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CHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF RIPE FRUIT, *n*-HEXANE FRACTION OF HALF-RIPE FRUIT, AND WATER FRACTION OF HALF-RIPE FRUIT OF SESOOT (*Garcinia picrorrhiza* Miq.) WITH LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

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Keywords: *Garcinia*, Sesoot (*Garcinia picrorrhiza* Miq.), Liquid Chromatography-Mass Spectrometry (LC-MS), Dictionary of Natural Product (DNP)

ABSTRACT

Garcinia is a plant belonging to the family of *Guttiferae* or *Cluciaceae* and has more than 180 famous species. Most of *Garcinia* species contains many prenelated xanthones, biflavonoids, and benzophenones. Using Liquid Chromatography - Mass Spectrometry (LC-MS), the development of electrospray ionization (ESI) could be detected and could be applied to a wide range of molecular biology such as xanthones, biflavonoids, and benzophenones and their derivatives. This method with a high speed of scanning at once could be measured a high degree of multiplexing and many components can be measured with a single analysis. These results were separated and analyzed using literature software of the Dictionary of Natural Product 2006. The ethanolic extract of the ripe fruit (IC), the *n*-hexane fraction of the half ripe (IIB), and the water fraction of the half ripe fruit (IVB) of sesoot (G. *picrorrhiza* Miq.) showed that the major compounds of them were xanthones, benzophenones with their derivatives, furanone and pyranone which were proven as cytotoxic to the brine shrimp nauplii using Brine

Shrimp Lethality Test (BSLT) and to the doxorubicin-resistant breast cancer cell line of MCF-7, the doxorubicin-sensitive breast cancer cell line of T47D.

INTRODUCTION

Garcinia is a plant belonging to the family of *Guttiferae* or *Cluciaceae* and has more than 180 famous species. *Garcinia* belongs to tropical plant, even though there are several species of *Garcinia* found in sub-tropical region such as Japan, Korea, and China. Several functions of consumable *Garcinia* such as G. *mangostana* and G. *dulcis* are as spices, traditional drugs, and cosmetics. From several researches that had been done to the *Garcinia* species, many active compounds having bioactivities such as antioxidant, antifungil, antivirus, antibacteri, anti-diabetic, anti-obesity and anticancer, are found. (Ilyas, Kamil, Parveen, & Khan, 1994).

According to Heyne (1987) *G. picrorrhiza* Miq. belongs to:

Division	:	*Spermatophyta*
Sub Division	:	*Angiospermae*
Class	:	*Dicotyledone*
Ordo	:	*Guttiferales*
Family	:	*Guttiferae*
Genus	:	*Garcinia*
Species	:	*G. picrorrhiza* Miq.
Tree's height	:	17 M
Stem's diameter	:	40,52 M
Canopy diameter	:	17 M
Canopy shape	:	round

Figure 1
***G. picrorrhiza* Miq. Tree**

Figure 2
Sesoot Fruits (*G. picrorrhiza* Miq.) with Its Seed (From Left to Right, Respectively: Unripe, Half-Ripe, Ripe)

Figure 3
Sesoot Fruits (*G. picrorrhiza* Miq.) which Have Been Separated from Its Seeds (I, Unripe; II, Half-Ripe; III, Ripe)

Based on the research done by Hanan and Sari (2000), the characteristic of sesoot (*G. picrorrhiza* Miq.) plant which is in Bogor Botanical Gardens as follows: *Garcinia* is famous containing yellow sap, which usually contains prenilated xanthones, biflavonoides, benzophenones (De Boer, Van Den Bosch, & Volberda, 1999).

