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BIOLOGICAL METABOLITE AND HISTOCHEMICAL MAPPING OF FIVE TRADITIONAL MEDICINAL PLANTS IN RIAU, INDONESIA

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SUMMARY

Calamus angustifolius Griff. (water rattan palm), *C. caesius* Blume (Sega rattan), *Champereia manillana* var. *manillana* (pucuk seminyak), *Cleome gynandra* L. (maman), and *Plukenetia corniculata* Sm. (pepina) traditionally served as food and herbal remedies in Riau, Indonesia. However, reports on scientific information on their bioactive compounds have yet to exist. This study aimed to investigate the histochemical traits and metabolite profiles of these medicinal plants to support their use in future medicinal applications. Leaves and petioles (pepina, maman, and pucuk seminyak) and young stems (water rattan and Sega rattan) underwent histochemical analysis and maceration using methanol, hexane, and ethyl acetate, followed by UPLC-QTOF MS/MS analysis. The histochemical staining identified alkaloids, flavonoids, tannins, and lipids, except for tannins in pucuk seminyak. Alkaloids emerged primarily in vascular tissues, while flavonoids were abundant in parenchyma. Metabolite profiling revealed a wide range of compounds. Epigallocatechin was notable across all species, whereas formononetin, isoliquiritigenin, and silybin were evident specifically in the Sega rattan and water rattan palm. Among the solvents, methanol yielded the most diverse metabolites through extraction. Based on these, the Sega rattan and water rattan palm have the greatest potential for therapeutic development, while methanol is the most effective solvent for extraction.

Keywords: Traditional medicinal plants, morphological and histochemical traits, metabolite profiles, herbal, UPLC-QTOF MS/MS, Sega rattan (*Calamus caesius* Blume), Riau

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Key findings: Vascular and parenchyma tissues were high in accumulated metabolites in the studied plants. Sega rattan and water rattan palm exhibited the highest metabolite diversity. Furthermore, methanol showed as the best organic solvent for metabolite extraction. The vascular and parenchymal tissues of Sega rattan and water rattan palm are potential targets for breeding programs.

INTRODUCTION

Phytochemicals are plant-derived secondary metabolites that help plants resist environmental stress conditions and offer therapeutic effects in treating humans (Twaji and Hasan, 2022). Major groups, such as alkaloids, phenolics, flavonoids, and terpenoids, exhibited anticancer, antioxidant, and immunomodulatory properties (Roy *et al.,* 2022). However, their distribution varies across plant organs, highlighting the importance of histochemical analysis to determine their specific sites of accumulation.

Histochemical analysis detects different phytochemicals within plant tissues by staining thin organ sections for microscopic observation to examine the compound localization. Recent applications in medicinal plants confirm their values (Konarska *et al.,* 2021; Sabila *et al.,* 2022). However, histochemical techniques cannot identify an individual compound, limiting their use in compound-specific characterization. In overcoming these limitations, the use of metabolite profiling has been progressive as a complementary method.

Metabolite profiling, also known as metabolomics, involves the analysis of low molecular weight compounds produced by metabolism in the organism. This approach commonly uses maceration for metabolite extraction due to its simplicity and cost-effectiveness. Maceration involves the use of organic solvents, such as methanol, hexane, and ethyl acetate, to release metabolite compounds from plant tissues (Barthwal and Mahar, 2024). The choice of solvent can considerably affect the composition of the extracted metabolites, highlighting the need for further research to identify the optimal organic solvents for metabolite extraction, particularly in medicinal plants (Kaur *et al.,* 2022).

Indonesia hosts numerous and diverse medicinal plants, including pepina (*Plukenetia corniculata*), maman (*Cleome gynandra*) (Roslim *et al.,* 2023), pucuk seminyak (*Champereia manillana* var. *manillana*) (Roslim *et al.,* 2024), Sega rattan (*Calamus caesius*), and water rattan palm (*C. angustifolius*). Similarly, these plant species' traditional uses are beneficial in Riau, Indonesia. Despite their ethnobotanical relevance, limited scientific information exists on their metabolite composition, tissues with high metabolite accumulation, and suitable extraction solvent. Therefore, the presented study combines histochemical and metabolite profiling to characterize the metabolite localization, compound diversity, and optimal extraction solvents used in these plant species. The findings intend to provide a scientific basis for breeding programs targeting herbal medicinal properties.

MATERIALS AND METHODS

Plant material and extraction procedure

Medicinal plants' collection of leaves and petioles, including pepina (*Plukenetia corniculata*), maman (*Cleome gynandra*), pucuk seminyak (*Champereia manillana* var. *manillana*), with young stems of Sega rattan (*Calamus caesius*) and water rattan palm (*Calamus angustifolius*), ensued in Riau Province, Indonesia. Plant samples underwent thorough washing, chopping, oven-drying, grinding, sieving, and storing at room temperature. Powdered materials sustained maceration in methanol, hexane, and ethyl acetate at 25 °C for three days with agitation (100 rpm). Filtrates' (Whatman No. 1) concentration continued via rotary evaporation (50 °C, 100 rpm) before subjecting them to LC-MS/MS (liquid chromatography/mass spectrometry) analysis.

Table 1. Information on histochemical analysis in five plant species from Riau, Indonesia.

