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LC-HRMS-Based Metabolomics for Profiling the Metabolites in Different Plant Parts of *Centella asiatica*

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ABSTRACT

***Centella asiatica*, or pegagan in Indonesia, is a perennial plant used in Indonesian traditional medicine (jamu) and functional food with many biological activities. Those biological activities come from the bioactive metabolites present in *C. asiatica*. Differences in metabolite pathways in each part of the plant affect the accumulation of metabolites contained, thus impacting its biological activity. Therefore, this study aims to identify and evaluate differences in the distribution of metabolites in each part of *C. asiatica*, namely leaves, stems, stolons, and roots. Each plant part was extracted using methanol and sonicated for 30 minutes. The metabolites in the samples were separated and detected using UHPLC-Q-Orbitrap HRMS. Differences in the distribution of metabolites in each part of the plant were evaluated using chemometrics analysis. UHPLC-Q-Orbitrap HRMS analysis could positively identify 37 metabolites, most of which belong to the phenylpropanoid, triterpenoid, triterpenoid saponin, and flavonoid groups. Principal component analysis was able to clearly distinguish each part of the plant using the peak intensity of the overall chromatogram and the peak area of the identified metabolites. The different biosynthetic pathways of metabolites in plants could cause a difference in the distribution of metabolites in each plant.**

1. Introduction

Centella asiatica is a stoloniferous plant from the Apiaceae family and is widely distributed in tropical and subtropical regions, such as Indonesia, Malaysia, India, Sri Lanka, China, South Africa, and Madagascar (Yousaf *et al.* 2020). These plant leaves and stems can be eaten as green leafy vegetables. In Indonesia, *C. asiatica* is known as pegagan and has been widely used by the community as a jamu (Indonesian traditional medicine) to treat fever, dysentery, diarrhea, leprosy, asthma, headaches, rheumatism, and tuberculosis (Roy and Bharadvaja 2017). *C. asiatica* is also reported to exhibit various biological activities, such as anticancer, antioxidant, antimicrobial, antidiabetic, and antiinflammatory (Kabir *et al.* 2014; Saha *et al.* 2013; Lian *et al.* 2018; Guo *et al.* 2020; Ariastuti *et al.* 2020; Mohapatra *et al.* 2021; Wong and Ramli 2021). Those biological activities of *C. asiatica* come from its bioactive compounds.

C. asiatica has been reported to contain bioactive compounds from alkaloids, flavonoids, phenols, tannins, and terpenoids (Mohapatra *et al.* 2021; Nav *et al.* 2021). Several studies have also reported that the main components that become biomarkers of *C. asiatica* are triterpenes, such as asiaticoside, asiatic acid, madecassoside, and madecassic acid (Rafi *et al.* 2018; Yinngam *et al.* 2020). These biomarker

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compounds are known to have the most significant contribution to the biological activity of *C. asiatica*. However, *C. asiatica* extract may contain different metabolites depending on the part of the plant used. This is due to differences in biosynthetic pathways and regulatory systems in organs and tissues that cause differences in the accumulation of metabolites (Li *et al.* 2020). Therefore, it is needed to identify the metabolites in each plant part as initial information to select the most effective *C. asiatica* plant part as herbal medicine.

In this study, we aimed to putatively identify the metabolites in each part of the *C. asiatica* plant using a UHPLC-Q-Orbitrap HRMS-based untargeted metabolomics approach. The untargeted metabolomics approach is an effective way to identify metabolites in *C. asiatica* extract comprehensively. This approach has been widely employed to evaluate the chemical characteristics of food and beverages and plant metabolite profiles (Tang *et al.* 2020; Zhang *et al.* 2022; Rafi *et al.* 2022; Karomah *et al.* 2023; Anggela *et al.* 2024; Gioktavian *et al.* 2024; Saputra *et al.* 2024). Several studies have reported the identification of metabolites in *C. asiatica* extract (Jiang *et al.* 2016; Alcazar *et al.* 2020; Kunjumon *et al.* 2022; Sabaragamuwa *et al.* 2022). However, no reported paper described the distribution of metabolites in each plant part of *C. asiatica*. Therefore, this study aims to identify the metabolites in each part of the *C. asiatica* plant, i.e., leaves, stems, stolons, and roots. In addition, a chemometric analysis, namely principal component analysis, was used to confirm the distribution of metabolites of *C. asiatica* plant parts based on their metabolite composition and concentration.

2. Materials and Methods

2.1. Plant Materials

C. asiatica plants were obtained from the medicinal plant experimental garden of Tropical Biopharmaca Research Center (TropBRC), IPB University, Bogor, Indonesia. A botanist from TropBRC, Taopik Ridwan, M.Si identified the sample. A voucher specimen number BMK0105082016 was documented in TropBRC. The samples were washed and separated between leaves, stems, stolons, and roots in five replicated. Each part of the plant was dried in the oven at a temperature of 45°C for three days, then ground into powder and filtered with an 80 mesh sieve.

