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Analysis of Metabolite Profile of Gambier Flower (*Uncaria gambir* (Hunter) Roxb.) in Various Solvent Fractions Using LC-MS

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Abstract: The gambier plant is known as a rich source of secondary metabolites with biological activities, including anticancer, antioxidant, and antimicrobial properties. The leaves of the gambier plant are reported to contain flavonoids, including catechins, and tannins. This study aims to analyze the metabolite profile of gambier flowers using Liquid Chromatography-Mass Spectrometry (LC-MS). Gambier flower samples were extracted by maceration using ethanol as the solvent. The resulting macerate was then fractionated using n-hexane, ethyl acetate, and n-butanol. The gambier flower extracts were tested for phytochemical content via thin-layer chromatography (TLC) and analyzed for secondary metabolite content via LC-MS for each fraction. TLC testing of the total extract yielded positive results for flavonoids, steroids, phenolics, and alkaloids. Analysis of the secondary metabolite profile of the total extract identified 6 compounds from the 14 compound chromatograms that appeared; the n-hexane extract showed 6 compounds successfully identified from a total of 20 compounds, the ethyl acetate extract showed 6 identified compounds from 17 compounds, and the n-butanol extract contained 7 known compounds from 35 compounds. From the metabolite profile analysis, it can be concluded that gambier flowers contain numerous bioactive compounds with potential for further development and can serve as a source of new knowledge in exploring plant potential. To the best of our knowledge, this is the first LC-MS-based metabolite profile of gambier flowers, an organ that has remained largely unexplored compared with the widely studied leaves; the flowers display a greater diversity of flavonoid glycosides and phenolic derivatives, highlighting their novelty and their promise as antioxidant-rich raw materials for the pharmaceutical and cosmetic industries.

Keywords: Cathechin; Gambier; Metabolic Profile; LC-MS; Secondary Metabolite.

Introduction

Secondary metabolites in plants play a crucial role in the fields of pharmaceuticals, cosmetics, and functional foods. In addition to their role in plant defense mechanisms against biotic and abiotic stress, these compounds have been widely reported to possess therapeutic activities, such as anticancer, anti-inflammatory, and antimicrobial properties. Technological advancements have also driven efforts to identify, isolate, and produce secondary metabolites more efficiently, thereby supporting the development of natural-product-based medicines [1].

Gambier (*Uncaria gambir* (Hunter) Roxb.) is a plant native to Indonesia from the Rubiaceae family that has long been used in various traditional practices, including as a herbal medicine, a tanning agent, and a component of betel mixtures. Gambier plants are distributed across several regions, including West Sumatra, South Sumatra, Riau, and Bangka Belitung. Among these locations, West Sumatra has the largest gambier plantations, particularly in the districts of Lima Puluh Kota and Pesisir Selatan [2][3].

Gambier leaves are rich in flavonoids, including catechins and tannins. Findings by Munggari et al. also confirm the presence of other groups of secondary metabolites, such as phenolics and alkaloids [4]. Several studies report that gambier leaf extract is rich in bioactive compounds with various biological activities, such as antioxidant [5], antibacterial [6], cytotoxic [7], antifungal [8], antidiabetic [9], and so on.

To date, exploration of gambier's potential has been dominated by studies on the leaves, while research on the flowers remains very limited. However, as a reproductive organ, the flower may exhibit a different composition of secondary metabolites, in both type and quantity. This opens opportunities to discover new compounds or unique metabolites that have not been widely reported. This study aims to elucidate the metabolite profile of gambier flowers using LC-MS analysis. This study is both interesting and important, not only to supplement existing scientific information but also to explore new potential applications in the development of active ingredients for the pharmaceutical and cosmetic industries. Despite the extensive pharmacological literature on gambier, previous work has focused almost exclusively on the leaves and on a narrow set of catechin-type markers, while comparatively little attention has been paid to how metabolites distribute across solvent fractions of differing polarity. More importantly, no study to date has applied an LC-MS-based metabolomic approach to the flowers, leaving the secondary-metabolite landscape of this reproductive organ largely uncharacterized. Addressing this gap, the present study provides the first fraction-resolved LC-MS metabolite profile of gambier flowers, thereby positioning the flower as a novel and potentially high-value source of bioactive compounds.

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Research Methods

Materials and Extraction of the Crude Sample

Gambier flowers were collected from gambier farmers in the Siguntur area, Pesisir Selatan, West Sumatra. Gambier flower samples were identified at the ANDA Herbarium, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. A total of 500 g of gambier flower crude drug (*Uncaria gambir* (Hunter) Roxb.) was dried, ground into a homogeneous powder, and extracted by stepwise maceration with 70% ethanol. The first extraction step was conducted at a 1:10 (w/v) ratio for 24 hours at room temperature, with stirring for the first 6 hours and standing for the next 18 hours, followed by filtration. The residue was re-extracted with 70% ethanol at a 1:5 (w/v) ratio for 24 hours under the same conditions. All filtrates were combined and evaporated under vacuum using a rotary evaporator until a concentrated extract was obtained.

