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Original Research Article

LC-MS Chemical Profiling of Dichloromethane Fraction of Methanol Extract of *Syzygium samarangense* Stem Bark

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ABSTRACT

Prior studies have demonstrated that *Syzygium samarangense* possesses antioxidant, antifungal, and anti-inflammatory properties as well as other advantages. The fruit is widely consumed, yet the medical benefits of the stem bark as an alternative therapeutic option have not been explored. This research was performed to determine the specific chemicals contained in the dichloromethane fraction of the methanol extract of this plant. The powdered plant sample was extracted with methanol by maceration and fractionated with dichloromethane. The resulting dichloromethane fraction was then concentrated, and its chemical constituents were identified using LC-MS based on the Willey and NIST libraries. The findings of this research showed the presence of 40 phytoconstituents in five main classes of secondary metabolites: phenolics, flavonoids, terpenoids, steroids, and others. This study explains the basic structures, compound derivatives, biological functions of the compounds identified in F2 with reference to relevant literature. The stem bark of *S. samarangense* predominantly contains compounds with significant bioactivity, including antioxidants, antifungal, anti-inflammatory, anticancer, and antiviral properties. This research offers valuable insight into the groundwork for the utilization of this plant and the selection of natural and synthetic substances.

***Keywords*:** Chemical profile, Liquid Chromatography-Mass Spectrometry, methanol extract, *Syzygium samarangense*.

Introduction

Syzygium samarangense or Jambu Semarang (in Indonesian), is one of Indonesia's local plants whose medical benefits still need further exploration. However, its mineral compositions, including iron, potassium, calcium, sodium, and magnesium, are higher than that of *S. aqueum*.[1] In addition to having higher magnesium levels, it also contains tannins and mild antibiotic properties against *S. aureus*, *M. smegmatis*, and *C. albicans*.[2] The plant also contains desmethoxymatteucinol, 5-*O*-methyl-4'-desmethoxymatteucinol, oleanic acid, and *β*-sitosterol as bioactive compounds.[3–5] Furthermore, the plant also has high nutritional content: protein (92.9%), carbohydrates (6%), crude fibre (0.46%), and ash content (0.21%).[6] Several studies have reported the leaves' analgesic, anti-inflammatory, and remarkable CNS effects.[1,6] The extract has shown potent antihelmintic properties.[4,5] Reports also showed that the stem bark extract of this plant has antidiabetic potential with inhibitory activity against the *α*-glucosidase enzyme.[7] Phytochemical analysis of the stem bark extract showed the presence of terpenoids, steroids, tannins, saponins, phenolics, and flavonoids, [8] and possessed excellent antioxidant properties.[9,10]. The LC-MS analysis of a fraction from the VLC of the dichloromethane extract showed the presence of four flavonoid compounds, namely pinocembrin, uvangoletin, sterkurensin, and aurethiacin.[11]

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There are no reports regarding the study of the chemical profile of the dichloromethane fraction resulting from the partitioning of the methanol extract of *S. samarangense* stem bark, making researchers interested in conducting this research.

The current aimed to investigate the chemical profile of the dichloromethane fraction of the crude methanol extract through LC-MS analysis, followed by an in-depth study of the diversity of chemical structures and bioactivity properties with a view of establishing its medicinal potential and use as a herbal agent/medicine.

Methods

Plant Collection and Identification

The plant stem bark sample of *S. samarangense* was collected from a local area in Kediri, East Java, located between Latitude 7.8480° S and longitude 112.0178° E, Indonesia, in October 2018. The plant was identified at the Herbarium-LIPI, Purwodadi, East Java, Indonesia, where voucher specimen no. 1498/IPH.06/HM/X/2018 was deposited.

Preparation, Extraction, and Fraction of Plant material

The stem bark of *S. samarangense* (c.a. 15 kg) was washed with tap water and dried under sunlight for one week. It was then dried in an oven at a reduced temperature (not more than 50 °C). The dried material was ground to a powder using an electric grinder to obtain 9.5 kg and transferred to an airtight container.[11] The powdered stem bark (5 kg) of *S. samarangense* was macerated using methanol (c.a. 15 L) at ambient temperature (25 °C). The container with its contents was sealed by foil and kept for 24 hours, accompanied by occasional shaking and stirring. The whole mixture was then filtered using a Buchner funnel, and the filtrate was concentrated at 50 °C with a vacuum rotary evaporator to obtain a thick, dark-brown crude extract (25 g), kept in a refrigerator at 4 °C until further use.

The crude extract was dissolved slightly in methanol and water (300 mL) and then partitioned using *n*-hexane (300 mL x 3, F1) in a separating funnel. The aqueous fraction was then separated with dichloromethane (300 mL x 3, F2) in a separating funnel. The fractions

were concentrated at 40 °C with a vacuum rotary evaporator. ~~under a vacuum using a rotary evaporator at 40℃~~. The dried fraction (F2) obtained was stored in a refrigerator until further analysis.

Identification Using LC-MS ESI

The chemical content of the F2 fraction was analysed using the LC-MS. LC-MS instrument (Shimadzu LC-MS 8040) equipped with a Shimadzu Shim Pack FC-ODS column (2 mm x 150 mm, 3 µm) and a column temperature of 35 ℃ was used. The sample injection volume was 1 µL with isocratic separation (the mobile phase used was 90% methanol with water) at a flow rate of 0.5 mL/min. The LC-MS analysis was conducted in negative ion mode based on the following parameters: source temperature of 100 ℃, cone sampling voltage of 23 eV, capillary voltage of 3.0 kV, solvent discharge temperature of 350 ℃, and solvent gas flow of 60 mL/hour. The mass spectrum detection range was set between m/z 10-1000 using ESI negative ion mode, with a scanning duration of 0.6 seconds per scan and a total running time of 80 minutes. LC-MS has been an effective method for identifying compounds in a matrix[13].