No.	Metabolite Test	Reagent	Positive Result
1	Flavonoid	10% NaOH	Yellow
2	Alkaloid	Wagner`s Reagent	Reddish brown
3	Tannin	$FeCl_3$	Blackish blue
4	Lipid	Sudan III	Pink

LC-MS/MS analysis

Filtration of plant extracts through 0.2 µm syringe filters proceeded to injecting 5 µL into an ACQUITY UPLC H-Class System coupled with a Xevo G2-QTOF mass spectrometer (Waters, USA). Separation continued on an ACQUITY UPLC HSS C18 column (1.8 µm, 2.1 × 100 mm) at 50 °C. The mobile phase comprised 5 mM ammonium formate in water (A) and acetonitrile with 0.05% formic acid (B), delivered at 0.2 mL/min over a 23-minute gradient: 0–2 min (95:5 A:B), 2–14 min (75:25), 14–19 min (0:100), and 19–23 min (95:5). Mass spectrometry proceeded in positive ESI mode (50–1200 m/z). The conditions included a source temperature of 100 °C, a desolvation temperature of 350 °C, cone gas at 0 L/h, desolvation gas at 793 L/h, collision energy at 4 V, and ramped collision energy at 25–70 V.

Histochemical analysis

Sections of petioles and young stems received treatments with specific reagents (Table 1) before rinsing with distilled water and examining under a bright-field microscope.

Data processing

Metabolite identification employed MzMine 4.3.0 (Schmid *et al.,* 2023). The MS1 and MS2 mass detection used centroid mode with noise thresholds of 10^3 and 10^2, respectively. MS1 chromatograms' building comprised ≥5 consecutive scans, intensity of ≥500, height of ≥1000, and m/z tolerance of 0.005 m/z (5 ppm). Peak resolution applied the local minimum resolver with a 70% threshold, relative height of ≥1%, absolute height of ≥100, and duration of 0.05–1 min. MS1 and MS2 data succeeded in pairing with retention time using a precursor tolerance of 0.005 m/z (5 ppm), and retention time filters refined feature boundaries. Features' alignment and annotation used a custom database compiled from HMDB, KEGG, and MassBank of North America (MoNA). Visualization, including heatmaps and Venn diagrams, continued in R Studio.

RESULTS

Metabolite distribution in plant tissues

Histochemical analysis revealed different phytochemicals, including alkaloids, flavonoids, and lipids, in all five plant species, while tannins were absent in the species pucuk seminya (Figure 1, Table 2). Alkaloid content was most abundant in water rattan palm, primarily localized in vascular tissues. Flavonoids were leading in the species maman, concentrating more in parenchyma. Tannins were the highest in water rattan palm; however, they were absent in the plant species pucuk seminyak. Lipids were most prominent in the Sega rattan, with a similar level across the remaining plant species.

Metabolite profiles of the species

The identified putative metabolites totaled 77 in the five plant species using organic solvents, such as methanol, ethyl acetate, and hexane. Fourteen metabolites were unique to methanol, five to ethyl acetate, and 27 were common to all the solvents (Figure 2, Tables 3A–C). Metabolites spanned 11 classes. The species-specific compounds included L-arginine, L-cystathionine, caffeic acid, rhein (Sega rattan), and scopoletin (pepina). The species Sega