Phytochemical Testing of Concentrated Gambier Flower Extract

Phytochemical screening was conducted following the procedure described by Maheshwaran [10] with minor modifications. Qualitative analysis of alkaloids, flavonoids, phenolics, terpenoids, and steroids was based on precipitation reactions and color changes, which are characteristic properties of the chemical constituents found in plants.

Metabolite Profile Analysis by LC-MS

Identification of the components of the fractionated gambier flower was performed using Liquid Chromatography-Mass Spectrometry (LC-MS) at the Forensic Laboratory Center (Puslabfor) of the Indonesian National Police's Criminal Investigation Agency (Bareskim) in Jakarta. LC-MS analysis with LC: UPLC® H-Class System (Waters, USA); column: C18 (1.8 µm, 2.1 × 100 mm) HSS; mobile phase: water + 5 mM ammonium formate (A) and acetonitrile + 0.05% formic acid (B); flow rate: 0.2 mL/min (step gradient) for 23 minutes, injection volume; 5 µL. MS; Xevo G2-S QTOF (Waters, USA), system; ES (electrospray ionization), mode; positive mode.

Results and Discussion

Phytochemical Test Result

Preliminary phytochemical screening results indicate that the total extract of gambier flowers (*Uncaria gambir*) contains several major classes of secondary metabolites. The presence of flavonoids was indicated by the appearance of yellow to orange colors on thin-layer chromatography (TLC) plates. Steroid and triterpenoid compounds were identified by a color change to reddish-brown, while the phenolic group was marked by the formation of red, purple, blue, or black colors. Additionally, the test results also indicate the presence of alkaloids, as evidenced by the formation of blue to black colors. These findings confirm that the total extract of gambier flowers contains a diverse array of bioactive compounds with potential for further development.

LC-MS analysis profile in various solvents

The results of the secondary metabolite profile analysis of gambier flower extract (*Uncaria gambir*) using LC-MS are shown in Figure 1. The metabolite profile of the total extract indicates the presence of 14 compounds, of which 6 were successfully identified. The names, formulas, and fragmentation patterns of the compounds in the total extract and the identified compounds are listed in Table 1.

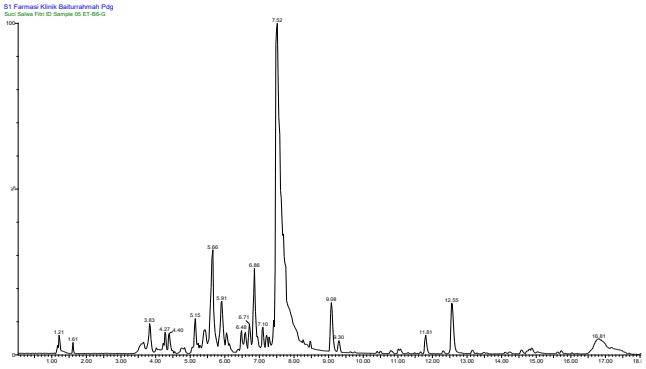

Figure 1. Chromatogram of the total extract of gambier flowers

Table 1. Compounds in the total extract of gambier flowers identified by LC-MS in positive mode

No	RT (min)	Compound	[M+H]+	Formula	Fragment (m/z)
1	1.21	*Aloeresin A*	541.2560	$C_{28}H_{29}O_{11}$	409,9321; 580,3471
2	3.83	*Catechin*	291.6811	$C_{15}H_{15}O_6$	139.3145; 580.3578
3	4.27	*Undetermined*	371.9589	$C_{21}H_{10}O_6$	227.6340; 372.9640
4	5.15	*Undetermined*	576,4243	$C_{26}H_{56}O_{13}$	355,9324; 574,4042
5	5.66	*Corynanthine*	355,9299	$C_{21}H_{26}N_2O_3$	353,9094; 356,9356
6	5.91	*Procyanidin*	395,1542	$C_{30}H_{27}O_{13}$	353,9094; 355,9258
7	6.71	*Undetermined*	353,9144	$C_{20}H_2O_7$	227,6368; 337,8853
8	6.86	*Dihydro-gambiertannine*	333,8445	$C_{21}H_{20}N_2O_2$	144,3671; 316,7814
9	7.52	*Undetermined*	327,0108	$C_{18}H_{15}O_6$	329,8029; 628,5812
10	9.08	*Undetermined*	666,7052	$C_{42}H_{82}O_5$	195,4805; 329,8039
11	9.30	*Undetermined*	664,6838	$C_{49}H_{76}$	227,6352; 329,8031
12	11.81	*Undetermined*	519,4033	$C_{26}H_{46}O_{10}$	318,9529; 520,401
13	12.55	*Undetermined*	521,4224	$C_{26}H_{49}O_{10}$	353,9953; 533,4577
14	16.81	*Safingol*	302,0464	$C_{18}H_{39}NO_2$	622,6045; 685,6344