2.2. Chemicals and Instruments

Methanol p.a was used as the solvent extraction and purchased from Merck (Darmstadt, Germany). Methanol, water, and acetonitrile (LC-MS grade) were obatined from Merck (Darmstadt, Germany) and were used as the mobile phase. The UHPLC-Q-Orbitrap HRMS instrument (Thermo Scientific, Germany) was used for the separation of metabolites, as well as software ThermoXCalibur (Thermo Scientific, Germany), Compound Discoverer 3.2 (Thermo Scientific, Germany) for metabolite separation and identification. The Unscrambler X version 10.1 (Camo, Oslo, Norway) was used for principal component analysis.

2.3. Sample Preparation

A total of 500 mg of sample powder *C. asiatica* leaves was extracted using 5 ml of methanol and sonicated for 30 minutes. The filtrate was then filtered using 0.2 µm nylon for UHPLC-Q-Orbitrap HRMS analysis. *C. asiatica* root, stolon, and stem was also extracted using the procedure described above.

2.4. UHPLC-Q-Orbitrap HRMS Analysis

Metabolites in each extract of *C. asiatica* were separated and detected using UHPLC-Q-Orbitrap HRMS using AccucoreTM C18 column (100 × 2.1 mm, 1.5 µm). The mobile phase used was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) in a gradient elution system: 0-1 min (5 %B); 1-20 min (5-45%B); 20-27min (45-95%B); 27-30 (95%B), 30-35min (5%B). The flow rate used was 0.2 mL/min and the injection volume was 2.0 µL. The ionization source is ESI with positive ionization mode with a spray voltage of 3.8 kV. Other mass spectrometer system parameters are set as follows: the m/z range used is 150-2,000, capillary temperature 320°C, collision energy for fragmentation used was 18, 35, and 53 eV, sheath and auxiliary gas 15 and 3 ml/min, respectively. Full MS/dd MS2 was used as the scan type and a resolving power of 70,000 FWHM.

2.5. Identification of Metabolites in *C. asiatica*

Raw data from UHPLC-Q-Orbitrap HRMS analysis was processed using Compound Discoverer 3.2 to putatively identify the metabolites in each plant part of *C. asiatica* extract. The metabolite identification stages are as follows: selected spectra, alignment retention time, detecting unknown compounds, grouping unknown compounds, predicting processes,

searching mass lists, filling gaps, normalizing areas, and marking background compounds. The detected metabolites were confirmed using the MS2 spectrum from the in-house database.

2.6. Multivariate Analysis

This study used multivariate principal component analysis (PCA) to distinguish extracts based on the part of the plant used. The PCA model was created using The Unscrambler X version 10.1 (CAMO, Oslo, Norway), using the peak intensity of the base peak chromatogram and the peak area of metabolites identified as the variable. The results of PCA are expected to distinguish the data set into four groups: leaves, stems, stolons, and roots. A heatmap of hierarchical cluster analysis (HCA) model was built using Metaboanalyst (https://www.metaboanalyst.ca/).

3. Results

3.1. Putative Identification of Metabolites *C. asiatica* Extracts

Untargeted metabolomics was used in this study for putative identification of metabolites in different plant parts of *C. asiatica*, namely leaves, stems, stolons, and roots. Figure 1 shows the chromatogram of the leaves, stems, stolons, and roots of *C. asiatica* with similar separation patterns but differences in their peak intensity. From this chromatogram, it can be seen that the distribution of metabolites is nearly similar, only different in their concentration. A total of 37 metabolites were putatively identified in all plant parts of *C. asiatica* (Table 1).

3.2. Clustering *C. asiatica* Extracts

Differentiations in the distribution of metabolites in each plant part of *C. asiatica* were evaluated using PCA. The PCA was performed using the base peak chromatogram and the area of the identified metabolite peaks. Before being subjected to PCA, pre-processing was carried out on the chromatogram peak intensity data using correlation optimized warping (COW) to align the retention time of each peak. Figure 2 and 3 showed the results of the PCA and heatmap of HCA, respectively. In the PCA, each plant part was classified according to its group using the intensity of the base peak chromatogram and peak area of the identified metabolites. Furthermore, the HCA heatmap analysis was performed using the peak area of each identified metabolite using the Euclidean distance measure and the Ward clustering