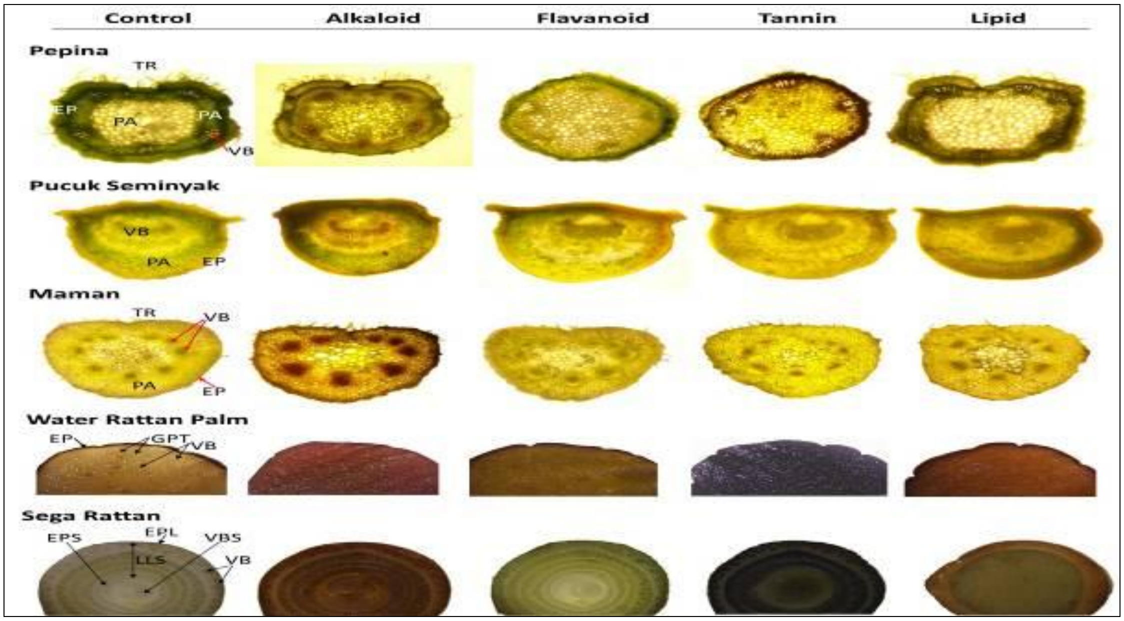

Figure 1. Cross-section of pepina (*Plukenetia corniculata*), maman (*Cleome gynandra*), and pucuk seminyak (*Champereia manillana* var. *manillana*) leaf petioles, as well as the young stems of sega rattan (*Calamus caesius*) and water rattan palm (*C. angustifolius*) from Riau, Indonesia in histochemical tests. TR: trichome, EP: epidermis, PA: parenchyma, GPT: ground parenchymatous tissue, EPL: epidermis of leaf sheath, VB: vascular bundle, LLS: layers of leaf sheath, EPS: epidermis of stem, and VBS: vascular bundle of stem.

Table 2. Histochemical analysis of petioles and young stems of five plants from Riau, Indonesia.

Tissue	Alkaloids	Flavonoids	Tannins	Lipids
Pepina				
Trichomes	-	++	-	+
Epidermis	+++	+	-	+
Parenchymal	-	+++	-	-
Vascular	+++	+	+	++
Pucuk Seminyak				
Trichomes	+	+	-	-
Epidermis	+	+	-	+
Parenchymal	+	++++	-	-
Vascular	++++	+	-	+
Maman				
Trichomes	++++	+++	-	-
Epidermis	++++	+++	+	+
Parenchymal	++	+++	-	+
Vascular	++++	+++	++	-
Water rattan palm				
Epidermis	++++	-	++++	-
Parenchymal	++++	++	++++	++
Vascular	++++	++	++++	-
Sega rattan				
Epidermis	++	++	++	+++
Parenchymal	++	+++	++	+++
Vascular	++++	+	++++	+++

(-): Not available, (+): Identified.

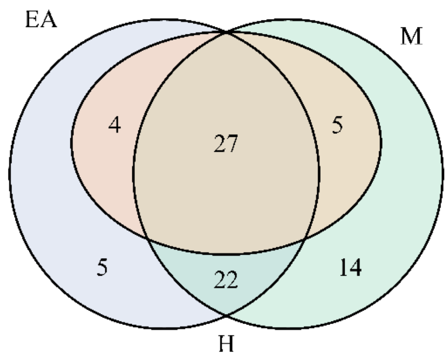

Figure 2. Venn diagrams of identified putative compounds in five plant species from Riau, Indonesia, using different solvents. M: methanol, EA: ethyl acetate, and H: hexane.

rattan had five fatty acids; meanwhile, palmitoleic acid was absent in the water rattan palm. Linolenic acid was universal; yet, elaidic acid was nonexistent in the species pucuk seminyak.

Among the nine flavonoids, only epigallocatechin was evident in all the species of medicinal plants. Tangeritin did not occur in the species Sega rattan, while luteolin, kaempferol, and rutin were also missing in the species water rattan palm. Gibberellic acid appeared in both rattan species, and kinetin appeared in the species maman, pepina, and pucuk seminyak. Betulinic acid and thymol (terpenoids) were visible in both rattan species, with thymol also found in the species maman and pucuk seminyak. The plant species pepina lacked the terpenoids. Three indoles and widespread phenolics, 1-phenylethanol and 4-aminobenzoate, also emerged, while caffeic acid and rhein were exclusive to the species Sega rattan. Five vitamins surfaced in the species pepina; D-pantothenic acid and niacin materialized in all the plant species. However, the species pepina stood out with the highest vitamin content.

Multivariate analysis of metabolite profiles

Heatmap visualization revealed metabolite distribution across the five plant species (Figure 3). Methanol extracts disclosed the most putative metabolites, followed by ethyl acetate and hexane. Fourteen compounds, including 4-coumarate, D-mannitol, linamarin, lobeline, rutin, and silybin, were unique to methanol extract (Figure 3A; Tables 3A–C). Ethyl acetate uniquely extracted isoliquiritigenin, maltotriose, N-acetyl-L-leucine, rotenone, and trehalose (Figure 3B). No metabolites were exclusive to extract hexane (Figure 3C), aligning with the Venn diagram results (Figure 2). The species Sega rattan showed the highest metabolite diversity, followed by the plant species water rattan palm, while the species pepina had the fewest. The 10 most abundant metabolites, such as amino acids, coumarins, fatty acids, flavonoids, and phenolics, were the predominant extracts with organic solvent methanol. Moreover, only phytosphingosine, an amino acid, entailed isolation via hexane (Table 4).

Solvent influence on flavonoid biosynthesis pathways

Flavonoid biosynthesis metabolites showed considerable solvent-dependent variation, and the methanol extracts revealed the highest concentrations. Key compounds, such as phenylacetic acid, phenylalanine, caffeoylquinic acid, kaempferol, luteolin, p-coumaric acid, epigallocatechin, and 3-(4-hydroxyphenyl)pyruvate, were the most abundant in methanol extract. Ethyl acetate and hexane extracted some of these metabolites at significantly lower levels.

Table 3A. Putative metabolites identified in five plant species from Riau, Indonesia, using different solvents: alkaloids, amino acids, and carbohydrates.

