CHEMICAL PROFILE BY LC-MS ANALYSIS FROM THE SELECTED FRACTION OF METHANOL EXTRACT OF *Syzygium malaccense*

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ABSTRACT

In our previous study, two compounds from the stem bark of *Syzygium malaccense* were isolated, namely palmitic acid and methyl oleate. In this article, we intend to display the chemical components' identification results in selected fractions of methanol extract of the plant. Through LC-MS analysis, twenty chemical components of the fraction were identified. Structurally, they can be grouped into phenolic acid derivatives such as gallic acid and casuarinin and flavonoid derivatives, including chalcones, flavanols or catechins, flavanones, and flavonols.

**Keywords:** Flavonoid Derivatives, LC-MS, Myrtaceae, Phenolic Acid Derivatives, *Syzygium malaccense*.

INTRODUCTION

In Indonesia, *Syzygium malaccense* (L.) Merr. & Perry (Family: Myrtaceae) was known as jambu bol.\(^1\) The fruit of this plant is generally eaten fresh or used as one of the ingredients of salad (Indonesian name: rujak). They are widely used in traditional medicines for treatments such as mouth infections, throat infections, etc.\(^2,3\) When *S. malaccense* leaves are pounded, they can be used as antieptic, tongue inflammation, dysentery, and other ailments.\(^4\) Besides that, these plants have antimicrobial and anthelmintic bioactivity\(^5\), antioxidants, and anti glycemic.\(^6,7\) At present, there are very few reports on the investigation of chemical components in this plant. Phytochemical screening test conducted toward methanol extract of *S. malaccense* stem bark was known that the plant contains alkaloids, steroids, phenolics, flavonoids, tannins, and saponins.\(^8\) Chemical components isolated from methylene chloride extract of the plant stem bark are palmitic acid\(^9\) and a mixture of ester compounds from stearic acid, namely 9,12-octadecadienoic acid and 10-octadecenoic acid.\(^10\) Meanwhile, the ethanol extract of the plant leaves is known to contain quer cetin, myricetin, and its derivative: myricitrin.\(^11\) Now, the investigation of phytocomponents on the methanol extract of the plant stem bark is continued.

EXPERIMENTAL

**Material and Methods**

Several organic solvents such as *n*-hexane, methylene chloride, ethyl acetate, and methanol were used in this study. The equipment such as filter paper, pipette, spatula, measuring glass, vial, container, separating funnel, Buchner funnel, Hirsch funnel, Erlenmeyer flask, and the Buchi R-215 rotary evaporator were used for extraction and fractionation. Meanwhile, chromatographic techniques to identify the chemical components of the methanol extract of *S. malaccense* stem bark were used vacuum liquid chromatography (VLC, silica gel 60, 0.040-0.063 mm) and gravitational column chromatography (GCC, silica gel 60, 0.200-0.500 mm or 70-230 mesh ASTM). In the VLC technique, an eluent with increasing polarity was used. The chromatographed compounds' homogeneity was examined using TLC on a Kieselgel 60 F254 (E.Merck) gel-coated sheet and monitored using a UV lamp at 254 or 366 nm. Using the Shimadzu LCMS-8040 LC/MS instrument, ion trap mass spectrometry in negative ion mode, the chemical components of the methanol extract's selected fractions can be detected. The plant's stem bark was obtained from Jombang, East Java, Indonesia, in February 2018 and the plant has been identified by Herbarium-LIPI in Purwodadi, East Java, Indonesia. The plant samples identified are stored in the LIPI herbarium, with an identification number. 276/IPH.06/HM/II/2018, February 21, 2018.

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General Procedure
The stem bark of *S. malaccense* (± 25 kg) was cleaned of dirt, cut into small pieces using a knife, dried in direct sunlight for one week, and obtained as much as 7 kg. Then, it was ground and yielded a dried powdered sample (c.a. 3 kg). The powder was then macerated using methanol as extracting solvent for a day and repeated thrice. Macerate (brown) was filtered using Buchner funnel and evaporated using vacuum rotavapor to yield methanol extract (103 g). The extract was further fractionated by *n*-hexane using a separating funnel to produce upper and lower two layers. The top layer as *n*-hexane fraction (light yellow) was separated from methanol fraction (brown) and this was repeated thrice. The methanol extract that has been fractionated by *n*-hexane is then fractionated by methylene chloride and obtained methylene chloride fraction (yellow) and methanol fraction (brown). Fractionation by methylene chloride was also repeated 3 times. The remaining methanol extract (52 g) was ready to be investigated for its chemical components.