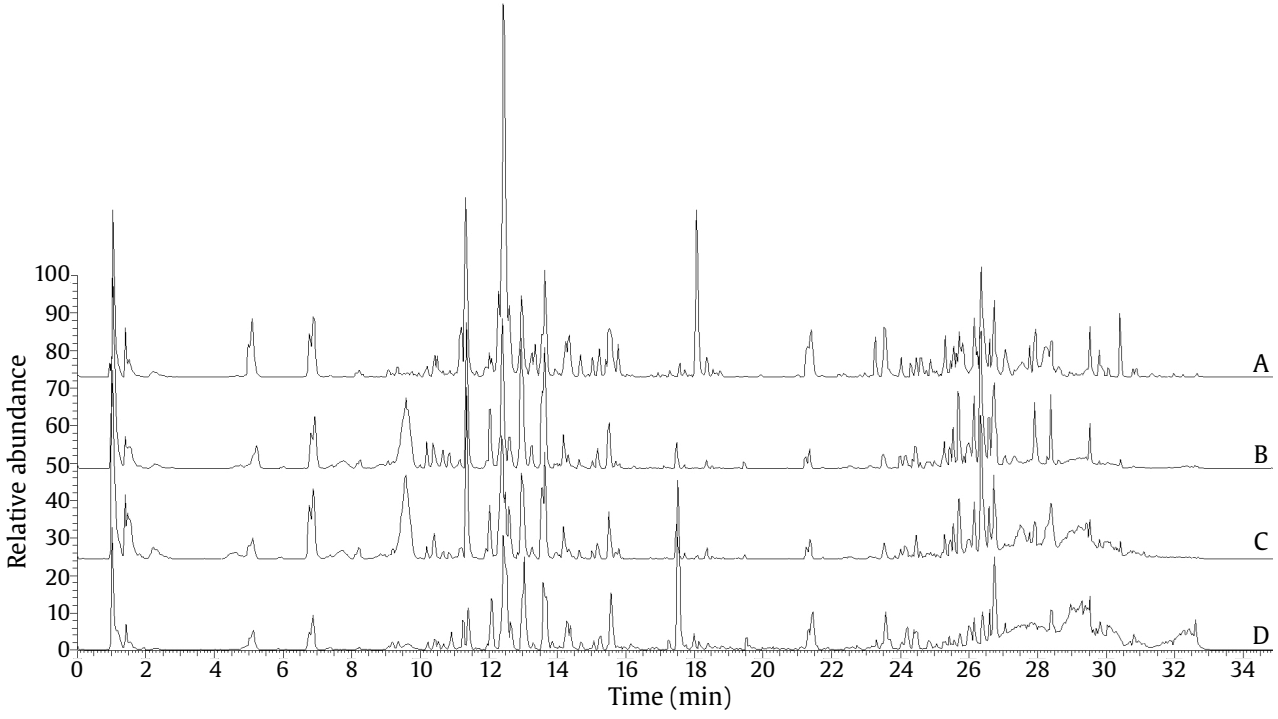

Figure 1. Base peak chromatogram of *C. asiatica* extract with different plant parts: leaves (A), stolons (B), stems (C), and roots (D) extract

Table 1. Putative metabolites in methanol extract of *Centella asiatica* species using UHPLC-Q-Orbitrap HRMS