No.	Compounds	RT	Formula	MW	Error mass (ppm)	Ion mode	MS-MS	Plant species	Solvent
Alkaloids									
1	Caffeine	6.50	$C_8H_{10}N_4O_2$	194.083	-0.77	[M+H]+	195, 138	CG, CA, CC	M, EA
2	Ephedrine	9.18	$C_{10}H_{15}NO$	165.116	2.17	[M+H]+	149, 131, 103	CA, CC	H, EA
3	Lobeline	4.13	$C_{22}H_{27}NO_2$	337.206	-0.27	[M]+	147, 103	CA, CC	M
4	Piperine	8.77	$C_{17}H_{19}NO_3$	285.110	-2.36	[M+H]+	131, 107	CC	H, EA
Amino acid									
5	5-Oxo-L-Proline	3.83	$C_5H_7NO_3$	129.043	2.26	[M+H]+	138, 84	CM, PC, CG, CA, CC	M
6	Betaine	2.18	$C_5H_{11}NO_2$	117.079	2.12	[M+H]+	118, 59, 58	CM, PC, CG, CA, CC	M, H, EA
7	Deoxyadenosine	4.99	$C_{10}H_{13}N_5O_3$	251.176	0.82	[M]+	252, 136, 117	CA, CC	M
8	Deoxycytidine	6.62	$C_9H_{13}N_3O_4$	227.093	0.64	[M]+	228, 117, 112, 99	CM, CC	M, H, EA
9	Guanosine	8.59	$C_{10}H_{13}N_5O_5$	283.093	3.26	[M+H]+	151, 134, 109	CA, CC	M, EA
10	Hypoxanthine	7.34	$C_5H_4N_4O$	136.039	-0.26	[M+H]+	109, 82, 55	CM, PC, CG, CC	M, EA
11	Isoleucine	4.99	$C_6H_{13}NO_2$	131.095	-1.09	[M+H]+	86, 69, 44, 41	CM, PC, CG, CC, CA	M, H, EA
12	L-Arginine	4.88	$C_6H_{14}N_4O_2$	174.113	0.43	[M]+	70, 60	CC	M, EA
13	L-Cystathionine	4.45	$C_7H_{14}N_2O_4S$	222.073	0.21	[M+H]+	88, 56	CC	M, EA
14	L-Proline	3.85	$C_5H_9NO_2$	115.063	1.92	[M+H]+	70, 43	CM, PC, CG, CC, CA	M, H, EA
15	N-Acetyl-L-Leucine	5.45	$C_8H_{15}NO_3$	173.106	0.53	[M+H]+	174, 157, 128, 114	CA, CC	EA
16	N-Acetylphenylalanine	16.20	$C_{11}H_{13}NO_3$	207.140	1.00	[M]+	119, 102	CM, PC, CA, CC	M, H, EA
17	Phenylalanine	18.61	$C_9H_{11}NO_2$	165.093	0.79	[M]+	119, 103, 77	CM, PC, CG, CC, CA	M, H, EA
18	Phytosphingosine	18.79	$C_{18}H_{39}NO_3$	317.294	-1.55	[M+H]+	318, 300, 282	CM, PC, CG, CC, CA	M, H, EA
19	Pipecolic Acid	2.51	$C_6H_{11}NO_2$	129.080	2.31	[M+H]+	84, 56	CM, PC, CA, CC	M, EA
20	Theanine	14.00	$C_7H_{14}N_2O_3$	198.077	0.67	[M+H]+	155, 136, 74	CM, PC, CA, CC	M, EA
21	Tyrosine	2.93	$C_9H_{11}NO_3$	181.075	0.4	[M+H]+	135, 118, 95, 91	CG, CA, CC	M
22	Uracil	1.63	$C_4H_4N_2O_2$	112.028	-1.17	[M+H]+	112, 95, 70, 68, 40	CG, CA, CC	M, EA
Carbohydrates									
23	D-mannitol	1.62	$C_6H_{14}O_6$	182.077	2.54	[M+H]+	130, 45, 43	CG, CA	M
24	Linamarin	4.68	$C_10H_{17}NO_6$	247.106	3.2	[M+H]+	103, 85	CM, CC	M
25	Maltotriose	11.92	$C_{18}H3_2O_{16}$	528.204	-0.11	[M+H]+	163, 145, 85	CA, CC	EA
26	Trehalose	14.56	$C_{12}H_{22}O_{11}$	342.169	0.17	[M]+	145, 97, 85	CA, CC	EA

MW: molecular weight, RT: retention time, MS-MS: mass spectrometry-mass spectrometry, CG: maman (*Cleome gynandra*), CA: water rattan palm (*Calamus angustifolius*), CC: Sega rattan (*Calamus caesius*), CM: pucuk seminyak (*Champereia manillana* var. *manillana)*, PC: pepina (*Plukenetia corniculata*), M: methanol, H: hexane, and EA: ethyl acetate.

Table 3B. Putative metabolites identified in five plant species from Riau, Indonesia, using different solvents: coumarins, fatty acids, flavonoids, hormones, and indoles.