Detection Method
The detection method used to determine the chemical components contained in fraction D3 (8-59) through LC-MS analysis using instruments Shimadzu LCMS-8040 can be explained as follows. Put 1 µL of a sample into an LC instrument equipped with a Shim Pack FC-ODS chromatographic column (2 mm x 150 mm, particle size 3μm) and a column temperature of 35 °C. LC-MS separation was carried out by isocratic elution with methylene chloride (as mobile phase) and a flow rate of 0.5 mL/minute. LC-MS analysis parameters using negative ion mode included: source temperature 100 °C, cone sampling voltage 23 eV, capillary voltage 3.0 kV, solvent discharge temperature 350 °C, and solvent gas flow 60 mL/hour. Mass spectrum detection between *m/z* 10-1000 in ESI negative ion mode, adjust scanning duration (0.6 seconds/scan) and running time (25 minutes).

Determination of Chemical Components in the Fraction D3 (8-59) by Using LC-MS Method
The chemical components of the fraction D3 (8-59) from the methanol extract of *S. malaccense* was further analyzed by the LC-MS method, the chromatogram profile is shown in Fig.-1. It can be seen that the spectrum shows 20 peaks with their respective abundance (composition%) and Table-1 lists all the compounds identified by chromatography: retention time and mass spectrum data, similarity index 92%.
### Table 1: The Identified Compounds of Fraction D3 (8-59) using LC-MS based on the Chromatogram Database Resume Report

| Comp. No. | Rt (Min) | Compositon (%) | Compound Result | Identified Compounds |
|-----------|----------|----------------|-----------------|---------------------|
| 1         | 3.042    | 10.55079       | Gallic acid     | CF: C7H6O5, EM: 170,0215, MW: 170,1200, m/z: 170.0215 (100.0%), 171.0249 (7.6%), and 172.0258 (1.0%) |
| 2         | 8.217    | 3.42009        | Pinocembrin     | CF: C15H12O4, EM: 256,0736, MW: 256,2570, m/z: 256.0736 (100.0%), 257.0769 (16.2%), and 258.0803 (1.2%) |
| 3         | 9.375    | 1.50650        | 8-Methylpinocembrin | CF: C16H14O4, EM: 270,0892, MW: 270,2840, m/z: 270.0892 (100.0%), 271.0926 (17.3%), and 272.0959 (1.4%) |
| 4         | 9.74     | 1.31442        | Uvangoletin     | CF: C16H16O4, EM: 272,1049, MW: 272,3000, m/z: 272.1049 (100.0%), 273.1082 (17.3%), and 274.1116 (1.4%) |
| 5         | 10.02    | 2.22895        | Stercurensin    | CF: C17H16O4, EM: 284,1049, MW: 284,3110, m/z: 284.1049 (100.0%), 285.1082 (18.4%), and 286.1116 (1.6%) |
| 6         | 10.336   | 0.80731        | 2',4'-Dihydroxy-6'-methoxy-3'-methylidihydrochalcone | CF: C17H18O4, EM: 286,1205; MW: 286,3270; m/z: 286.1205 (100.0%), 287.1239 (18.4%), and 288.1272 (1.6%) |
| 7         | 11.015   | 0.95633        | Aurentiacin     | CF: C18H18O4, EM: 298,1205; MW: 298,3380, m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%) |
| 8         | 11.017   | 0.63396        | 2',4'-Dihydroxy-6'-methoxy-3',5'-Dimethylchalcone | CF: C18H18O4, EM: 298,1205; MW: 298,3380; m/z: 298.1205 (100.0%), 299.1239 (19.5%), and 300.1272 (1.8%) |
| 9         | 11.02    | 0.44227        | (+)-6,8-Dimethyl-5-methoxypinocembrin | CF: C18H18O4, EM: 298,1205; MW: 298,3380; m/z: 298.1205 (100.0%), 299.1239 (19.5%) |
| Comp. | Rt  | Compositi | Compound Result |
|-------|-----|-----------|-----------------|
| 10    | 11.514 | 8.99225 | Myricetin  
CF: C15H10O8; EM: 318.0376;  
MW: 318.2370; m/z: 318.0376 (100.0%), 319.0409 (16.2%), 320.0418 (1.6%), and 320.0443 (1.2%) |
| 11    | 23.194 | 12.01997 | Quercitrin  
CF: C21H20O11; EM: 448,1006; MW: 448,3800;  
m/z: 448.1006 (100.0%), 449.1039 (22.7%), 450.1073 (2.5%), and 450.1048 (2.3%) |
| 12    | 23.705 | 7.57609 | Epigallocatechin gallate  
CF: C22H18O11; EM: 458,0849; MW: 458,3750;  
m/z: 458.0849 (100.0%), 459.0883 (23.8%), 460.0916 (2.7%), and 460.0892 (2.3%) |
| 13    | 24.119 | 13.48729 | Myricitrin  
CF: C21H20O12; 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%) |
| 14    | 25.839 | 3.91405 | Mearnsitrin  
CF: C22H22O12; 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%) |
| 15    | 35.646 | 6.56918 | Desmanthin 1  
CF: C28H24O16; 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%) |
| Comp. | Rt      | Composi  | Compound Result                                                                 |
|-------|---------|----------|---------------------------------------------------------------------------------|
| 16    | 35.649  | 7.56693  | Myricetin-3-(3''-galloyl)rhamnoside                                               |
|       |         |          | CF: C28H24O16; 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%) |
| 17    | 46.577  | 5.78627  | Samarangenin A                                                                  |
|       |         |          | CF: C37H28O18; EM: 760,1276; MW: 760,6130; m/z: 760.1276 (100.0%), 761.1309 (40.0%), 762.1343 (7.8%), 762.1318 (3.7%), and 763.1352 (1.5%) |
| 18    | 60.009  | 3.56650  | Samarangenin B                                                                  |
|       |         |          | CF: C44H32O22; EM: 912,1385; MW: 912,7180; m/z: 912.1385 (100.0%), 913.1419 (47.6%), 914.1452 (11.1%), 914.1428 (4.5%), and 915.1461 (2.2%) |
| 19    | 60.039  | 6.06644  | 3-O-Galloylepigalloy-catechin (4β→8)-epigallocatechin-3-O-gallate                |
|       |         |          | CF: C44H34O22; EM: 914,1542; MW: 914,7340; m/z: 914.1542 (100.0%), 915.1575 (47.6%), 916.1609 (11.1%), 916.1584 (4.5%), and 917.1618 (2.2%) |
| 20    | 60.063  | 2.69440  | Casuarinin                                                                       |
|       |         |          | CF: C41H28O26; EM: 936,0869; MW: 936,6490; m/z: 936.0869 (100.0%), 937.0902 (44.3%), 938.0936 (9.6%), 938.0911 (5.3%), and 939.0945 (2.4%) |