Results and Discussion

The chemical profile of the dichloromethane fraction (F2) of *S. samarangense* investigated using LC-MS is shown in Figure 1. There are 40 compounds peaks identified with the MS detector in Table 1. Polyphenolic compounds (flavonoids, flavanones, flavonols), phytosterols, etc, were identified in F2. The basic structure of flavonoid and its derivatives were shown in Figure 2. Those with simple structures include 1, 3, and 5. Compound 1 (methyl salicylate) is mainly found in *Gynura procumbens*, which has a spicy taste commonly called mint.[14] When making deep heating liniments (like Bengay) to relieve joint and muscular pain, methyl salicylate is utilized in high doses as an analgesic and rubefacient. On the other hand, this substance in the body metabolizes into salicylates, including salicylic acid, a well-known NSAID.[15,16] Compound 3 (gallic acid) is widely found in many parts of plants[17] and offers antioxidant activity. Compound 3 is also widely used to measure the total phenolic content of an extract [18]. This compound can be utilized as an antioxidant[19], antineoplastic agent[20], astringent[21], and cyclooxygenase-2 (COX-2) inhibitor.[22] Finally, compound 5 (eugenin) is mainly produced by *Cylindropuntia bigelovi* [23] and *Daucus carota* plants.[24] This compound is well-known as a potential HIF-1-α inhibitor.[23]

Other phenolic derivatives identified in the fraction were tannins and phenylpropanoid groups. Tannins are phenolic compounds with more than one hydroxyl group and a more complex structure, generally called polyphenols.[25] Compound 37 (strictinin) is a tannin belonging to the ellagitannin group, predominantly in *Alnus japonica*[26] and *Balanophora japonicsssa*,[27] was identified in this study.

A phenylpropanoid group is a group of compounds with an aromatic ring substituted with a propene tail, which is generally synthesized from the amino acid phenylalanine and tyrosine pathways.[28] Compounds belonging to the phenylpropanoid group are compounds 2 and 6, classified as phenylpropene. Compounds 2 (eugenol) and 6 (eugenol acetate) are mainly found in clove plants and are commonly called clove oil.[29,30] Compound 6 is a derivative of compound 2 substituted by an acetyl group; both of these compounds have antioxidant, insecticidal [30], antimicrobial [31], and potency as antiviral inhibitor HIV-1 protease.[32]

Flavonoids are polyphenolics found in plants with a C6-C3-C6 basic structure with carbon rings.[33] Some flavonoids, namely the chalcone and flavanone groups, were identified in the dichloromethane fraction (F2) of *S. samarangense*.

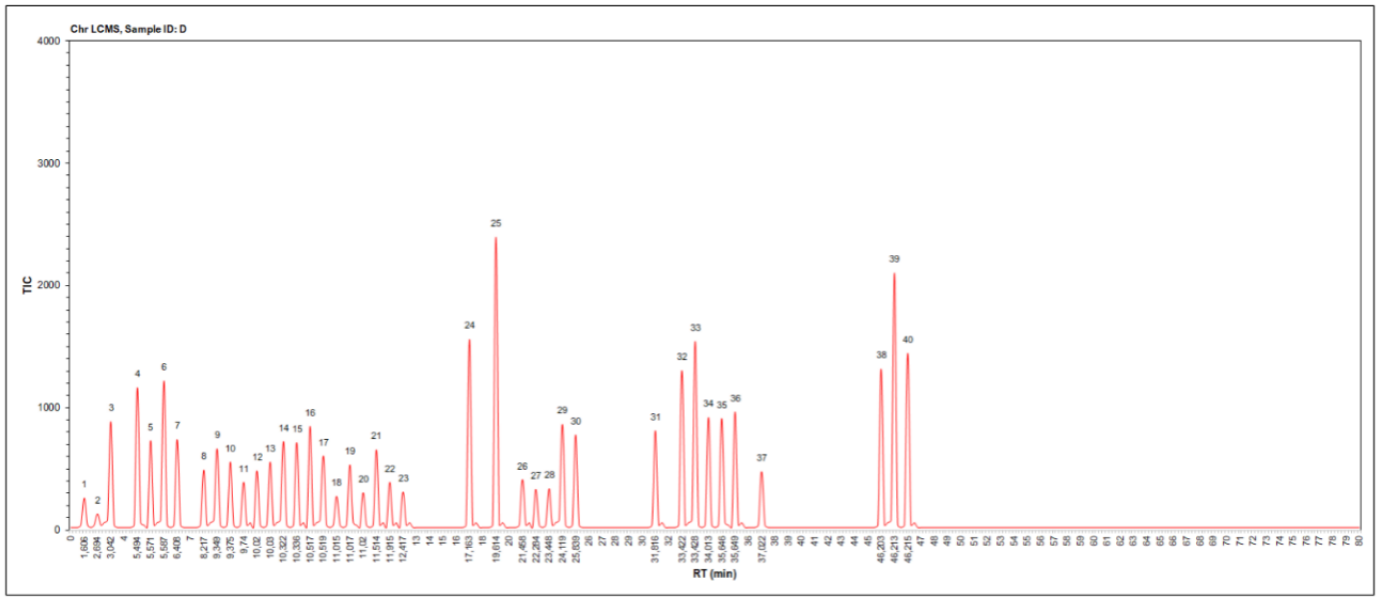


Figure 1: LC-MS Chromatogram of F2 Fraction (Dichloromethane Fraction)