No.	Compounds	RT	Formula	MW	Error mass (ppm)	Ion mode	MS-MS	Plant species	Solvent
Coumarins									
27	4-Methylumbelliferone	17.36	$C_{10}H_8O_3$	176.047	-2.71	[M+H]+	177, 121, 105, 103, 91, 77	CM, PC, CG, CA, CC	M, H, EA
28	Coumarin	14.59	$C_9H_6O_2$	146.037	0.36	[M+H]+	147, 103	CM, PC, CG, CA, CC	M, H, EA
29	Scopoletin	5.21	$C_{10}H_8O_4$	192.043	0.92	[M+H]+	193, 178, 150, 137, 133, 122	PC	M, EA
30	Umbelliferone	13.95	$C_9H_6O_3$	162.032	1.38	[M+H]+	163, 135, 119, 107	PC, CG, CA, CC	M, H, EA
Fatty Acids									
31	Elaidic Acid	16.95	$C_{18}H_{34}O_2$	282.257	-0.18	[M+H]+	283, 241, 121, 107, 69, 57, 43	PC, CG, CA, CC	M, H, EA
32	Linolenic acid	13.68	$C_{18}H_{30}O_2$	278.247	-0.91	[M]+	279, 95, 81, 67	CM, PC, CG, CA, CC	M, H, EA
33	Palmitoleic acid	17.38	$C_{16}H_{30}O_2$	254.247	2.82	[M]+	237, 219, 171, 97	CG, CC	M, EA
34	Sebacate	10.39	$C_{10}H_{18}O_4$	202.121	2.34	[M+H]+	203, 139, 121, 93	CA, CC	M, H, EA
35	Tomatine	9.04	$C_{50}H_{83}NO_{21}$	1033.521	-0.81	[M+H]+	1034, 416	CA, CC	M, H
Flavonoids									
36	3',4',5,7-Tetrahydroxyflavone	7.94	$C_{15}H_{10}O_6$	286.048	0.39	[M+H]+	287, 153	CM, PC, CG, CC	M, EA
37	Epigallocatechin	13.94	$C_{15}H_{14}O_7$	308.171	-2.67	[M+H]+	308, 139, 138, 123	CM, PC, CG, CA, CC	M, H, EA
38	Formononetin	12.03	$C_{16}H_{12}O_4$	268.168	0.38	[M+H]+	269, 254, 181, 156, 118	CA, CC	M, H, EA
39	Isoliquiritigenin	11.32	$C_{15}H_{12}O_4$	256.092	3.79	[M+H]+	137, 119, 91	CA, CC	EA
40	Kaempferol	8.42	$C_{15}H_{10}O_6$	286.209	-3.97	[M]+	286, 164, 152, 120	CM, PC, CC	M, H
41	Rotenone	10.99	$C_{23}H_{22}O_6$	394.234	0.31	[M+H]+	395, 367, 213	PC, CA, CC	EA
42	Rutin	4.67	$C_{27}H_{30}O_{16}$	610.155	0.8	[M+H]+	611, 610, 303, 84	CG, CC	M
43	Tangeritin	13.76	$C_{20}H_{20}O_7$	370.335	-1.86	[M+H]+	358, 343, 312	CG, CA	M, H
44	Silybin	4.95	$C_{25}H_{22}O_{10}$	482.239	0.14	[M]+	505, 366, 325	CA, CC	M
Hormone									
45	Gibberellic acid	13.59	$C_{19}H_{22}O_6$	346.333	1.56	[M]+	329, 311, 285, 239,	CA, CC	H, EA
46	Kinetin	4.14	$C_{10}H_9N_5O$	215.080	1.24	[M+H]+	216, 81	CM, PC, CG	M
Indoles									
47	Indole-3-acetate	7.98	$C_{10}H_9NO_2$	175.063	1.43	[M+H]+	129, 102	CA, CC	M, H, EA
48	Indoleacetaldehyde	18.77	$C_{10}H_9NO$	159.069	1.39	[M+H]+	118, 117	CG, CA, CC	M, H
49	N-Acetylserotonin	4.72	$C_{12}H_{14}N_2O_2$	218.103	1.03	[M]+	160, 132, 115	CM, PC, CG, CC	M

MW: molecular weight, RT: retention time, MS-MS: mass spectrometry-mass spectrometry, CG: maman (*Cleome gynandra*), CA: water rattan palm (*Calamus angustifolius*), CC: Sega rattan (*Calamus caesius*), CM: pucuk seminyak (*Champereia manillana* var. *manillana)*, PC: pepina (*Plukenetia corniculata*), M: methanol, H: hexane, and EA: ethyl acetate.

Table 3C. Putative metabolites identified in five plant species from Riau, Indonesia, using different solvents: phenolics, terpenoid, and vitamins.