**RESULTS AND DISCUSSION**

Based on LC-MS analysis, 20 compounds were identified from fraction D3 (8-59) of the methanol extract of *S. malaccense*. The 20 compounds identified can be classified into two big groups of compounds, namely 1) phenolic acid and 2) flavonoid derivatives. The structural features and presence of these compounds in plants other than *S. malaccense* can be reported.

**Phenolic Acid Derivatives**

Of the 20 compounds identified above, the compounds belonging to phenolic acid derivatives are gallic acid (GA) (1) and casuarinin (20). They have at least one galloyl group unit in their structures.
Compound 1, gallotannins and ellagitannins are hydrolyzable tannins that can be hydrolyzed by treatment with dilute acid. Compound 1 (3,4,5-trihydroxybenzoates) is a type of simple phenolic compound found commonly in plant tissues. According to reports, compound 1 has also been found in other Syzygium plants, such as stem bark of S. littorale, stem bark of S. polyanthum, stem bark of S. jambos, fruits of S. cumini, S. aromaticum, fruits of S. samarangense, and leaves of S. polyanthum. Besides, compounds 1 and two flavonoid glycosides (quercetin and myricetin, which are glycosylated by O-rhamnosyl group at position 3 in ring C) had also been found in the ethanol extract of the Santaloides afzelii leaves (Connnaraceae). Generally, compound 1 is used as a standard or reference for determining the total phenol content of plant extracts.

As reported, tannins can be hydrolyzed to become monomers, dimers, and oligomers of gallic acid (1), such as compound 20, nobotanins B, D, G, H, and J, strictinin, casuarictin, pedunculagin, (-)-epicatechin gallate, stachyurin, brevifolinicarboxylic acid, and others. Compound 20 is a monomeric hydrolyzable tannin composed of five monomer units of gallic acid. Compound 20 and acutissimin A, castalagin, eugenigrandin A, eugeniin, etc. had been found in S. aquaeum leaves. Also, compound 20 together with alunusnin A, syzyginin A, platycaryanin A, bicornin, rugosin C, tellimagrandin II, casuarictin, etc. had been found in S. aromaticum flower buds. Then, 15 compounds that are still related to hydrolyzable tannins included compound 20 have also been identified from the ethanol extract of S. cumini leaves.