The chalcone group has an open flavonoid basic structure with two rings (A and B) attached by an *α,β*-unsaturated carbonyl system with different substituents.[33] The chalcone group has significant bioactivity and inhibitory activity against NF-kB receptors implicated in cancers.[34] The chalcone compounds identified in the LC-MS chromatogram include compounds 11, 12, 13, 15, 16, 18, 19, and 22. Compound 11 (uvangoletin) is also found in *Sarcandra glabra* [35] and *Monanthotaxis trichocarpas*.[36] Several researchers reported that this compound has anticancer and antiviral activities. Compound 12 (stercurensin), also found in *Cleistocalyx operculatus*[37] and *Comptonia peregrina*[38], has neuraminidase antiviral activity against two influenza viruses, H1N1 and H9N2.[37] Similar to compound 12, is compound 13 (demethoxymatteucinol), commonly found in *Pentarhizidium orientale*[39] and *Cleistocalyx operculatus*[37], also exhibits the antiviral properties against the influenza viruses.[37,39] Compound 15, generally called myrigalone H, can be found in *Myrica gale*[40] and is an mTOR inhibitor in cancer cells.[41] Meanwhile, compound 16 (4',6'-dihydroxy-3',5'-dimethyl-2'-methoxychalcone) can be found in *S. nervosum*[42] and has antiviral neuraminidase function against two influenza viruses, H1N1 and H9N2.[37,39] Compound 18 (aurentiacin) found in *Myrica pensylvanica*[38] possesses an anti-inflammatory activity tested on mouse macrophages.[43,44] Compound 19 (2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone) can be found in *S. nervosum*[42] with similar bioactivity as compound 16.[37,39] Compound 22 (syzygiol) can be isolated from *S. polycephaloides*, where this compound has antitumor activity against skin tumours.[45]

Flavanones are the other flavonoid derivative identified in the fraction. The flavanone group generally has 15 carbon rings with two phenyl groups (A and B), a heterocyclic ring (oxygen-embedded carbon ring), and a ketone group on carbon number 4. This group has other derivatives with several substituted hydroxyl groups in its basic structure. Flavanones that are substituted with one hydroxyl group are called hydroxyflavanones. Compound 17 (7-hydroxy-5-methoxy-6,8-dimethylflavanone) is a dihydroxyflavone derivative of pinocembrin where the hydroxyl group attached at C-5 is methylated. This compound can be found in *S. samarangense*[46] and *Couroupita guianensis*.[47] The next in this group is a substituted compound with two hydroxyl groups called dihydroxyflavone[33], in which compounds belonging to the dihydroxyflavone groups are compounds 8, 9, 10, and 20 (Table 1). Pinocembrin and its derivatives can be found predominately in *Piper sarmentosum* [48] and *Cryptocarya chartacea.*[49] In general, this group exhibits antioxidant[50], anticancer[51,52], anti-inflammatory[12], antineoplastic[53] activities, and vasodilatory and neuroprotective properties.[54] The next hydroxylated flavone group with four hydroxyl groups is tetrahydroxyflavones. A compound belonging to this group found in the fraction is compound 14 (kaempferol), which is often extracted from Witch-hazel[55], grapefruit[56], and *Lotus ucrainicus.*[57] Kaempferol is a well-known antioxidant that works by reducing oxidative stress.[58] It is often used in cancer treatment[59] and has antibacterial activity.[60]

The next group is that with five hydroxyl groups, commonly referred to as pentahydroxyflavone. Compound 29 (myricitrin), a pentahydroxyflavone myricetin derivative (Figure 4), belongs to this group. The hydroxyl group attached at C-3 is substituted with an α-L-rhamnopyranosyl group. This compound is found and has been isolated from *Myrica cerifera*[61], *S. levinei*[62], and *Limonium aureum.*[63,64] This compound has antiallergic properties[65], and its protein kinase C inhibitors have a significant role in cancer treatment.[66]

Another group, characterized by six hydroxyl groups, is commonly referred to as hexahydroxyflavone. The compounds found in this group are myricetin and its derivatives, compounds 21, 31, and 36. Many myricetins and their derivatives are found in *Morella rubra* [67] and *Ficus auriculata* [68]. These compounds have anti-inflammatory (COX-1 inhibitors)[69, 70], antineoplastic[20,71], and antioxidant[67] activities.