Based on the results of positive-mode LC-MS screening of total gambier flower extracts, six compounds were identified, each belonging to a different class of compounds. Catechins, procyanidins, and dihydrogambiertannin belong to the pocorylyphenol group; aloeresin A is a group of phenolic compounds derived from chromones; while safingol belongs to the sphingolipid derivative group [11]. Catechins are among the main active compounds characteristic of gambier products, as reported in several previous studies [12][13]. Procyanidins, also known as condensed tannins, are compounds formed by the combination of two or more units such as catechin/epicatechin, epigallocatechin, epicatechin gallate, and catechin dimers. The formation of these compounds

generally occurs through a polymerization process triggered by heating to the boiling point during the gambier extraction process [14].

Analysis of the secondary metabolite profile of the n-hexane extract revealed the presence of 20 compounds (Figure 2). Based on the fragmentation pattern analysis, 6 compounds were identified (Table 2) as corynoxinic acid, quinovic acid, atropuroside, epigallocatechin 3-O-p-coumarate, and 2 compounds identified as aloeresin a. The six successfully identified compounds belong to a more diverse group. Corynoxinic acid belongs to the alkaloid group; quinovic acid and atropuroside belong to the triterpenoid group; epigallocatechin 3-O-p-coumarate belongs to the flavonoid group; and aloeresin A belongs to the phenolic group.

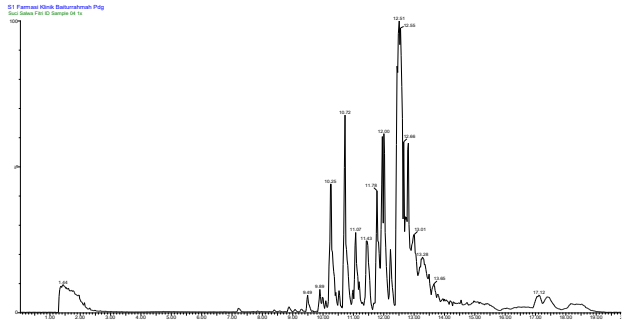

Figure 2. Chromatogram of the n-hexane extract of gambier flowers

Table 2. Metabolite profile of the n-hexane extract

No	RT (min)	Compound	[M+H]+	Formula	Fragment (m/z)
1	1.21	*Undetermined*	147,114+	$C_{11}H_{15}$	74.0609, 75.0643
2	9.47	*Corynoxinic acid*	371.1971+	$C_{21}H_{27}N_2O4$	353.1889, 398.1356
3	9.49	*Undetermined*	398,1362+	$C_{22}H_{22}O_7$	130.0661, 368.1252
4	9.89	*Undetermined*	424,149+	$C_{24}H_{24}O_7$	2193.2166, 392.1593
5	10.25	*Undetermined*	311,128+	$C_{19}H_{19}O_4$	130.0654, 177.0550
6	10.72	*Quinovic acid*	487.3127+	$C_{30}H_{47}O_5$	423.3082, 512.1828
7	11.07	*Undetermined*	462,2033	$C_{28}H_{30}O_6$	269.1180, 434.1967
8	11.42	*Atropuroside*	595.3179	$C_{30}H_{47}O_5$	349.1450, 596.2404
9	11.43	*Undetermined*	349,145	$C_{22}H_{21}O_4$	268.2647, 350.1477
10	11.77	*Epigallocatechin 3-o-p-coumarate*	452,1896	$C_{24}H_{21}O_9$	295.1342, 296.1317
11	11.78	*Undetermined*	295,1331	$C_{19}H_{19}O_3$	296.1375, 526.1993
12	12.00	*Undetermined*	526,1968	$C_{32}H_{30}O_7$	311.1271, 498.1901
13	12.48	*Aloeresin A*	541.2173	$C_{28}H_{29}O_{11}$	541.2173
14	12.51	*Undetermined*	540,2142	$C_{33}H_{32}O_7$	311.1287, 521.2077
15	17.10	*Aloeresin A*	541.1710	$C_{24}H_{29}O_{11}$	339.2894, 413.3781
16	17.12	*Undetermined*	413,371	$C_{29}H_{49}O$	313.2745, 337.2740

LC-MS analysis of the ethyl acetate extract of gambier flowers yielded chromatogram profiles and mass spectra indicating the presence of various metabolites. The LC-MS spectrum of the ethyl acetate extract showed 17 peaks, as shown in Figure 3, with compound identification details in Table 3. Six compounds were successfully identified as 3-(3,4-dihydroxycinnamoyl)quinic acid, uncarechin, quercetin 3-(4”-acetylrhamnoside) 7-rhamnoside, catechin, epigallocatechin 3-O-p-coumarate, and arecatannin A1. In general, this profile is dominated by polyphenols, particularly flavonoids and their derivatives, which are known for their strong antioxidant activity.