No.	Compounds	RT	Formula	MW	Error mass (ppm)	Ion mode	MS-MS	Plant species	Solvent
Phenolics									
50	1-Phenylethanol	19.16	$C_8H_{10}O$	122.073	1.23	[M+H]+	124, 107, 105	CM, PC, CG, CA, CC	M, H, EA
51	3-(4-Hydroxy-phenyl)pyruvate	10.53	$C_9H_8O_4$	180.043	1.86	[M+H]+	182, 163, 145, 135, 107	CM, CA, CC	M, EA
52	3-Hydroxybenzoate	10.27	$C_7H_6O_3$	138.033	-0.55	[M+H]+	140, 95, 93, 77	CM, CA, CC	M, EA
53	4-Allylpyrocatechol	8.77	$C_9H_{10}O_2$	150.068	-0.85	[M+H]+	152, 147, 133, 41	CM, CC	M, EA
54	4-Aminobenzoate	4.09	$C_7H_7NO_2$	137.048	-0.01	[M+H]+	138, 94, 77	CM, PC, CG, CA, CC	M
55	4-Coumarate	12.61	$C_9H_8O_3$	164.048	1.96	[M+H]+	165, 147, 119, 91	CG, CA, CC	M, H
56	4-Hydroxybenzaldehyde	4.61	$C_7H_6O_2$	122.036	-3.08	[M+H]+	123, 93, 81, 79	CM, CG, CA	M
57	Arbutin	16.17	$C_{12}H_{16}O_7$	272.090	1.23	[M+H]+	273, 255, 163, 145, 111	PC, CG, CA, CC	M, H, EA
58	Benzaldehyde	14.51	C_7H_6O	106.043	-0.24	[M+H]+	107, 91, 81, 51	CA, CC	M, H, EA
59	Caffeic acid	5.41	$C_9H_8O_4$	182.045	2.04	[M+H]+	179, 163, 145, 117, 89	CC	M, EA
60	Caffeoyl quinic acid	3.92	$C_{16}H_{18}O_9$	355.102	0.53	[M+H]+	355, 193, 175, 163	PC, CG, CA, CC	M, H, EA
61	Cinnamate	11.42	$C_9H_8O_2$	148.053	-0.67	[M+H]+	149, 131, 103	CA, CC	M, EA
62	D-(-)-Salicin	14.53	$C_{13}H_{18}O_7$	281.266	-1.25	[M]+	131, 107	CA, CC	M, H, EA
63	Ellagic acid	5.30	$C_{14}H_6O_8$	302.043	1.12	[M+H]+	302, 284, 274	CM, CG, CA, CC	M, EA
64	Emodin	7.64	$C_{15}H_{10}O_5$	270.065	0.16	[M+H]+	268, 263, 258, 255	PC, CC	M, EA
65	Estragole	15.72	$C_{10}H_{12}O$	148.089	0.25	[M+H]+	148, 136	CM, PC, CA, CC	M, H, EA
66	Eugenol	17.78	$C_{10}H_{12}O_2$	164.084	0.47	[M+H]+	137, 124, 109, 105	CM, PC, CA, CC	M, H, EA
67	Kynurenic acid	3.93	$C_{10}H_7NO_3$	189.043	3.39	[M+H]+	143, 116, 89	PC, CG	M
68	Phenylacetic acid	11.38	$C_8H_8O_2$	136.052	0.45	[M+H]+	91, 65, 39	CM, CG, CA, CC	M, H, EA
69	Resveratrol	9.48	$C_{14}H_{12}O_3$	228.161	-0.22	[M]+	229, 211, 165, 135, 107	CA, CC	M, EA
70	Rhein	8.07	$C_{15}H_8O_6$	284.081	-2.91	[M+H]+	285, 241	CC	M, EA
Terpenoid									
71	Betulinic Acid	17.49	$C_{30}H_{48}O_3$	456.360	2.94	[M+H]+	457, 439, 421	CA, CC	M, H, EA
72	Thymol	14.72	$C_{10}H_{14}O$	150.104	-3.28	[M+H]+	151, 98, 69	CM, CG, CA, CC	M, H, EA
Vitamins									
73	D-Pantothenic Acid	4.75	$C_9H_{17}NO_5$	219.113	0.73	[M+H]+	220, 202, 90	CM, PC, CG, CA, CC	M, H, EA
74	Folic Acid	14.39	$C_{19}H_{19}N_7O_6$	441.153	2.92	[M]+	442, 294	PC, CA	H, EA
75	Niacin	1.41	$C_6H_5NO_2$	123.032	2.33	[M+H]+	80, 78, 53	CM, PC, CG, CA, CC	M, EA
76	Pyridoxine	4.14	$C_8H_{11}NO_3$	169.074	1.39	[M+H]+	133, 79, 77	CM, PC, CG, CC	M, EA
77	Riboflavin	4.35	$C_{17}H_{20}N_4O_6$	376.139	0.47	[M+H]+	243, 242, 171, 70	CM, PC	M

MW: molecular weight, RT: retention time, MS-MS: mass spectrometry-mass spectrometry, CG: maman (*Cleome gynandra*), CA: water rattan palm (*Calamus angustifolius*), CC: Sega rattan (*Calamus caesius*), CM: pucuk seminyak (*Champereia manillana* var. *manillana)*, PC: pepina (*Plukenetia corniculata*), M: methanol, H: hexane, and EA: ethyl acetate.