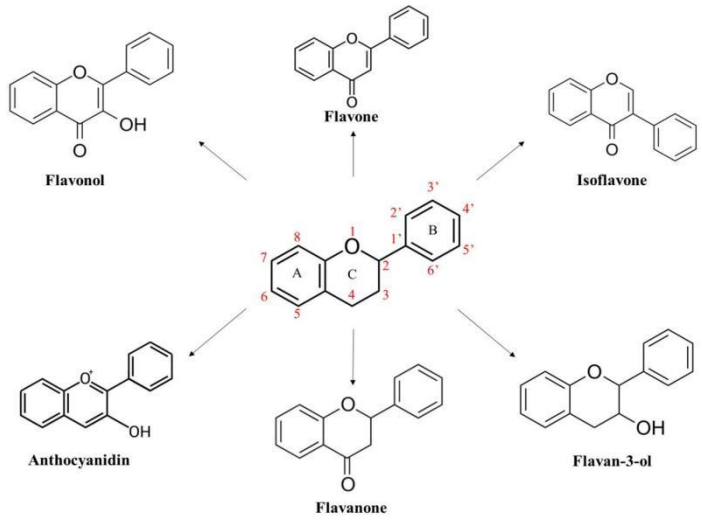
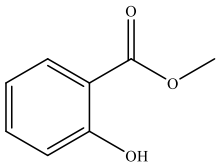
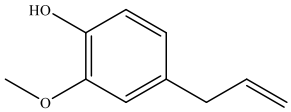
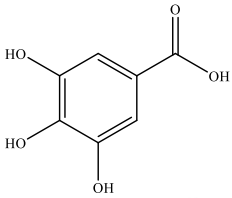
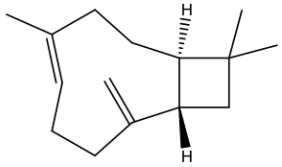
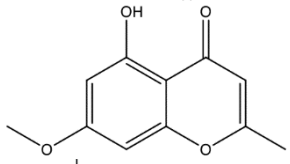
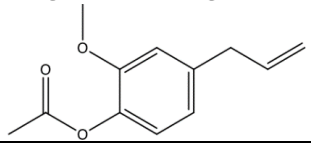


Figure 2: Basic structure of flavonoid and its derivatives

Table 1: Identified Compounds based on LC-MS Chromatogram of F2 Fraction (Dichloromethane Fraction)

Comp. No.	RT (Min)	Composition (%)	Compound Results Analysis	Identified Compound
1	1.606	0.81312	Methyl salicylate CF: $C_8H_8O_3$; EM: 152.0473; MW: 152.490; m/z: 152.0473 (100%), and 153.0507 (8.7%)	
2	2.694	0.40981	Eugenol CF: $C_{10}H_{12}O_2$; EM: 164.0837; MW: 164.2040; m/z: 164.0837 (100%), and 165.0871 (10.8%)	
3	3.042	2.75891	Gallic acid CF: $C_7H_6O_5$; EM: 170.0215; MW: 170.1200; m/z: 170.0215 (100%), 171.0249 (7.6%), and 172.0258 (1.0%)	
4	5.494	3.62398	β-Caryophyllene CF: $C_{15}H_{24}$; EM: 204.1878 ; MW: 204.3570 ; m/z: 204.1878 (100%), and 205.1912 (1.2%)	
5	5.571	2.27671	Eugenin CF:$C_{11}H_{10}O_4$; EM: 206.0579; MW:206.1970; m/z: 206.0579 (100%), and 207.0613 (11.9%)	
6	5.587	3.79729	Eugenol acetate CF:$C_{12}H_{14}O_3$; EM: 206.0943; MW:206.2410; m/z: 206.0943 (100%), and 207.0976 (13.0%)	

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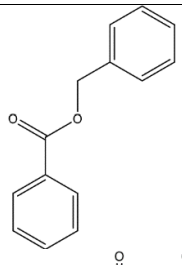
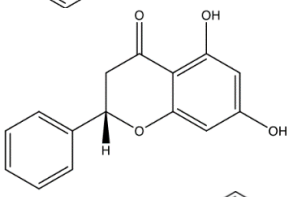
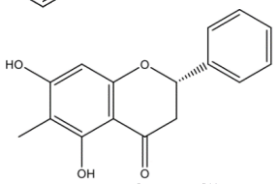
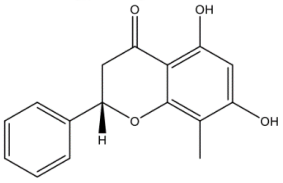
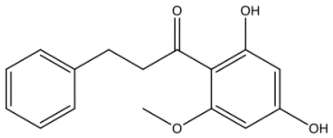
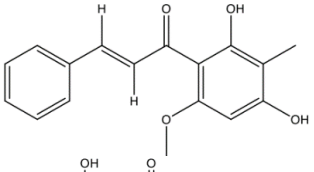
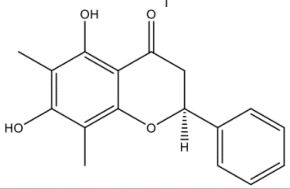
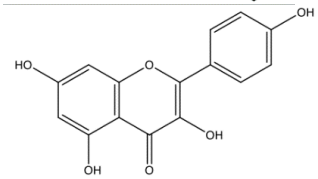
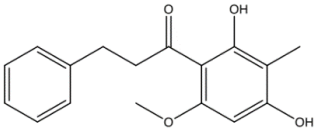
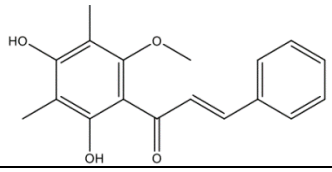
7	6.408	2.30441	Benzyl benzoate CF:$C_{14}H_{12}O_2$; EM: 212.0837; MW: 212.2480; m/z: 212.0837 (100%), 213.0871 (15.1%), and 213.0871 (1.1%)	
8	8.217	1.52868	Pinocembrin CF: $C_{15}H_{12}O_4$; EM: 256.0736; MW: 256.2570; m/z: 256.0736 (100.0%), 257.0769 (16.2%), and 258.0803 (1.2%)	
9	9.349	2.06804	(-)-Strobopinin CF:$C_{16}H_{14}O_4$; EM: 270.0892; MW: 270.2840; m/z: 270.0892 (100.0%), 271.0926 (17.3%), and 272.0959 (1.4%)	
10	9.375	1.73286	8-Methylpinocembrin CF: $C_{16}H_{14}O_4$; EM: 270.0892; MW: 270.2840; m/z: 270.0892 (100.0%), 271.0926 (17.3%), and 272.0959 (1.4%)	
11	9.74	1.21678	Uvangoletin CF: $C_{16}H_{16}O_4$; EM: 272.1049; MW: 272.3000; m/z: 272.1049 (100.0%), 273.1082 (17.3%), and 274.1116 (1.4%)	
12	10.02	1.50924	Stercurensin CF: $C_{17}H_{16}O_4$; EM: 284.1049; MW: 284.3110; m/z: 284.1049 (100.0%), 285.1082 (18.4%), and 286.1116 (1.6%)	
13	10.03	1.73178	Demethoxymatteucinol CF: $C_{17}H_{16}O_4$; EM: 284.1049; MW: 284.3110; m/z: 284.1049 (100.0%), 285.1082 (18.4%), and 286.1116 (1.6%)	
14	10.322	2.25465	Kaempferol CF: $C_{15}H_{10}O_6$; EM: 286.0477; MW: 286.2390 m/z: 286.0477 (100.0%), 287.0511 (16.2%), 288.0520 (1.2%), and 288.0544 (1.2%)	
15	10.336	2.22455	2',4'-Dihydroxy-6'-methoxy-3'-methyldihydrochalcone CF: $C_{17}H_{18}O_4$; EM: 286.1205; MW: 286.3270; m/z: 286.1205 (100.0%), 287.1239 (18.4%), and 288.1272 (1.6%)	
16	10.517	2.64109	4',6'-Dihydroxy-3',5'-dimethyl-2'-methoxychalcone CF: $C_{18}H_{18}O_4$; EM: 298.1205 ; MW: 298.3380 m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%)	