Metabolite	Rt (min)	Formulas	MW	Errors (ppm)	MS2	Sample			
						roots	Stems	Leaves	Stolons
Phenylpropanoid									
Dicaffeoylglucaric	9.597	$C_{24}H_{22}O_{14}$	534.09901	-3.65	535, 287, 137	√	√	√	√
Chlorogenic acid	6.853	$C_{16}H_{18}O_9$	354.09377	-3.69	355, 163, 154, 135	√	√	√	√
Dihydroferulicacid-4-O-glucuronide	9.194	$C_{16}H_{20}O_{10}$	372.10445	-3.21	373, 197, 179	√	√	√	√
Feruloylquinic acid	9.34	$C_{17}H_{20}O_9$	368.10956	-3.19	369, 177, 145	√	√	√	√
Prenyl Caffeate	11.541	$C_{14}H_{16}O_4$	248.10428	-2.34	249, 221, 159		√	√	√
p-Coumaroylferuloyltartaric acid	11.919	$C_{23}H_{20}O_{11}$	472.10191	2.85	473, 279, 163		√	√	√
Tricaffeoylquinic acid	12.333	$C_{34}H_{30}O_{15}$	678.15618	-3.37	679, 163, 135	√	√	√	√
5-Caffeoylshikimic acid	12.347	$C_{16}H_{16}O_8$	336.08321	-3.89	337, 163, 145	√	√	√	√
1,3-Dicaffeoylquinic acid	12.378	$C_{25}H_{24}O_{12}$	516.1248	-3.84	517, 163, 145	√	√	√	√
Dicaffeoylshikimic acid	12.388	$C_{25}H_{22}O_{11}$	498.11423	-3.99	499, 145, 135	√	√	√	√
Feruloylcaffeoylquinic acid	14.185	$C_{26}H_{26}O_{12}$	530.14057	-3.5	531, 177, 163, 145	√	√	√	√
Flavonoids (Flavonols)									
Quercetin-glucoronide	11.343	$C_{21}H_{18}O_{14}$	478.07299	-3.67	479, 303,153	√	√	√	√
Quercetin	11.345	$C_{15}H_{10}O_7$	302.04139	-4.18	303, 153, 137	√	√	√	√
Quercetin-3-O-(6'-O-malonyl)-glucoside	12019	$C_{24}H_{22}O_{15}$	550.09383	-3.71	551, 303, 153	√	√	√	√
Quercetin dirhamnosylhexoside	9.763	$C_{33}H_{40}O_{20}$	756.2083	-3.96	757, 303, 153	√		√	
Rutin	10.408	$C_{27}H_{30}O_{16}$	610.15101	-3.89	611, 303	√	√	√	
Kaempferol-3-O-rutinoside	11.21	$C_{27}H_{30}O_{15}$	594.15607	-4.03	595, 287	√	√	√	√
Kaempferol 7-C-glucoside	12.646	$C_{21}H_{20}O_{11}$	448.09876	-4.02	449, 287			√	
Kaempferol	12.439	$C_{15}H_{10}O_6$	286.04663	-3.88	287, 153, 137	√	√	√	√
Flavonoids (Flavones)									
Luteolin-7-O-glucuronide	11.239	$C_{21}H_{20}O_{12}$	464.09372	-3.78	465, 303, 153			√	
Luteolin 6-C-glucoside	12.586	$C_{19}H_{18}O_{11}$	422.08302	-4.47	423, 163, 145	√	√	√	√
Flavonoids (Flavones)									
Luteolin-7-O-glucuronide	12.441	$C_{21}H_{18}O_{12}$	462.07803	-3.89	463, 287	√	√	√	√
Luteolin 6-C-glucoside	12.424	$C_{21}H_{20}O_{11}$	448.09863	-4.31	449, 287	√		√	√
Coumarins									
Isofraxidine	11063	$C_{11}H_{10}O_5$	222.05214	-3.06	223, 179, 163			√	
Stilbenes									
trans-Resveratrol	23.657	$C_{14}H_{12}O_3$	228.07803	-2.7	229, 187, 175, 159	√			
Triterpenoids									
Asiatic acid	15.519	$C_{30}H_{48}O_5$	488.34833	-3.77	489, 453, 435, 407	√	√	√	√
Corosolic acid	18.746	$C_{30}H_{48}O_4$	472.35407	-2.53	473, 437, 409			√	
Brahmic acid	21.368	$C_{30}H_{48}O_6$	504.34347	-3.21	505, 469, 459	√		√	
Triterpenoid saponins									
Centellasaponin H	12.526	$C_{54}H_{88}O_{25}$	1136.55784	-3.19	1137, 487, 469	√	√	√	√
Centellin	13.638	$C_{15}H_{22}O_3$	250.15595	-3.77	251, 233	√	√	√	√
Asiaticoside A	14.268	$C_{48}H_{78}O_{20}$	974.50419	-4.57	975, 487	√	√	√	√
Centelloside E	14.272	$C_{48}H_{76}O_{19}$	956.49389	-4.38	957, 487, 469	√	√	√	√
Quadranoside IV	14.28	$C_{36}H_{58}O_{10}$	650.40064	-3.62	651, 451	√	√	√	√
Centellasaponin B	14.298	$C_{42}H_{68}O_{16}$	828.44745	-3.97	829, 487, 469			√	
Asiaticoside	14.681	$C_{48}H_{78}O_{19}$	958.50904	-4.89	959, 489, 471, 453	√	√	√	√
Asiaticoside C	17.128	$C_{50}H_{80}O_{20}$	1000.52201	-2.29	1001, 513, 453	√	√	√	√
Asiaticoside D	18.738	$C_{48}H_{78}O_{18}$	942.5159	-3.09	943, 455	√	√	√	√

