine Drugs.* **17** (6): 331-1. 10.3390/md17060331.

[57] M. Naveed. (2018). "Chlorogenic Acid (CGA): A Pharmacological Review and Call for Further Research". *Biomedicine and Pharmacotherapy.* **97** : 67-74. 10.1016/j.biopha.2017.10.064.

[58] A. A. Ganai and H. Farooqi. (2015). "Bioactivity of Genistein: A Review of In Vitro and In Vivo Studies". *Biomedicine and Pharmacotherapy.* **76** : 30-38. 10.1016/j.biopha.2015.10.026.

[59] O. H. Kang. (2012). "Anti-Inflammatory Effect of Oleanolic Acid 28-O-\beta-D-Glycopyranosyl Ester Isolated From Aralia cordata in Activated HMC-1 Cells". *African Journal of Pharmacy and Pharmacology.* **6** (46): 3206-3214. 10.5897/AJPP12.1156.

[60] A. R. Oladipupo. (2024). "Structure-Based Molecular Networking, Molecular Docking, Dynamics Simulation and Pharmacokinetic Studies of Olax subscorpioidea for Identification of Potential Inhibitors Against Selected Cancer Targets". *Journal of Biomolecular Structure and Dynamics.* **42** (3): 1110-1125. 10.1080/07391102.2023.2198032.

[61] X. Pei, H. Yao, L. Shen, Y. Yang, L. Lu, and J. Xiao. (2020). "Alpha-Cyperone Inhibits the Proliferation of Human Cervical Cancer HeLa Cells via ROS-Mediated PI3K/Akt/mTOR Signaling Pathway". *European Journal of Pharmacology.* **883** : 173355. 10.1016/j.ejphar.2020.173355.

[62] R. Marinelli. (2020). "Garcinoic Acid Prevents Beta-Amyloid (Aβ) Deposition in the Mouse Brain". *Journal of Biological Chemistry.* **295** (15): 11866-11876. 10.1074/jbc.RA120.013303.

[63] F. N. Hamzah, Subandi, W. Sujarwo, A. W. Septama, and T. Mozel. (2020). "Antioxidant and Xanthine Oxidase Inhibitory Activities of Kecapi (Sandoricum koetjape (Burm. f) Merr.) Leaf Extract". *IOP Conference Series: Materials Science and Engineering.* **833** : 012012. 10.1088/1757-899X/833/1/012012.

[64] S. Poeaim and N. Pedklang. (2024). "The Biological Activities of the Methanolic Extract of Santol (Sandoricum koetjape) Fruits". *International Journal of Agricultural Technology.* **20** (2): 749-760.

[65] R. Reymon, S. Sofyan, A. W. M. Yodha, and M. Musdalipah. (2021). "The Toxicity of Meistera chinensis Rhizome Fraction by Shrimp Larvae With the BSLT Method". *Natural Science: Journal of Science and Technology.* **10** (2): 53-58. 10.22487/25411969.2021.v10.i02.15673.

[66] E. B. Aksono, A. C. Latifah, L. T. Suwanti, K. U. Haq, and H. Pertiwi. (2022). "Clove Flower Extract (Syzygium aromaticum) Has Anticancer Potential Effect Analyzed by Molecular Docking and Brine Shrimp Lethality Test (BSLT)". *Veterinary Medicine International.* **2022** : 5113742. 10.1155/2022/5113742.

[67] S. Pisutthanan, P. Plianbangchang, and N. Pisutthanan. (2004). "Brine Shrimp Lethality Activity of Thai Medicinal Plants in the Family Meliaceae". *Naresuan University Journal.* **12** (2): 13-18.