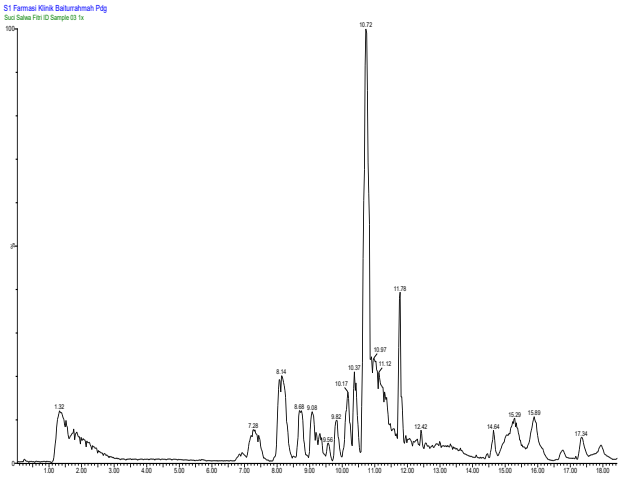

Figure 3. Chromatogram of the ethyl acetate extract of gambier flowers

Table 3. Metabolite profile of ethyl acetate extract

No	RT (min)	Compound	[M+H]+	Formula	Fragment (m/z)
1	1.34	*Undetermined*	147,1142	$C_{11}H_{15}$	74.0610, 144,9835
2	7.28	*Undetermined*	354,1116	$C_{20}H_{18}O_6$	130.0666, 174.0570
3	7.43	*3-(3, 4-Dihyroxycinnamoly) Quinic Acid*	355,1146	$C_{16}H_{18}O_9$	174.0570, 326.1052
4	8.14	*Undetermined*	394,1444	$C_{23}H_{22}O_6$	130.0667, 193.0882
5	9.19	*Uncarechin*	327.0869	$C_{18}H_{15}O_6$	297.1124, 498.1664
6	9.08	*Undetermined*	434,1317	$C_{30}H_{26}O_7$	297.1123, 470.1598
7	9.82	*Undetermined*	498,1664	$C_{43}H_{32}O_{11}$	528.1763, 701.2243
8	10.17	*Undetermined*	311,1288	$C_{19}H_{19}O_4$	144.9827, 2971128
9	10.37	*Undetermined*	512,1833	$C_{31}H_{28}O_7$	285.2909, 311.1284
10	10.72	*Quersetin 3-(4”-acctylrhamnoside) 7-rhamnoide*	637.1769	$C_{29}H_{33}O_{16}$	512.1830, 575.222,
11	11.37	*Catechin*	291.1013	$C_{15}H_{14}O_6$	137.0596, 145.0283
12	11.78	*Undetermined*	295,1325	$C_{19}H_{19}O_3$	115.0546, 187.0754
13	12.42	*Undetermined*	354,1705	$C_{18}H_{26}O_7$	295.1329, 304.3001
14	14.64	*Epigallocatechin 3-o-p-coumrate*	453,1186	$C_{24}H_{21}O_9$	324.3263, 799.2285
15	15.29	*Arecatannin A1*	867,2136	$C_{45}H_{39}O_{18}$	696,5400, 324.3263
16	15.89	*Undetermined*	696,5398	$C_{37}H_{76}O_{11}$	128.0627, 534.4879
17	17.34	*Undetermined*	698,5535	$C_{37}H_{78}O_{11}$	264.2685, 536.5034

The LC-MS analysis of the *n*-butanol extract of gambier flowers revealed a diverse range of metabolites, as reflected in the resulting chromatogram profiles and mass spectra. A total of 17 distinct peaks were observed in the LC-MS spectrum (Figure 4), indicating the presence of multiple compounds within the extract. Detailed identification of these compounds is further presented in Table 4.