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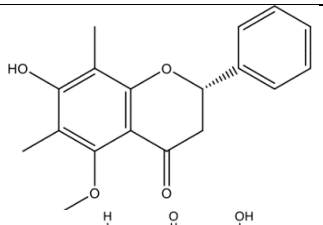
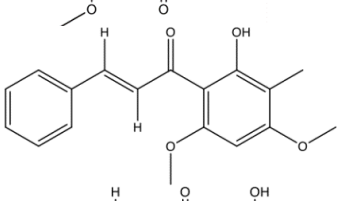
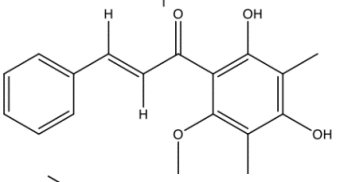
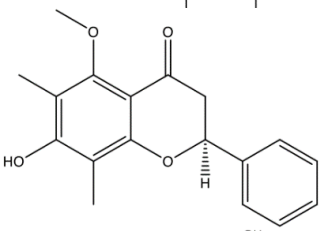
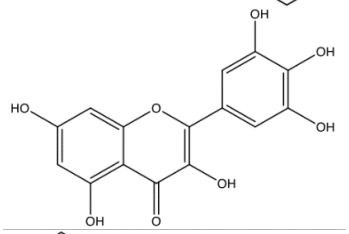
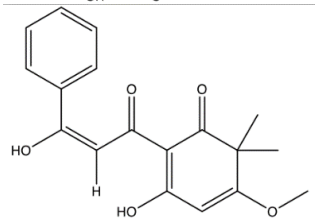
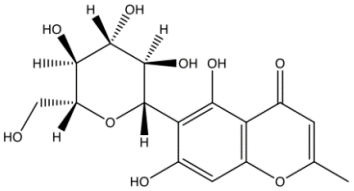
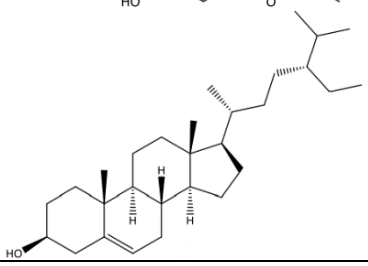
17	10.519	1.88630	7-Hydroxy-5-methoxy-6,8-dimethylflavanone CF: $C_{18}H_{18}O_4$; EM: 298.1205 ; MW: 298.3380 m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%)	
18	11.015	0.85809	Aurentiacin CF: $C_{18}H_{18}O_4$; EM: 298.1205; MW: 298.3380; m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%)	
19	11.017	1.66310	2',4'-Dihydroxy-6'-methoxy3',5'-dimethylchalcone CF: $C_{18}H_{18}O_4$; EM: 298.1205; MW: 298.3380; m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%)	
20	11.02	0.94841	(+)-6,8-Di-C-methylpinocembrin-5-methylether CF: $C_{18}H_{18}O_4$; EM: 298.1205; MW: 298.3380; m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%)	
21	11.514	2.04365	Myricetin CF: $C_{15}H_{10}O_8$; EM: 318.0376; MW: 318.2370; m/z: 318.0376 (100.0%), 319.0409 (16.2%), 320.0418 (1.6%), and 320.0443 (1.20%)	
22	11.915	1.21666	Syzygiol CF: $C_{18}H_{18}O_5$; EM: 314.1154 ; MW: 314.3370 m/z: 314.1154 (100.0%), 315.1188 (19.5%), 316.1221 (1.8%), and 316.1197 (1.0%)	
23	12.417	0.96577	Biflorin CF: $C_{16}H_{18}O_9$; EM: 354.0951 ; MW: 354.3110; m/z: 354.0951 (100.0%), 355.0984 (17.3%), 356.0993 (1.8%), and 356.1018 (1.4%)	
24	17.163	4.85456	*β*-Sitosterol CF: C29H50O ; EM: 414.3862 ; MW: 414.7180 m/z: 414.3862 (100.0%), 415.3895 (31.4%), 416.3929 (2.7%), and 416.3929 (2.0%)	