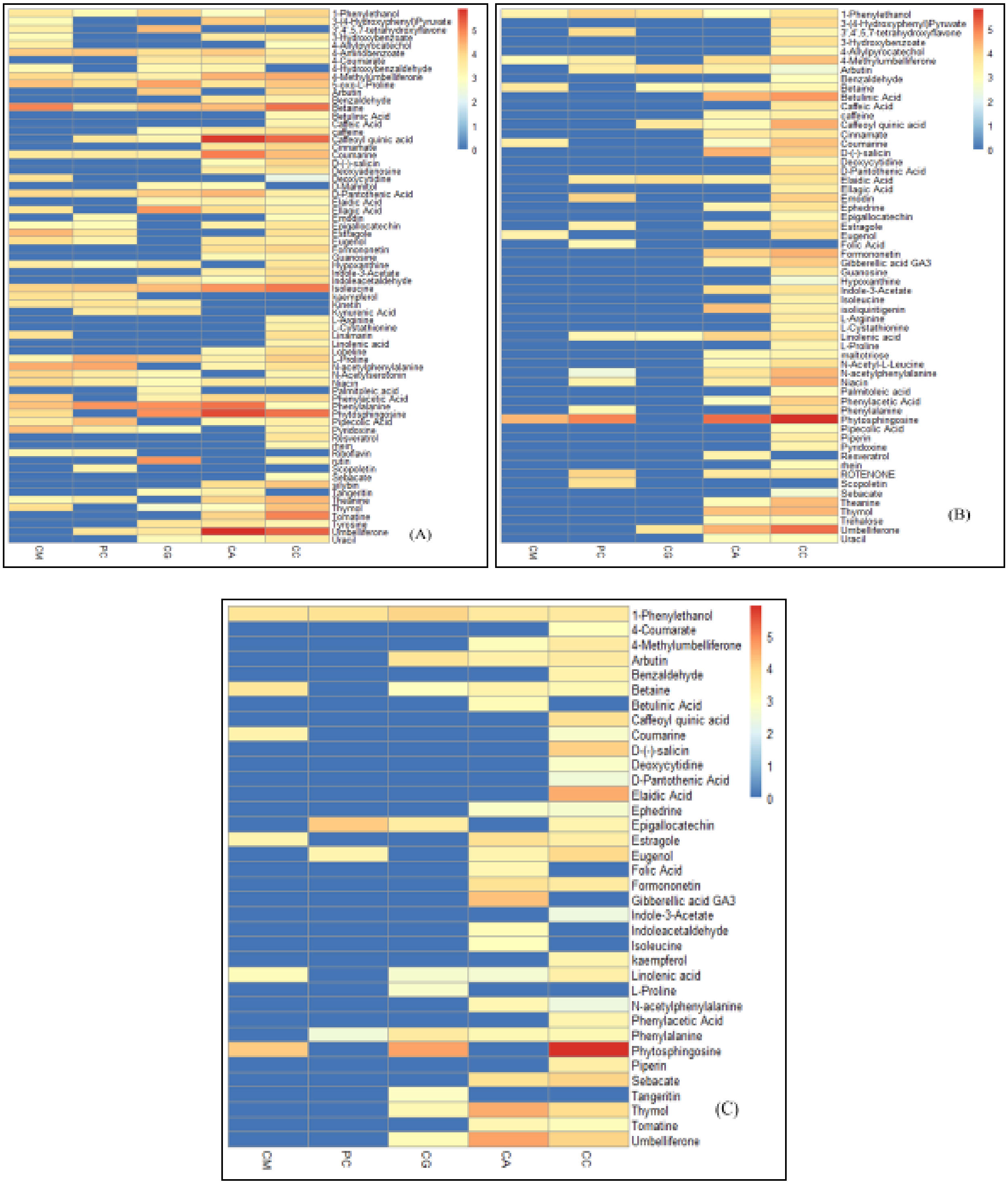

Figure 3. Heatmap analysis of identified putative metabolites in five plant species from Riau, Indonesia, using different solvents. (A) Methanol, (B) Ethyl acetate, and (C) Hexane, CG: maman (*Cleome gynandra*), CA: water rattan palm (*Calamus angustifolius*), CC: Sega rattan (*Calamus caesius*), CM: pucuk seminyak (*Champereia manillana* var. *manillana)*, and PC: pepina (*Plukenetia corniculata*).

DISCUSSION

Higher plants typically produce over 50,000 secondary metabolites, shaped by environmental stressors, such as drought, salinity, temperature, heavy metals, and radiation. These stress conditions considerably modulate the physiological and metabolic responses, including secondary metabolite biosynthesis (Reshi *et al.,* 2023). The five investigated plant species in Riau, Indonesia—pepina (*P. corniculata*), maman (*C. gynandra*), pucuk seminyak (*C. manillana* var. *manillana*), water rattan palm (*C. angustifolius*), and Sega rattan (*C. caesius*)—proved well adapted to the region's peatlands, nutrient-poor acidic soils, and seasonal dryness. The Malay community traditionally uses these medicinal plant species for food, medicines, and furniture; yet, these plant species face progressive threats from deforestation. Therefore, their conservation is crucial, although no studies have addressed their secondary metabolite physiology. In this study, we combined metabolite profiling and histochemical analysis to fill that gap and support their sustainable use.

Histochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, and lipids in all the plant species (Figure 1, Table 2). Alkaloids revealed primary localization in vascular tissues, as confirmed via Wagner's reagent, aligning with earlier studies in *Rhodomyrtus tomentosa* (Kuntorini *et al.,* 2023), referring to the plant's adaptive response to nutrient-poor acidic soils in Riau. These nitrogen-containing compounds support plant defense and offer therapeutic properties for human health (Reddy *et al.,* 2024). LC-MS profiling identified alkaloids like caffeine, ephedrine, lobeline, and piperine (Table 3A), though they were not found in the species pepina and pucuk seminyak, likely due to lower concentrations.