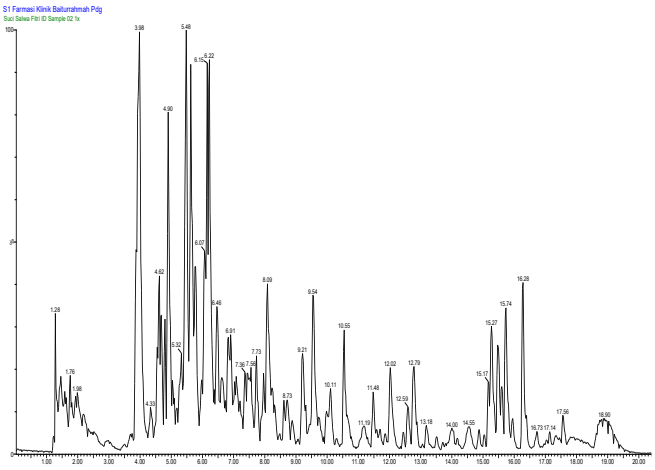

Figure 4. Chromatogram of the n-butanol extract of gambier flowers

Table 4. Metabolite profile of n-butanol extract

No	RT (min)	Compound	[M+H]+	Formula	Fragment (m/z)
1	1.28	*Unknown*	136,0632^{+}	$C_7H_4O_3$	95.0613, 118.0875
2	1.76	*Unknown*	268,1052	$C_{12}H_{28}O_6$	123.0563, 136.0628, 154.0508
3	1.98	*Unknown*	159,0780	$C_6H_7O_5$	128.0719, 136.0632, 154.0513
4	3.98	*Unknown*	146,0732	$C_{10}H_{10}O$	91.0550, 113.0605
5	4.62	*Quercetin-3-o-rhamnoside*	449,1084	$C_{21}H_{21}O_{11}$	287.0556, 450.1140
6	4.90	*Quersetin 3-Glukoside*	465,1038	$C_{21}H_{20}O_{12}$	313.0709, 433.1132
7	5.03	*Kaempferol*	287.0549	$C_{15}H_{11}O_6$	449.1084, 595.1656
8	5.48	*Unknown*	211,0609	$C_{10}H_{11}O_5$	163.0405, 447.1302
9	6.22	*Unknown*	431,1353	$C_{22}H_{11}O_9$	395.1141, 432.1394, 577.1943
10	6.46	*Unknown*	461,1456	$C_{23}H_{25}O_{10}$	311.0564, 425.1243
11	6.91	*Unknown*	431,1351	$C_{22}H_{23}O_9$	309.2079, 251.1653
12	7.37	*3-(3, 4-Dihydroxycinnamoyl) Quinic Acid*	335,1029	$C_{16}H_{18}O_9$	283.0609, 438.2409
13	7.56	*Unknown*	561,1981	$C_{28}H_{33}O_{12}$	275.2012, 291.1961
14	7.73	*Unknown*	308,2233	$C_{15}H_{32}O_6$	271.0616, 275.2019
15	8.09	*Unknown*	325,2299	$C_{21}H_{29}N_2O$	233.1661, 277.2179
16	8.73	*Unknown*	277,2174	$C_{18}H_{29}O_2$	149.1336, 249.1863
17	9.21	*Unknown*	251,2019	$C_{16}H_{27}O_2$	251.1808, 233.1911
18	9.54	*Unknown*	291,1967	$C_{18}H_{27}O_3$	115.0552, 273.1852
19	10.11	*Unknown*	289,1805	$C_{18}H_{25}O_3$	159.0812, 271.1697
20	10.55	*Unknown*	820,6317	$C_{45}H_{88}O_{12}$	423.3234, 748.5435
21	11.48	*Unknown*	830,6317	$C_{16}H_{36}N_4O_2$	149.0609, 298.2751
22	12.02	*Unknown*	316,2867	$C_{18}H_{40}NO_3$	265.2183, 311.2236
23	12.61	*3', 4', 7-Trihydroxyflavanone*	741,2869	$C_{33}H_{41}O_{19}$	478.2942, 520.3412
24	12.79	*Unknown*	318,3026	$C_{22}H_{46}NO_8$	221.1183, 317.1964
25	13.18	*Unknown*	496,3425	$C_{28}H_{48}O_7$	277.2180, 409.3114
26	14.55	*Unknown*	688,5225	$C_{49}H_{68}O_2$	556.4472, 600.4719
27	15.17	*Unknown*	609,2825	$C_{38}H_{41}O_7$	161.0632, 380.3227
28	15.24	*3'4', 7-Trihydroxyflavanone*	271.0565	$C_{15}H_{13}O_5$	581.1626, 637.3107
29	15.27	*Unknown*	625,2816	$C_{33}H_{53}O_{11}$	161.0622, 609.2763
30	15.74	*Unknown*	593,2860	$C_{35}H_{45}O_8$	38.4009, 427.3642
31	16.58	*Unknown*	639,2849	$C_{35}H_{43}O_{11}$	395.3698, 535.2728
32	16.73	*Unknown*	607,2957	$C_{36}H_{47}O_8$	411.3651, 532.4761
33	17.12	*Norcimifugin*	293.1190	$C_{15}H_{17}O_6$	313.2760, 579.2710
34	17.56	*Unknown*	954,6239	$C_{52}H_{86}O_{10}$	671.5190, 741.5500
35	18.90	*Unknown*	429,3826	$C_{28}H_{49}N_2O$	184,0787, 306.2854

In the n-butanol extract, the highest number of peaks (35) was detected, indicating greater chemical complexity than in the other fractions. This is related to the semipolar nature of n-butanol, which allows it to dissolve and extract polar-to-semipolar compounds more effectively, particularly phenolic and flavonoid compounds. These compounds are known to have high solubility in solvents with medium polarity, resulting in a greater number of metabolites detected in the LC-MS analysis [15]. In addition, the choice of solvent significantly influences the quantity and type of metabolites extracted; as the polarity of the solvent increases, so does the amount of bioactive compounds obtained [16].