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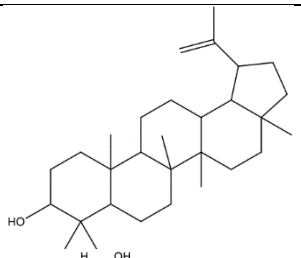
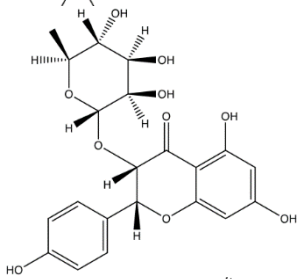
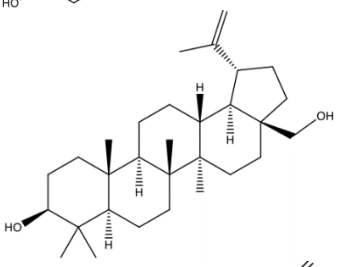
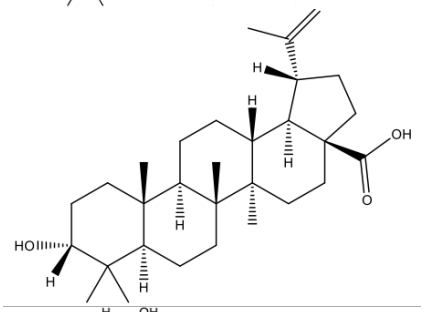
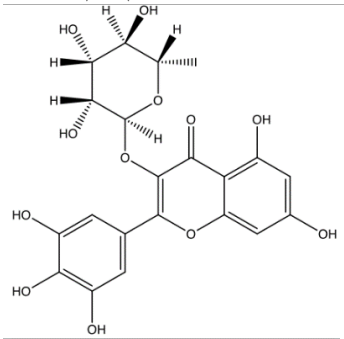
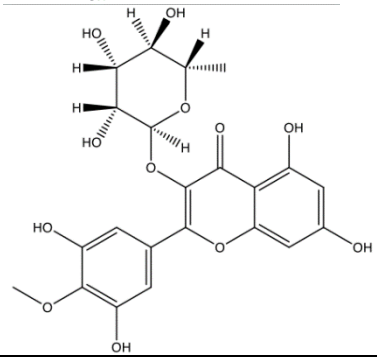
25	19.614	7.45610	Lupeol CF: $C_{30}H_{50}O$; EM: 426.3862 ; MW: 426.7290 m/z: 426.3862 (100.0%), 427.3895 (32.4%), 428.3929 (2.7%), and 428.3929 (2.4%)	
26	21.458	1.28346	Isoengeletin CF: $C_{21}H_{22}O_{10}$; EM: 434.1213 ; MW: 434.3970 m/z: 434.1213 (100.0%), 435.1247 (22.7%), 436.1280 (2.5%), and 436.1255 (2.1%)	
27	22.284	1.03783	Betulin CF: $C_{30}H_{50}O_2$; EM: 442.3811 ; MW: 442.7280 m/z: 442.3811 (100.0%), 443.3844 (32.4%), 444.3878 (2.7%), and 444.3878 (2.4%)	
28	23.448	1.05291	Epibetulinic acid CF: $C_{30}H_{48}O_3$; EM: 456.3603 ; MW: 456.7110 m/z: 456.3603 (100.0%), 457.3637 (32.4%), 458.3671 (2.7%), and 458.3671 (2.4%)	
29	24.119	2.69024	Myricitrin CF: $C_{21}H_{20}O_{12}$; EM: 464.0955; MW: 464.3790; m/z: 464.0955 (100.0%), 465.0988 (22.7%), 466.0997 (2.5%), and 466.1022 (2.5%)	
30	25.839	2.42486	Mearnsitrin CF: $C_{22}H_{22}O_{12}$; EM: 478.1111; MW: 478.4060; m/z: 478.1111 (100.0%), 479.1145 (23.8%), 480.1178 (2.7%), and 480.1154 (2.5%)	