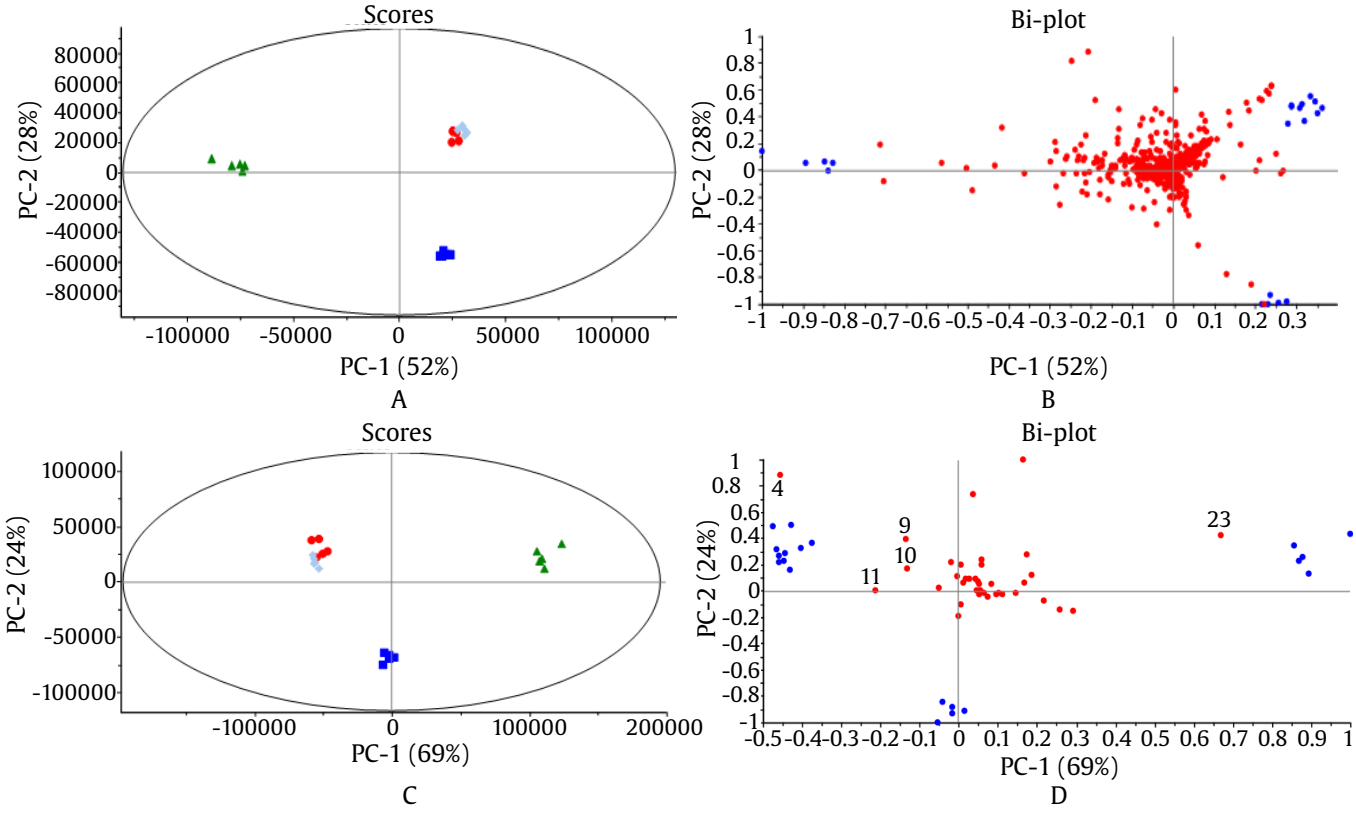

Figure 2. PCA of *C. asiatica* extract with different plant parts (■: roots, ▲: leaves, ●: stems, and ●: stolons) using base peak chromatogram (score plot (A) using intensity peak and biplot of PCA (B)) and peak area of identified metabolites (score plot (C) and PCA biplot (D))

method. From the result obtained, the HCA heatmap analysis also grouped the sample using the peak area of the identified metabolites. Also, the differences in the identified metabolite's composition in each extract are described in this HCA heatmap analysis, so this model illustrates the variation of metabolites present in each plant part of *C. asiatica*.

4. Discussion

An untargeted metabolomics approach by UHPLC-Q-Orbitrap HRMS was used to putatively identify metabolites in the *C. asiatica* plant part. A total 37 metabolites were putatively identified in this study. The identified metabolite consists of 13 flavonoids, 11 phenylpropanoids, 7 triterpenoid saponins, 3 triterpenoids, and 1 stilbene compound. Flavonoids are the most dominant compounds identified in *C. asiatica*. This study's results agree with those reported by Kandasamy *et al.* (2023) and Magana *et al.* (2020), who state that *C. asiatica* contains large amounts of flavonoid compounds. Flavonoid compounds that have been reported contained in *C. asiatica* i.e., kaempferol, rutin, quercetin, mangiferin, and flavonoid glycosides (Ondeko *et al.* 2020). Yang *et al.* (2023) also reported that several triterpenoid compounds, namely asiaticoside, madecassoside, asiatic acid, and madecassic acid