Flavonoid Derivatives

Flavonoids are secondary metabolites and have been identified as broad classes of polyphenols widely found in plants. In structure, flavonoids are arranged by C6-C3-C6 pattern with the C ring carbon attached by B ring as shown in Fig.-2. Therefore, the flavonoid derivatives identified above can be grouped into chalcone, flavanone or catechin, flavanone, and flavonol derivatives.

![Fig.-2; The Basic Skeleton of Flavonoids](image)

Chalcone Derivatives

Chalcone, a group of flavonoids, is a compound of the polyphenol group found in plants. Structurally, chalcone is an aromatic ketone that can form the nucleus of many biologically important compounds (called chalcones). In other words, chalcone is similar to flavonoids, and the basic skeleton does not contain a C ring, as shown in Fig.-3. In this study, several compounds such as uvangoletin (4), stercurensin (5), 2',4'-dihydroxy-6'-methoxy-3'-methyldihydrochalcone (6), aurentiacin (7) and 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (8) can be classified as a chalcone derivative because they have a chalcone basic skeleton (Fig.-3). If observed structurally, these five compounds are included in an organic compound called 2'-hydroxychalcones.

![Fig.-3; Basic Skeleton of Chalcones](image)

Compound 4 had been isolated from the roots of Uvaria angolensis for the first time, along with angoletin and the known C-benzylidihydrochalcones, uvaretin, and isouvangletin. Compound 4 (2',4'-dihydroxy-6'-methoxydihydrochalcone) had also been found the seeds of Myrica gale (Myrtaceae), the roots of Uvaria acuminata (Annonaceae), and the rhizomes of Boesenbergia pandurata (Zingiberaceae). Compound 5 had been isolated from the leaves of S. samarangense. Compound 6 that is called C-methylated chalcone has a methyl group attached directly to ring A. Subsequently, compound 6 and 8 have been identified from the fruit of S. samarangense and the leaves of S. samarangense.
Compound 7 and flavokawin B were isolated from *Pityrogramma triangularis* leaves.\(^{37}\) Also, compound 7 in conjunction with myrigalon-D, was found in leaf glands of *Myrica pensylvanica*\(^{38}\) and was also isolated from *S. samarangense*.\(^{39}\) Compound 8 is natural chalcone, which is the main compound isolated from *S. samarangense* leaves.\(^{35,40}\) In nature, compound 8 (also called dimethylcardamonin (DMC)), is a major compound found in *S. samarangense* fruits\(^ {19}\) and had also been found in *S. campanulatum* leaves.\(^ {41}\) Ragasa *et al.*\(^ {35}\) reported that compounds 5, 7, 8, along with non-flavonoids: squalene, betulin, lupeol, sitosterol, lupenyl stearate, β-sitosteryl stearate, and 24-methylenecycloartenyl stearate, had been identified from methylene chloride extract of *S. samarangense* leaves.

### Flavanol or Catechin Derivatives

As well known that flavanols, which are also called flavan-3-ol possess a hydroxyl group attached to 3 positions in C ring. Unlike most flavonoid compounds, this compound does not have a double bond between positions 2 and 3. In this research, compounds 12, 17, 18, and 19 can be grouped to be flavanol derivatives because they possess at least catechin units, as shown in Fig.-4.

![Fig.-4: Catechin Unit](image)

In nature, flavanols can be linked with GA (1) to become aglycone forms of catechin such as epicatechin gallate, epigallocatechin (EGC), and epigallocatechin gallate (12) as shown in Fig.-5. Epigallocatechin gallate (12) (also called to be EGCG) and catechin derivatives were the main polyphenols found in the natural resources belonging to *Camellia sinensis* L. (white tea).\(^ {42}\) Many alkyl derivatives of GA (1) are found previously such as compound 12, lauryl gallate, propyl gallate, and theaflavin-3-gallate.\(^ {37}\) Compound 12 along with epigallocatechin (EGC), vescalagin, castalagin, and samarangenins A and B were also isolated *S. aqueum*.\(^ {33}\)

![Fig.-5: Aglycone Forms of Catechin Derivatives](image)

| Name                                      | R1  | R2      |
|-------------------------------------------|-----|---------|
| (-)-Epicatechin (EC)                      | H   | H       |
| Epigallocatechin (EGC)                    | OH  | H       |
| Epicatechin gallate (ECG)                 | H   | galloyl =|
| Compound 12 (EGCG)                       | OH  | galloyl =|

### Flavanone Derivatives

Slightly different from flavones, flavanones, also known as dihydroflavonoids, do not have double bonds at positions 2 and 3 in ring C; as a result, flavanones are saturated\(^ {44}\), as presented in Fig.-6.