From the total spectrum, seven compounds were successfully identified: quercetin-3-O-rhamnoside, quercetin 3-glucoside, kaempferol, 3-(3,4-dihydroxycinnamoyl) quinic acid, 3',4',7-trihydroxyflavanone, and norcimifugin. The presence of these compounds indicates the dominance of flavonoids and phenolic derivatives in the n-butanol extract, which are generally known to possess biological activities such as antioxidant properties and have potential for cosmetic and pharmaceutical applications.

The results of the LC-MS analysis indicate that the choice of solvent significantly influences the profile of metabolites detected in gambier flower (Uncaria gambir) extracts. Variations in the types and quantities of compounds across fractions reflect differences in the polarity of the solvents used, which directly affect extraction selectivity. The basic principle of extraction states that compounds dissolve optimally in solvents with similar polarity; thus, the use of a gradient of solvents allows for a more specific and comprehensive separation of metabolites [17][18]. In addition, the polarity of the solvent also affects the extraction efficiency of phenolic compounds and flavonoids, which are generally polar to semipolar [19].

In the total extract, identified compounds such as aloeresin A, catechin, procyanidin, corynanthine, dihydro-gambiertannin, and safingol indicate that gambier flowers contain various classes of metabolites, including flavonoids, condensed tannins, alkaloids, and lipid derivatives. Catechin and procyanidin are characteristic components of gambier, widely recognized as the primary compounds with high antioxidant activity [20]. The presence of these compounds indicates that the total extract comprehensively reflects the diversity of metabolites, albeit without specific selectivity toward particular classes. The presence of these compounds indicates that the total extract provides a comprehensive picture of metabolite diversity, even though it lacks specificity for certain classes.

In the n-hexane fraction, which is nonpolar, compounds such as corynoxinic acid and quinovic acid demonstrate this solvent's ability to extract lipophilic compounds such as triterpenoids and nonpolar alkaloids. Quinovic acid is known to possess biological activities, including anti-inflammatory and antimicrobial properties. However, the detection of phenolic compounds such as epigallocatechin 3-O-p-coumarate indicates that the extraction is not entirely selective, which is likely influenced by matrix interactions or co-extraction of compounds [21].

The ethyl acetate extract exhibited a more diverse metabolite profile, with the identification of 3-(3,4-dihydroxycinnamoyl)quinic acid, uncarechin, quercetin derivatives, catechin, epigallocatechin 3-O-p-coumarate, and arecatannin A1. Ethyl acetate, as a semipolar solvent, is known to be effective in extracting phenolic compounds with moderate polarity, including aglycone flavonoids and some glycosides. Compounds such as hydroxycinnamoyl quinic acid play an important role as natural antioxidants capable of neutralizing free radicals [17]. Meanwhile, the n-butanol extract showed a predominance of flavonoid glycosides, including quercetin-3-O-rhamnoside, quercetin-3-glucoside, and kaempferol. This indicates that n-butanol is highly effective at extracting polar compounds with numerous hydroxyl groups. Flavonoid glycosides have high solubility in polar solvents due to the presence of sugar groups that increase the molecule's hydrophilicity [3]. Additionally, compounds such as 3',4',7-trihydroxyflavanone and norcimifugin demonstrate the presence of more complex flavonoid structures. The presence of quercetin and kaempferol is also associated with antioxidant activity and protection against oxidative stress in cells, including skin cells [22].

Compared to gambier leaves, which are generally dominated by catechin, epicatechin, and procyanidin, the metabolite profile of gambier flowers shows greater diversity, particularly in flavonoid glycoside derivatives. This difference can be explained by variations in physiological functions among plant organs. Flowers tend to possess a more diverse array of secondary metabolites to support reproductive functions and interactions with the environment, such as attracting pollinators and providing protection against both biotic and abiotic stresses [23].

Beyond the descriptive profile, the distribution of compound classes carries a clear biological meaning. The flavonoid glycosides and phenolic acids that dominate the ethyl acetate and n-butanol fractions, quercetin and kaempferol glycosides, hydroxycinnamoyl quinic acids, and catechin derivatives, are well-established radical scavengers, which suggests that these two fractions are the most promising for antioxidant-driven pharmaceutical and cosmetic applications. By contrast, the triterpenoids and alkaloids recovered in the less polar n-hexane fraction, such as quinovic acid and corynoxinic acid, are more commonly associated with anti-inflammatory and antimicrobial activity, indicating that a polarity-graded fractionation could be used to tailor extracts toward specific bioactivities. A recent fraction-based LC-MS study of gambir similarly found that the more polar fractions concentrate the characteristic bioactive constituents and exhibit the strongest cellular activity [24], consistent with the present findings. It should also be noted that a large proportion of the detected peaks remained undetermined (8 of the 14 peaks in the total extract and most of the 35 peaks in the n-butanol fraction). On the basis of their accurate masses and fragmentation, many of these likely correspond to higher-order proanthocyanidin oligomers, glycosylated flavonoids, and lipid derivatives that are under-represented in the spectral databases used; their reliable annotation would require MS/MS acquisition in both ionization modes and comparison with authentic standards [25]. The principal limitations of the present study are therefore its reliance on database matching without isolated reference compounds, the use of positive-ion mode only, and the absence of absolute quantification—constraints that should be addressed in future work.