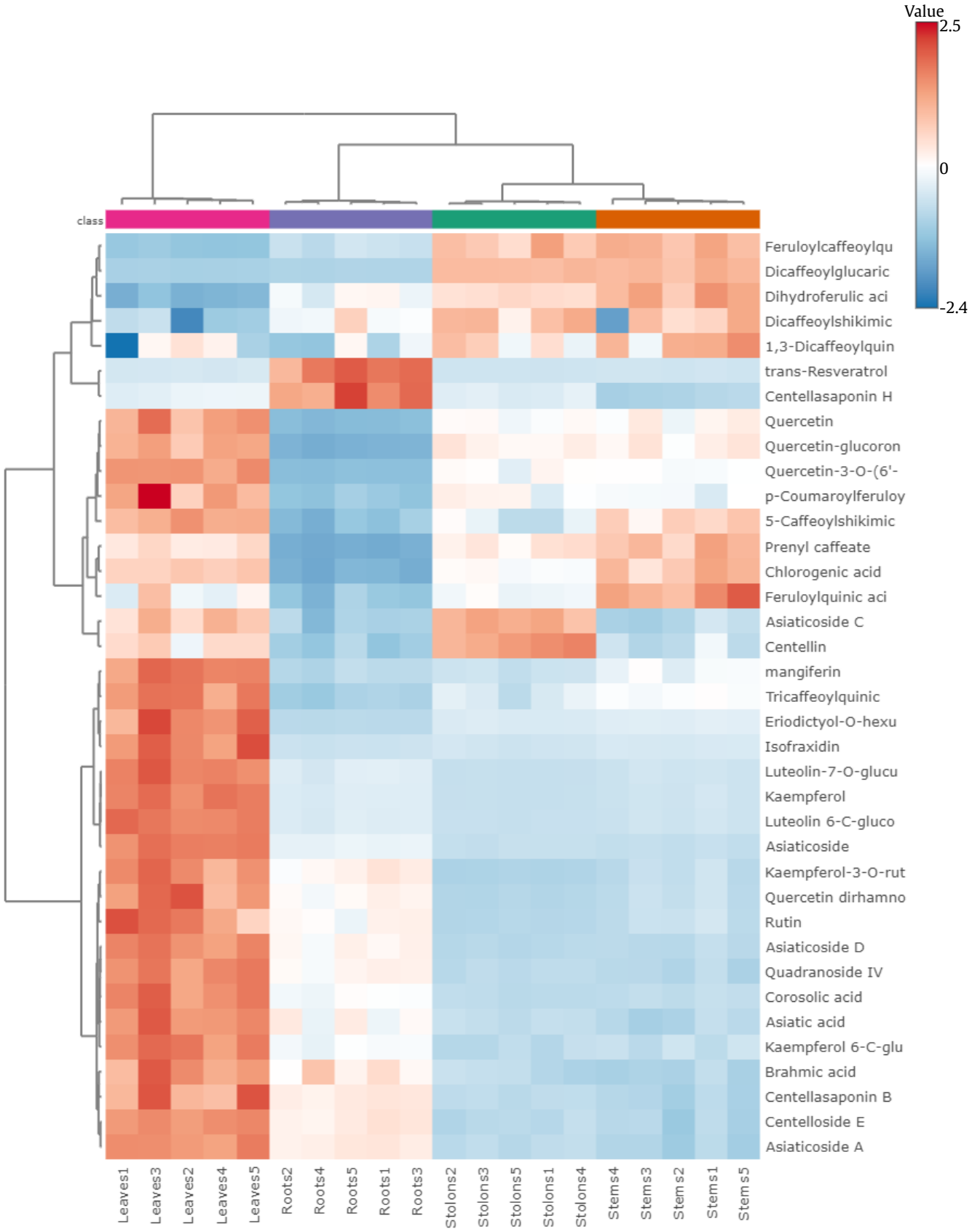

Figure 3. Heatmap of *C. asiatica* extract with five replications in four groups of heatmap compound in four groups with five replication

are biomarker compounds in *C. asiatica*. This compound has also been reported to contribute to the pharmacological properties of *C. asiatica* extract. Apart from flavonoids and triterpenoids, *C. asiatica* is also reported to contain several phenylpropanoid compounds, such as 1,3-di-caffeoylquinic acid and 1-caffeoyl-5-feruloylquinic acid (Magana *et al.* 2020). In this study, to putatively identify metabolites in *C. asiatica*, metabolite annotations were confirmed from MS2 fragmentation and compared with the literature.

Phenylpropanoid compounds are characterized by a phenyl group that binds three carbon chains. Dicaffeoylglucaric (1) was fragmented and released $C_6H_8O_8$, CO, and $C_9H_7O_3$ generated fragment ion m/z 137 $[M+H-208-28-163]^+$ release mass 399 Da. Chlorogenic acid (2) was fragmented by releasing $C_7H_{11}O_6$ to produce fragment ion m/z 163 $[M+H-191]^+$, followed by releasing CO at m/z 135 $[M+H-191-28]^+$. Dihydroferulicacid-4-O-glucuronide (3) lost its hexose glycone ($C_6H_8O_6$) to produce fragment ion at m/z 197 $[M+H-176]^+$, followed by releasing H_2O at m/z 179 $[M+H-176-18]^+$.

Feruloylquinic acid (4) loses one glycone heptose ($C_7H_{10}O_5$), followed by releasing H_2O and producing a fragment ion at m/z 177 $[M+H-174-18]^+$. The fragmented compound with m/z 177 was further fragmented to m/z 145 after losing CH_3OH $[M+H-42-32]^+$. Prenyl caffeate (5) was detected to give a fragmentation by releasing CO at m/z 221 $[M+H-28]^+$ and $C_5H_{14}O$ at m/z 159 [M+H-89]+. Tricaffeoylquinic acid (7) fragmented, releasing $C_{25}H_{23}O_{12}$ to produce fragment ion m/z 163 [M+H-515]+, followed by losing CO to generate fragment ion m/z 135 $[M+H-163-28]^+$ releasing mass 28 Da.

5-caffeoylshikimic acid (8) releasing a glycone $C_7H_9O_5$ followed by H_2O to produces fragment ion at m/z 163 $[M+H-173]^+$ and 145 $[M+H-173-18]^+$. Fragmentation of 1,3-dicaffeoylquinic acid (9) losing glycone heptose ($C_7H_{10}O_5$) and $C_9H_7O_4$ to give fragment ion at m/z 163 $[M+H-353]^+$, followed by releasing H_2O to produce fragment ion 145 $[M+H-353-18]^+$. Dicaffeoylshikimic acid (10) was fragmented by releasing $C_{16}H_{15}O_8$ and H_2O to produce fragment ions at m/z 145 $[M+H-354]^+$. Feruloylcaffeoylquinic acid (11) was fragmented by releasing $C_{16}H_{15}O_8$ and H_2O to produce a fragment ion m/z 177 $[M+H-353]^+$. This compound is followed by releasing CH_3OH to create a fragment ion m/z 145 $[M+H-32]^+$, releasing a mass of 32 Da. This study detected most of the phenylpropanoids in all *C. asiatica* plant parts. Only prenyl caffeate (5) and p-Coumaroylferuloyltartaric acid (6) were not detected in the root.