![Fig.-6: The Basic Skeleton of Flavanones](image)
In this study, the compounds that included flavanone derivatives are pinocembrin (2), 8-methylpinocembrin (3), and (+)-6,8-dimethyl-5-methoxypinocembrin (9). From S. samarangense fruits, it had been found compound 2 along with GA (1) and ellagic acid.\(^1\) Compound 9, a 5,7-dihydroxy-6,8-dimethylflavanone (also called 6,8-dimethylpinocembrin), seemed to have never been found in other plants in the family Myrtaceae, but compound 9 without the methoxyl group in position 5 had been isolated from the methanol extract of S. aqueum (water apple).\(^4\)

**Flavonol Derivatives**

Structurally, flavonoids, which are also flavonoid groups, have a hydroxyl group attached to position 3 in ring C where this group can also undergo methylation or glycosylation. The methylation and glycosylation of these various flavonoids become the most common and largest subgroup of flavonoids in fruits and vegetables.\(^4\) The basic skeleton of flavonoids can be demonstrated as displayed in Fig.-7. In this study, the identified compounds belonging to flavonol derivatives are myricetin (10), quercetin (11), myricitrin (13), mearnsitrin (14), desmanthin 1 (15), and myricetin-3-(3''-galloylhamnoside) (16).

![Fig.-7: The Basic Skeleton of Flavonols](image)

Myricetin (10) is a common plant-derived flavonoid. Structurally, the compound is related to several phenolic compounds such as kaempferol, quercetin, morin, and fisetin.\(^6\) Compound 10 and myricitrin (13) had been obtained in the leaf extract from S. malaccense.\(^6\) Both compounds and GA (1) had also been separated from the ethanol extract of S. jambos leaves.\(^4\)

Furthermore, quercetin (11) together with myricitrin (13), reynoutrin, hyperin, quercetin, guaijaverin, pinocembrin (2), GA (1), and ellagic acid had been found from the methanol extracts of S. samarangense fruits.\(^19\) It should be noted that myricitrin (13) has been identified in the leaves of S. jambos\(^4\), as well as in the fruit of S. samarangense.\(^19\) This compound is a myricetin 3-O-L-rhamnioside, which is also found in S. malaccense leaf extract together with other glycosylated myricetin.\(^6,9\) Also, compound 13 together with desmanthin-1 (15) and guaijaverin have been isolated from Myrcia multiflora\(^49\) in the same family (Myrtaceae). Compound 13 and 3,5-di-O-methylgossypetin had also been found from S. samarangense leaves.\(^50\) The compound was the major constituent from several Myrcia (Myrtaceae) such as M. uniflora\(^\text{51}\), M. bella\(^\text{52}\), M. splendidens, and M. palustris.\(^53\)

Then, mearnsitrin (14), a glycosylated flavonoid, had been isolated from the leaves of S. samarangense.\(^54,55\) This compound had also been isolated from plants that are not from the Myrtaceae family, namely Sorindeia juglandifolia leaves.\(^56\) Meanwhile, desmanthin-1 (15) (a myricetin 3-(2''-galloylhamnoside)), quercitrin (11), myricitrin (13), and eight other compounds had been isolated from Eugenia uniflora leaves (Myrtaceae).\(^57\) Compound 15 and three known flavonoid glycosides, quercitrin (11), myriciacitinin, and guaijaverin were isolated from M. multiflora.\(^49\) Myricetin 3-(3''-galloylhamnoside) (16), its isomeric of desmanthin-1 (15), seemed that there are no literature sources that report the existence of the compound in nature or plants.

**CONCLUSION**

Chemical profile of the selected fraction of methanol extract of S. malaccense stem bark using LC-MS analysis indicated twenty compounds with molecular masses ranging from 170-936. In structure, these compounds can be classified into two types of phenolic acids (gallic acid and casuarinin) and types of flavonoid derivatives included chalcones, flavanols, flavanones, and flavonols as explained above.

**ACKNOWLEDGMENT**

The authors extend thanks to M. Ariesandy PT. Djarum Kudus Malang, East Java, Indonesia for help in LC-MS measurements.

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