Conclusion

The findings of this study indicate that the use of solvents with medium-to-high polarity, such as ethyl acetate and n-butanol, yields better extraction results for bioactive compounds from gambier flowers. Both solvents proved more effective at extracting flavonoids and phenolic compounds, which are known to possess biological activity, particularly for applications in the cosmetic and pharmaceutical industries. These results also indicate that the flowers of the Uncaria gambir plant are a promising source of secondary metabolites but remain relatively under-explored compared to the leaves, which have traditionally been more widely utilized; thus, gambier flowers hold potential for further development as a high-value alternative raw material. More broadly, this work presents the first fraction-resolved LC-MS metabolite map of gambier flowers and demonstrates how a polarity-graded fractionation strategy can enrich specific metabolite classes for natural-product development. Future studies should focus on isolating and structurally confirming the major flavonoid glycosides and the currently undetermined peaks, quantifying them using validated targeted metabolomics, and evaluating their antioxidant, anti-inflammatory, and antimicrobial activities, thereby substantiating the pharmaceutical and cosmetic potential of gambier flowers beyond metabolite profiling alone.

Author's Contribution

A. Ferdian: Conceptualization, Methodology, Investigation, Data Curation, Writing-Original Draft, Supervision. A. Bakhtiar: Methodology, Validation, Formal Analysis, Writing-Review & Editing. S.S. Fitri M: Investigation, Data Curation, Visualization. N. Sandrawati: Supervision, Project Administration, Resources, Writing-Review & Editing.

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References

[1] B. Kaushik, J. Sharma, P. Kumar, and A. Shourie,

"Phytochemical properties and pharmacological role of plants: Secondary metabolites," *Biosci. Biotechnol. Res. Asia*, vol. 18, no. 1, p. 23, 2021, doi: 10.13005/bbra/2894.

[2] H. L. Hilmi and D. Rahayu, "Review artikel: Aktivitas farmakologi gambir (*Uncaria gambir* Roxb.)," *Farmaka*, vol. 16, no. 2, 2018, doi: 10.24198/jf.v16i2.17643.

[3] S. Sabarni, "Teknik pembuatan gambir (*Uncaria gambir* Roxb.) secara tradisional," *Elkawnie J. Islam. Sci. Technol.*, vol. 1, no. 1, pp. 105–112, 2015.

[4] I. P. Munggari, D. Kurnia, Y. Deawati, and E. Julaeha, "Current research of phytochemical, medicinal and non-medicinal uses of *Uncaria gambir* Roxb.: A review," *Molecules*, vol. 27, no. 19, p. 6551, 2022, doi: 10.3390/molecules27196551.

[5] A. T. Wibowo *et al*., "Metabolomic study and valuation of ethyl acetate extract of *Uncaria gambir* (W. Hunter) Roxb. as a natural antioxidant against lead acetate-induced free radicals," *Braz. J. Biol.*, vol. 86, p. e303070, 2026, doi: 10.1590/1519-6984.303070.

[6] D. Mahdiyah, M. Theana, A. Sari, and B. H. Mukti, "The antibacterial activity of gambir extract (*Uncaria gambir* (Hunter) Roxb.) against *Salmonella typhi*," *KnE Soc. Sci.*, pp. 747–757, 2023, doi: 10.18502/kss.v8i9.13389.

[7] N. Yunarto, H. Fadhilah, R. Purnamasari, and U. N. Reswandaru, "Cytotoxic activities of bioactive fraction of *Uncaria gambir* (Hunter) Roxb. on MCF-7 human breast cancer cell line," *J. Kefarmasian Indones.*, vol. 15, no. 1, pp. 107–115, 2025, doi: 10.22435/jki.v15i1.6717.

[8] R. Nugroho, D. Iskandar, R. Delyani, and D. I. Yama, "Antifungal study of *Uncaria gambir* Roxb. extract against *Ganoderma* sp. *in vitro*," *ROCE J. Pertan. Terap.*, vol. 2, no. 1, 2025, doi: 10.71275/roce.v2i1.52.

[9] I. Fajarwati, D. D. Solihin, T. Wresdiyati, I. Batubara, and S. S. Mariya, "Antidiabetic effects and mechanisms of action of *Uncaria gambir* Roxb. in diabetic Sprague–Dawley rats," *J. Am. Assoc. Lab. Anim. Sci.*, vol. 64, no. 1, pp. 35–43, 2025, doi: 10.30802/AALAS-JAALAS-24-117.