Compounds from flavonols, flavanone, and flavones that belong to the flavonoid group were identified in *C. asiatica*. Flavonols belong to the 1,3-diarylpropane group, which binds the -OH group to the C2. The identified compounds classified as flavonols are quercetin-glucuronide (12), quercetin (13), quercetin-3-O-(6'-O-malonyl)-glucoside (14) that have structural similarities in the framework of quercetin. In contrast, their differences are in the substituents that bind it, such as glucoronide in compound (12), malonyl-glucoside, and polyacetyl.

Quercetin-glucuronide (12) was fragmented by releasing one hexose glycone indicated by the peak at m/z 303 $[M+H-176]^+$. The m/z 303 further fragmented to m/z 153 after the loss of $C_8H_6O_3$ through the retro-Diels–Alder reaction $[M+H-176-150]^+$. Meanwhile, quercetin (13) was fragmented through the rDA by releasing $C_8H_6O_3$ at m/z 153 $[M+H-150]^+$, followed by losing the hydroxyl group at m/z 137. Quercetin-3-O-(6'-O-malonyl)-glucoside (14) is fragmented by releasing malonylglucoside ($C_9H_{12}O_8$) at m/z 303 $[M+H-248]^+$, followed by losing $C_8H_6O_3$ through the rDA reaction at m/z 153 $[M+H-248-150]^+$. Quercetin-dirhamnosylhexoside (15) fragmented, releasing three hexose glycones ($C_{18}H_{30}O_{13}$) to produce 303 fragment ions $[M+H-454]^+$ followed by losing $C_8H_6O_3$ through the rDA reaction at m/z 153 [M+H-454-150]+.

Rutin (16) is fragmented by releasing two hexose glycones ($C_{12}H_{20}O_9$) to produce a fragment ion m/z 303 $[M+H-309]^+$. Kaempferol-3-O-rutinoside (17) fragmented, releasing two hexose glycones ($C_{12}H_{20}O_9$) to produce a fragment ion m/z 287 $[M+H-308]^+$. Kaempferol 7-C-glucoside (18) is fragmented by releasing a glycone hexose ($C_6H_{10}O_5$) to produce a fragment ion m/z 287 $[M+H-162]^+$. Kaempferol (19) undergoes an rDA reaction to generate fragment ions at m/z 153 $[M+H-133]^+$.

The flavanone compounds include eriodictyol-O-hexuronide (20) and mangiferin (21), grouped into flavonoid compounds. These two compounds are similar in that they have a ketone group which forms a chelate due to interactions with the -OH group and a glycone group in ring B. The difference between eriodictyol-O-hexuronide (20) and mangiferin (21) is that there is a carbon bridge that connects ring A and ring B in eriodictyol-O-hexuronide (20). There

are three carbons (-C-CH_2-CH), while in mangiferin (21) there is only one carbon (-C-). The eriodictyol-O-hexuronide compound (20) is fragmented by releasing $C_6H_{10}O_5$ and giving fragment ions at m/z 303 $[M+H-162]^+$. Mangiferin (21) fragmented by releasing $C_{13}H_8O_6$ to provide a fragment ion at m/z 163 $[M+H-260]^+$, followed by releasing H_2O to produce a fragment ion at m/z 145 [M+H-260-18].

Flavones are characterized by a double bond on the C2 carbon. The two flavones identified, namely Luteolin-7-O-glucuronide (22) and Luteolin-6-C-glucoside (23), only differ in the glycone bond in ring A. The two compounds fragmented by undergoing an ester reduction reaction, releasing one glycone hexose to generate a fragment ion at m/z 287.

The coumarins compound is a flavonoid derivative compound characterized by the absence of chelation, which means that there is no -OH group adjacent to the carbonyl group, isofraxidine (24) had an m/z of 222. Isofraxidine (24) is fragmented by releasing methyl (-CH_3) and hydroxyl (-OH) groups, followed by CO at m/z 163 $[M+H-60]^+$.

The stilbenes compound belongs to the phenolic group, characterized by a prenyl group on rings A and B and two carbons connecting the two rings. Trans-resveratrol (25) had an m/z of 229 and fragmented by freeing three H_2O molecules at m/z 175 $[M+H-54]^+$. The fragment ion at m/z 187 was generated from the C_2H_2O loss from the ion at m/z 229 $[M+H-42]^+$. The fragmented molecule with m/z 185 further fragmented to m/z 159 after a loss of CO $[M+H-42-28]^+$ (Wang *et al.* 2005).