Flavonoids displayed localization in parenchymal tissues, as evidenced by yellow coloration with 10% NaOH, consistent with previous studies in *Centratherum punctatum* (Chitra *et al.,* 2014). The presence of flavonoid acts as a secondary barrier during stress and aids reactive oxygen species (ROS) scavenging (Panche *et al*., 2016). Nine flavonoids, such as epigallocatechin, luteolin, formononetin, isoliquiritigenin, kaempferol, rotenone, rutin, tangeritin, and silybin, were also notable (Table 3B). These phenolic compounds were remarkable for their antioxidant and anti-aging properties (Shen *et al.,* 2022). Tannins, also vascularly localized, entailed identification via histochemical and metabolite profiling. The identified phenolic compounds included epigallocatechin, ellagic acid, resveratrol, cinnamate, eugenol, and caffeoylquinic acid (Table 3C), known for antimicrobial and antidiabetic potential (Miara *et al.,* 2023).

Lipids were sparse, localized in parenchymal tissues, as confirmed via Sudan II staining. Terpenoids (thymol and betulinic acid) and fatty acids (Table 3B) reflect the modest lipid accumulation. Terpenoids aid stress resilience and improve human cardiovascular health (Wang *et al.,* 2023). Other identified classes included amino acids, carbohydrates, indoles, hormones, and vitamins, which are vital for plant metabolism (Trovato *et al.,* 2021). Coumarins (4-methylumbelliferone, scopoletin) also showed therapeutic relevance, as reported in past studies (Sharifi-Rad *et al.,* 2021).

Four metabolites, such as N-acetyl-L-leucine, maltotriose, trehalose, and rotenone, were exclusive to ethyl acetate extracts and to the species water rattan palm and Sega rattan (Tables 3A–B). These compounds offer notable benefits, i.e., N-acetyl-L-leucine is neuroprotective (Sarkar and Lipinski, 2022), trehalose and maltotriose serve in food and pharma (Bláhová *et al*., 2023), isoliquiritigenin aids oxidative stress defense (Zgodova *et al.,* 2022), and rotenone is a natural insecticide but neurotoxic (Lin *et al.,* 2022).

In the species Sega rattan, the L-arginine, L-cystathionine, caffeic acid, and rhein were unique, while scopoletin was specific to the species pepina (Table 3B). L-arginine supports plants photosynthesis under drought stress conditions (Sun *et al.,* 2023). The L-cystathionine functions both in plants and human methionine pathways, affecting embryogenesis and cell survival (Jurkowska and Wrobel, 2018). Caffeic acid and rhein

Table 4. The 10 most abundant metabolites detected based on the heatmap analysis.

No.	Metabolites	Compounds	Species	Solvent	Concentration (mAU)
1	Phytosphingosine	Amino acid	CC	H	827200,0
2	Umbelliferone	Coumarin	CA	M	732420,0
3	Caffeoyl quinic acid	Phenolic	CA	M	506720,0
4	Phenylalanine	Asam amino	CA	M	139238,6
5	Betaine	Asam amino	CC	M	136100,0
6	Isoleucine	Asam amino	CC	M	110900,6
7	Coumarin	Coumarin	CA	M	106114,0
8	Tomatine	Fatty acid	CC	M	94723,0
9	Rutin	Flavonoid	CG	M	59910,0
10	Ellagic acid	Phenolic	CG	M	55340,0

CG: maman (*Cleome gynandra*), CA: water rattan palm (*Calamus angustifolius*), CC: Sega rattan (*Calamus caesius*), M: methanol, and H: hexane.

provide anti-inflammatory and anticancer effects (Cheng *et al.,* 2021). Rhein also acts as a novel herbicide (Twitty *et al.,* 2024), while scopoletin displayed broad pharmacological activities (Antika *et al.,* 2022).

Organic solvent types significantly affected the extraction profiles. Methanol extracts clearly differed from the two other solvents, ethyl acetate and hexane (Figure 2), yielding nine of the 10 most abundant metabolites (Table 4) and consistent with its polarity (Maphari *et al.,* 2024). Ethyl acetate and hexane solvents showed the same and limited profiles due to low polarity (Nkwocha *et al.,* 2024). Among the five species studied, Sega rattan and water rattan palm exhibited the highest phytochemical diversity, particularly flavonoid. Based on these findings, the combination of histochemical and metabolite profiling provides comprehensive insights into the localization and diversity of metabolites, which is valuable for understanding plant adaptation and bioactivity. Such knowledge can raise awareness among the community regarding the importance of conserving these plant species through propagation and sustainable use. Furthermore, these results support therapeutic validation and offer potential strategies for biofortification.

CONCLUSIONS

The assessed five medicinal plant species notably displayed richness in alkaloids and flavonoids, especially evident in vascular and parenchymal tissues of petioles and young stems. Using UPLC-QTOF MS/MS, several phytochemical compounds, such as coumarin, epigallocatechin, 1-phenylethanol, and D-pantothenic acid, emerged in all species. Methanol was most effective in extracting metabolites, which proved optimal for flavonoid-rich profiles. Among the plants studied, Sega rattan and water rattan palm exhibited the highest phytochemical diversity, highlighting their potential as promising sources of natural therapeutic agents. The integration of histochemical and metabolite profiling approaches further revealed the vascular and parenchymal tissues of these two species are key sites of metabolite accumulation, regarding their potential utility in targeted breeding programs.

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