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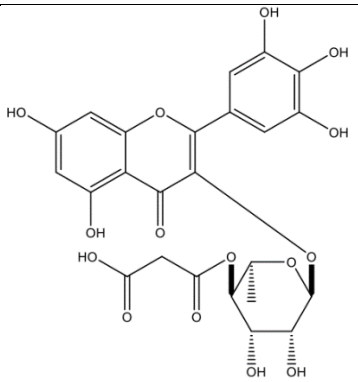
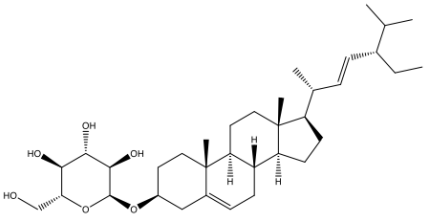
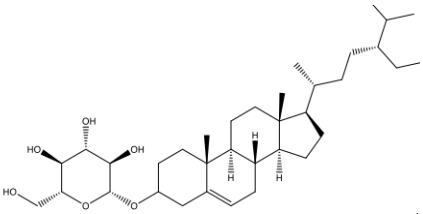
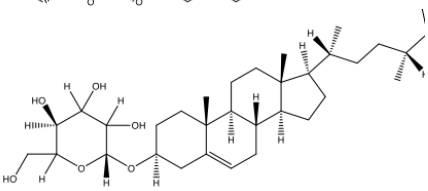
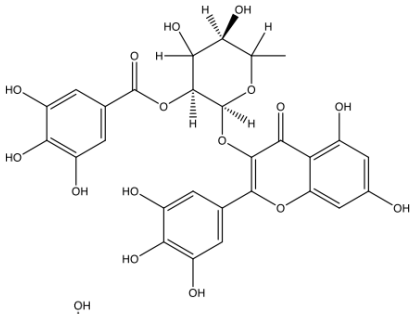
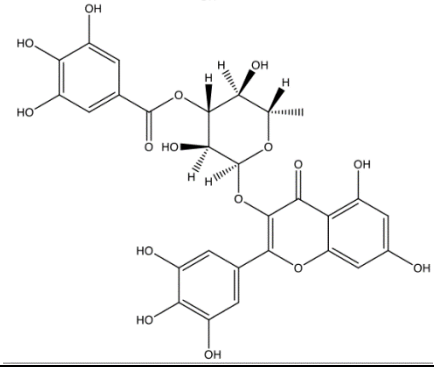
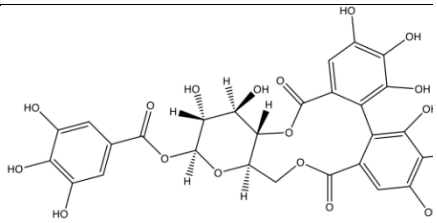
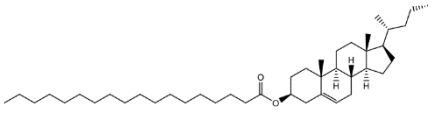
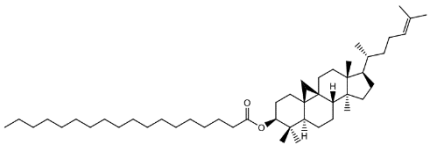
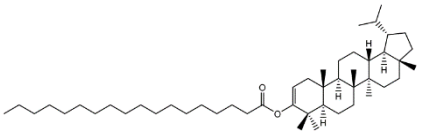
31	31.816	2.52852	Myricetin-3-*O*-(4"-*O*-malonyl)-*α*-L-rhamnopyranoside CF: $C_{24}H_{22}O_{15}$; EM: 550.0959 ; MW: 550.4250 m/z: 550.0959 (100.0%), 551.0992 (26.0%), 552.1026 (3.2%), and 552.1001 (3.1%)	
32	33.422	4.05880	Stigmasterol-3-*O*-*β*-D-glucoside CF: $C_{35}H_{58}O_6$; EM: 574.4233 ; MW: 574.8430 m/z: 574.4233 (100.0%), 575.4267 (37.9%), 576.4300 (4.3%), 576.4300 (2.7%), and 576.4276 (1.2%)	
33	33.428	4.80307	*β*-Sitosterol-D-glucoside CF: $C_{35}H_{60}O_6$; EM: 576.4390 ; MW: 576.8590 m/z: 576.4390 (100.0%), 577.4423 (37.9%), 578.4457 (4.3%), 578.4457 (2.7%), and 578.4432 (1.2%)	
34	33.013	2.86462	Campesterol glucoside CF: $C_{34}H_{58}O_6$; EM: 562.4233 ; MW: 562.8320 m/z: 562.4233 (100.0%), 563.4267 (36.8%), 564.4300 (6.6%), and 564.4276 (1.2%)	
35	35.646	2.83992	Desmanthin 1 CF: $C_{28}H_{24}O_{16}$; EM: 616.1064; MW: 616.4840; m/z: 616.1064 (100.0%), 617.1098 (30.3%), 618.1131 (4.4%), and 618.1107 (3.3%)	
36	35.649	3.00663	Myricetin-3-(3"-galloylrhamnoside) CF: $C_{28}H_{24}O_{16}$; EM: 616.1064 ; MW: 616.4840 m/z: 616.1064 (100.0%), 617.1098 (30.3%), 618.1131 (4.4%), and 618.1107 (3.3%)	

37	37.022	1.48540	Strictinin CF: $C_{27}H_{22}O_{18}$; EM: 634.0806 ; MW: 634.4550 m/z: 634.0806 (100.0%), 635.0840 (29.2%), 636.0873 (4.1%), 636.0849 (3.7%), and 637.0882 (1.1%)	
38	46.203	4.09850	Sitosteryl stearate CF: $C_{47}H_{84}O_2$; EM: 680.6471 ; MW: 681.1870 m/z: 680.6471 (100.0%), 681.6505 (50.8%), 682.6538 (12.6%), and 683.6572 (1.2%)	
39	46.213	6.54282	Cycloartenyl stearate CF: $C_{48}H_{84}O_2$; EM: 692.6471 ; MW: 693.1980 m/z: 692.6471 (100.0%), 693.6505 (51.9%), 694.6538 (13.2%), and 695.6572 (1.4%)	
40	46.215	4.49788	Lupenyl stearate CF: $C_{48}H_{84}O_2$; EM: 692.6471 ; MW: 693.1980 m/z: 692.6471 (100.0%), 693.6505 (51.9%), 694.6538 (13.2%), and 695.6572 (1.4%)	