Compounds belonging to the triterpenoids group are asiatic acid (26), corosolic acid (27), and brahmic acid (28). These three compounds share the same structural framework, a triterpenoid framework, but differ in their substituents at C_6 and C_{24} carbon. Asiatic acid (26) is fragmented by freeing three H_2O molecules at m/z 453 $[M+H-36]^+$ and 435 $[M+H-54]^+$, followed by releasing CO at m/z 407 $[M+H-54-28]^+$. Corosolic acid (27) is fragmented by releasing two H_2O molecules at m/z 437 $[M+H-36]^+$, followed by releasing CO at m/z 409 $[M+H-36-28]^+$. Brahmic acid (28) is fragmented by releasing two H_2O molecules at m/z 469 $[M+H-36]^+$ and releasing COOH at m/z 459 $[M+H-45]^+$.

This compound is characterized by a triterpenoid structural framework that binds glycone to the A ring. Compounds belonging to the triterpenoid saponin group are asiaticoside A (31), centelloside E (32), quadranoside IV (33), centellasaponin B (34), and asiaticoside (35), asiaticoside C (36), and asiaticoside D (37). Those five compounds differ in the number of glycones attached at the A ring and the -OH substituents attached at the D and E rings.

Asiaticoside A (31) is fragmented by releasing three glucose molecules, i.e., mannopyranose and two glucopyranoses at m/z 487 $[M+H-488]^+$. Centelloside E (32) is fragmented by releasing three glucose molecules, i.e. mannopyranose and two glucopyranoses at m/z 469 $[M+H-488]^+$. Quadranoside IV (33) is fragmented by releasing $C_{11}H_{20}O_3$ molecules at m/z 451 $[M+H-200]^+$. Centellasaponin B (34) is fragmented by releasing two molecules of glucopyranose at m/z 487 $[M+H-342]^+$ and followed by releasing H_2O at m/z 469 $[M+H-C_{12}H_{22}O_{11}-H_2O]^+$.

Asiaticoside (35) is fragmented by releasing three glucose molecules, i.e., mannopyranose and two glucopyranoses at m/z 471 $[M+H-488]^+$ and followed by releasing H_2O at m/z 453 $[M+H-488-18]^+$. Asiaticoside C (36) is fragmented by releasing three glucose molecules, i.e., mannopyranose and two glucopyranoses at m/z 513 $[M+H-488]^+$, followed by releasing CH_3COOH at m/z 453 $[M+H-488-60]^+$. Asiaticoside D (37) is fragmented by releasing three glucose molecules, i.e., mannopyranose and two glucopyranoses at m/z 455 $[M+H-488]^+$.

A combination of metabolomics approaches and chemometrics were used to identify differences in metabolite distribution in each part of the *C. asiatica* plant. PCA is used to reduce data to describe relationships between samples, for example, the grouping patterns of each sample (Dadwal *et al.* 2023). The PCA score plot using a variable from the peak intensity of base peak chromatogram and identified metabolites peak area showed a similar pattern. Overall, the PCA score plot can differentiate the sample into three groups, namely (i) leaves, (ii) roots, and (iii) stems and stolons. PC1 of the two score plots (52% and 69%) depicts clear differences between leaves, stems, stolons, and roots. Likewise, PC2 (28% and 24%) could differentiate between roots and leaves, stems, and stolons. However, neither PC could clearly distinguish between stems and stolons. Stolons are modifications of stems that grow sideways, so the two parts of this plant have the same metabolic biosynthetic pathway. This resulted in the similarity of metabolite content between stems and stolons. The PCA biplot

(Figure 2D) showed that dicaffeoylglucaric (4), 1,3-dicaffeoylquinic acid (9), dicaffeoylshikimic acid (10), and feruloylcaffeoylquinic acid (11) were the most abundant metabolites accumulated in stems and stolons. At the same time, luteolin-7-O-glucuronide (22) is the most abundant metabolite accumulated in leaves. The PCA analysis results illustrate that plant parts' differences can affect the distribution of metabolites, which will impact the biological activity caused.

Apart from that, the HCA heatmap is also used to describe variations in metabolite composition in each part of the plant. The HCA heatmap illustrates each sample's metabolite variation (Figure 3). *C. asiatica* leaves have the most varied metabolite level compared to other plant parts, mainly from flavonoid and triterpenoid compounds. Meanwhile, the stem and stolon are dominated by phenylpropanoids. Also, clustering with HCA also shows results similar to those of PCA, which can divide the sample into three large clusters. The differences in metabolite variations indicate that the part of the plant extracted can influence the metabolites contained in an extract.

In conclusion, a metabolomics approach based on UHPLC-Q-Orbitrap HRMS combined with chemometrics was successfully used to identify and evaluate metabolite differences in *C. asiatica* roots comprehensively, stems, stolons, and leaves extracts. Identification of metabolites using UHPLC-Q-Orbitrap HRMS with an in-house database successfully identified 37 metabolites putatively. The identified metabolites consist of phenylpropanoids, triterpenoids, triterpenoid saponins, and flavonoids. PCA can clearly distinguish the metabolites contained in each part of the plant using the peak intensity of the chromatogram and the area of the peaks of the identified metabolites. This indicates that differences in plant parts can affect the distribution of metabolites contained therein due to differences in plant metabolic pathways.

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