[10] C. B. Ranaweera, L. Maheshwaran, L. Nadarajah, S. Senadeera, A. K. Chandana, and R. N. Pathirana, "Phytochemical testing methodologies and principles for preliminary screening/qualitative testing," *Asian Plant Res. J.*, vol. 12, no. 5, pp. 11–38, 2024, doi: 10.9734/APRJ/2024/v12i5267.

[11] P. M. Dewick, *Medicinal Natural Products: A Biosynthetic Approach*, 2nd ed. Chichester, U.K.: John Wiley & Sons, 2002, doi: 10.1002/9780470742761.

[12] N. Rahmawati, A. Bakhtiar, and D. P. Putra, "Isolasi katekin dari gambir (*Uncaria gambir* (Hunter) Roxb.) untuk sediaan farmasi dan kosmetik," *Penelit. Farm. Indones.*, vol. 1, no. 1, pp. 6–10, 2012.

[13] N. N. Sazwi, T. Nalina, and Z. H. A. Rahim, "Antioxidant and cytoprotective activities of *Piper betle*, *Areca catechu*, *Uncaria gambir*, and betel quid with and without calcium hydroxide," *BMC Complement. Altern. Med.*, vol. 13, no. 1, p. 351, 2013, doi: 10.1186/1472-6882-13-351.

[14] J. Shi, Y. Wang, H. Wei, J. Hu, and M.-T. Gao, "Structure analysis of condensed tannin from rice straw and its inhibitory effect on *Staphylococcus aureus*," *Ind. Crops Prod.*, vol. 145, p. 112130, 2020, doi: 10.1016/j.indcrop.2020.112130.

[15] L. Derbak *et al*., "Extraction process and phenolic profiling of *Glebionis coronaria*: Insight into antioxidant and cytotoxic activities with ADME-based therapeutic potential," *Food Anal. Methods*, vol. 18, no. 8, pp. 1621–1636, 2025, doi: 10.1007/s12161-025-02818-7.

[16] N. Ghaffar and A. Perveen, "Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: A comparative study," *N. Z. J. Bot.*, vol. 63, no. 4, pp. 627–637, 2025, doi: 10.1080/0028825X.2024.2392705.

[17] H. Nawaz, M. A. Shad, N. Rehman, H. Andaleeb, and N. Ullah, "Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds," *Braz. J. Pharm. Sci.*, vol. 56, p. e17129, 2020, doi: 10.1590/S2175-97902019000417129.

[18] W. P. Jones and A. D. Kinghorn, "Extraction of plant secondary metabolites," in *Natural Products Isolation*, 2nd ed., S. D. Sarker and L. Nahar, Eds. Totowa, NJ, USA: Humana Press, 2012, pp. 341–366, doi: 10.1007/978-1-61779-624-1_13.

[19] A. Jurinjak Tušek, D. Šamec, and A. Šalić, "Modern techniques for flavonoid extraction—To optimize or not to optimize?," *Appl. Sci.*, vol. 12, no. 22, p. 11865, 2022, doi: 10.3390/app122211865.

[20] P. Buzzini *et al*., "Catechins and proanthocyanidins: Naturally occurring O-heterocycles with antimicrobial activity," in *Bioactive Heterocycles IV*, Berlin, Germany: Springer, 2007, pp. 239–263, doi: 10.1007/7081_2007_065.

[21] V. Arbona, D. J. Iglesias, M. Talón, and A. Gómez-Cadenas, "Plant phenotype demarcation using nontargeted LC-MS and GC-MS metabolite profiling," *J. Agric. Food Chem.*, vol. 57, no. 16, pp. 7338–7347, 2009, doi: 10.1021/jf9009137.

[22] T. Okselni *et al*., "Quercetin as a therapeutic agent for skin problems: A systematic review and meta-analysis on antioxidant effects, oxidative stress, inflammation, wound healing, hyperpigmentation, aging, and skin cancer," *Naunyn Schmiedebergs Arch. Pharmacol.*, vol. 398, no. 5, pp. 5011–5055, 2025, doi: 10.1007/s00210-024-03722-3.

[23] N. He, G. Yu, C. Liu, Y. Li, and R. Wang, "Variation, adaptation, and optimization mechanism of plant functional traits at the organ level," in *Plant Functional Ecology: From Organ to Ecosystem*. Singapore: Springer, 2025, pp. 33–70, doi: 10.1007/978-981-96-8264-5.

[24] F. Fadilah *et al*., "Unraveling potential compounds of *Uncaria gambir* (W. Hunter) Roxb. as antikeloid agent: *In silico*, *in vitro*, and *ex vivo* experimental validation," *Biologics*, vol. 5, no. 3, p. 18, 2025, doi: 10.3390/biologics5030018.

[25] K. Wei *et al*., "LC-MS/MS-based metabolic profiling: Unraveling the impact of varying degrees of curing on metabolite transformations in tobacco," *Front. Plant Sci.*, vol. 15, p. 1473527, 2024, doi: 10.3389/fpls.2024.1473527.