Note = CF: Chemical Formula; EM: Exact Mass; MW: Molecular Weight; m/z: mass per charge

Beyond the chalcone and flavanone groups, other groups of polyphenols identified in F2 include the flavonol group, notably compound 30 (mearncitrin) and its derivatives featuring two hydroxyl groups, commonly referred to as dihydroflavonols, such as compound 26 (isoengeletin). Compound 30 (mearncitrin) is a glycoside derived from mearnsetin substituted by the α-L-rhamnopyranosyl. This compound has been isolated from *Byrsonima coccolobifolia*[72] and *Myrsine africana*[73], known as natural antioxidants from plants[74]. Meanwhile, compound 26 (isoengeletin) is a dihydroflavonol derivative substituted by the α-L-rhamnopyranosyl at the third position. This compound can be found in *Smilax glabrae* [75] and *Iryanthera lancifolia* [76] and is being researched for psoriasis, hyperuricemia, and gout.[77]

Terpenoids are a group of compounds commonly identified as oils derived from 5-carbon isoprene, namely monoterpenes, diterpenes, triterpenes, etc. The isoprene structure as the basic structure for the terpenoid group is presented in Figure 6.[78] In general, terpenoids are compounds with different bioactivity in the pharmaceutical field, especially in medicinal chemistry.[79] In this fraction, compounds belonging to the terpenoids are compounds 25, 27, 28, and 40. These compounds can be grouped into triterpene-type terpenoids because of the presence of 3 basic structures of isoprenes. Compound 25 (lupeol) is a pentacyclic triterpenoid with anticancer[80,81] and anti-inflammatory activities.[80] This compound can be found in mango[82], *Camellia japonica* [83], *Acacia visco* [84], and *Abronia villosa.* [85] Compound 27 (betulin) is a triterpene commonly isolated from birch trees.[86] This compound is a lupeol derivative with hydroxyl groups attached at C-3 and C-28 known to have anticancer activity.[87] Compound 28 (epibetulinic acid) is a derivative of betulic acid (also a lupeol derivative) found in *Microtropis fokienensis* [88], *Hypericum lancifolium* [89], and birch trees.[90] Epibetulinic acid has anti-inflammatory, antineoplastic [91], and anti-HIV [92] activities. Compound 40 (lupenyl stearate) is a lupeol derivative substituted with a stearyl group at the β-position, commonly found in *S. Samarangense*.[93].

Steroids are compounds with a basic structure of seventeen C atoms bonded in four rings (with three cyclohexane rings and one cyclopentane ring).[94,95] The compounds within this group are steroid derivatives with a hydroxyl group, namely sterols [96]. Sterols found in plants are called phytosterols, while the ones found in animals are called zoosterols. These derivative compounds have many uses, such as inhibiting human cholesterol absorption. Compounds classified as phytosterols include compounds 24, 32, 33, 34, 38, and 39. Compound 24 (β-sitosterol) is a sterol derivative substituted with a β-hydroxyl group at the third position. This compound can be found in *Typhonium trilobatum* [97] and *Elodea canadensis* [98], used as an antioxidant [99], anticholesteremic drug [100], and reduces the toxic effects of radiation.[97] Compound 32 (stigmasterol-3-*O*-β-D-glucoside) is a glycoside compound derived from stigmasterol, which is substituted with β-D-glucopyranosyl at position 3 with glycosidic bonds, which are also classified as steroid saponins. This compound is often found in *Symplocos lancifolia*, which is used as an antioxidant and antibacterial.[101] Compound 33 (β-sitosterol-D-glucoside) is a glycoside compound derived from sitosterol, which is substituted with D-glucopyranosyl found in *Dimocarpus longan* [102] and *Erigeron canadensis* [103] having antioxidant [104] and anti-inflammatory properties.[48] Compound 38 (sitosteril stearate) is a sterol compound derived from sitosterol, substituted by a stearyl group at the β-position. This compound is commonly found in *S. samarangense*, which has antioxidant and anti-inflammatory properties.[93] Compound 39 (cycloartenyl stearate) is a cycloartenol derivative compound substituted with a stearyl group found in *S. samarangense*, with analgesic and anti-inflammatory activities.[93]

In addition to the main compound groups described above, the ester and quinone groups were detected in this fraction. The ester group found in this fraction is compound 7 (benzyl benzoate), a benzoic acid derivative. This compound is easily found and isolated from the genus *Polyalthia* [105] plants and *Populus tremula* [106] as a scabicide and acaricide.[107] Meanwhile, the quinone group found in this fraction is compound 23 (biflorin), belonging to the naphthoquinone group. This compound is found in *S. aromaticum* [108] and *Capraria biflora* [109] and has antimicrobial, antitumor, and antimutagenic activities.[109]

Conclusion

The findings of this study indicated that the dichloromethane fraction of methanol extract of the stem bark of *S. samarangense* contains 40 phytoconstituents categorised into phenolics, flavonoids, terpenoids, and steroids, each with distinct bioactivities. This information is insightful for further research and understanding of the properties of these compounds. Additional research on isolating compounds from F2 is necessary to identify lead compounds for the development of pharmaceutical products.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgments

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