From the phytochemical study, it is known that *Garcinia* species is rich of oxygenated and prenilated xanthones. Since most of xanthones have phenolic function group at linear trisiclic ring, hence that xanthones show often biological

activities and broad pharmacological effects, such as in vitro cytotoxic, in vivo anti-tumor, anti-inflammation, anti-microbe, antifungi, xanthine oxidase inhibition, monoammine oxidase inhibition, (antidepressant) (Sordat-Diserens, Rogers, Sordat, & Hostettmann, 1992). Antioxidant effect had been proved that antioxidant could muffle free-radical and superoxide anion and lipid superoxidation inhibition (Minami et al., 1994). It had been reported above that *Garcinia*xanthone A dan B dari G. Subelliptica had neurotrophyc. The uses of xanthone such as: allergic prevention, bronchodilator in healing asthma, antileuchemia, (Balasubramanian, Manohar, & Mathan, 1988) and antipyretic (Likhitwitayawuid, Klongsiriwet, Jongbunprasert, Sritularak, & Wongseripipatana, 2006).

Liquid chromatography – Mass Spectrometry (LC-MS) is a technique with the development of electrospray ionization (ESI) providing strong and simple interface. This method can be applied to the broad range of biological molecule. With high scanning velocity, followed by high grade multiplexing, many components can be measured at the same time. (Pitt, Tucker, Riley, & Longden, 2009).

Based on (Utami & Widiasavitri, 2013), the ethanolic extract of the ripe fruit (IC), the *n*-hexane fraction of the half-ripe fruit (IIB), and the water fraction of the half-ripe fruit (IVB) of sesoot (G. *picrorrhiza* Miq.) containing saponins, tannin, and total phenol, had been proved having bioactivity in Brine Shrimp Lethality Test (BSLT) at each extract and fractions 21.110 µg/mL; 22.120 µg/mL; and 26.090 µg/mL, respectively.

RESEARCH METHODS

Experimental

Tools:

Liquid Chromatography Hitachi L 6200, LC-MS: Mariner Biospectrometry, ESI (Electrospray Ionisation) system, positive ion mode, Supelco Column 5µ C18. 250 × 2 mm i.d., (RP 18) and Dictionary of Natural Product (DNP) 2006.

Materials:

Ethanolic extract of sesoot (G. *picrorrhiza* Miq.) ripe fruit (IC), *n*-hexane fraction of sesoot (G. *picrorrhiza* Miq.) half-ripe fruit (IIB), and water fraction of sesoot (G. *picrorrhiza* Miq.) half-ripe fruit (IVB), methanol, water, acetate acid, and acetonitrile used for HPLC.

Procedure:

Sample Preparation.

The sample preparation was published on the Jurnal Bahan Alam Indonesia (JBAI) Vol. 8, No. 4 January 2013. The extraction of the herbs (raw/A, half ripe/B, and ripe

fruit/C) with 5 kinds of solvent: the ethanol extract/I, the fractions of *n*-hexane/II, ethanol/III, water/IV and ethyl acetate/V), and test of their phytochemistry. The ethanolic extract of the A produced 64.17% of yield, the B was 17.95%, and the C was 31.50%. The yields of the extract and fractions can be affected by the process of the fruit ripening. They contained the total phenols, tannins, and saponins. The concentration ranges for Brine Shrimp Lethality Test (BSLT) to measure the LC_{50} were 5 – 50 µg/mL for I (ABC) or 10 - 1000 µg/mL for other kinds of solvent. The best LC_{50} was IC with 21.11 µg/mL; the LC_{50} of IIB was 22.12 µg/mL, and the LC_{50} of IVB was 26.09 µg/mL. The three samples of IC, IIB, and IVB were determined their compounds qualitatively using LC-MS.

LC-MS Analysis.

For the LC-MS analysis, it was used Mariner Biospectrometry with a pair of pumps. The HPLC was interpreted by the mass spectrometer Q-tof which was suited with the ES source. The full-scan mode was started with the m/z value of 100 up to 1200, and it was done at 140 °C. For the analysis, it was used HPLC column using the solvent A is water with 0.3% acetate acid, the solvent B is acetonitrile with 0.3% acetate acid. The solvents were distributed in its chromatograph column with flow average 1 mL/min using isocratic elution (changing of eluent during separating did not happen). Eluent used was methanol and water (95:5), sample injected was 20 µL.

Data Analysis.

The result of the LC-MS analysis was the chromatogram which gave m/z data in each peak which was the molecule mass (mass molecule = M) of a compound, added by 1 ion H^+ (M + H). That m/z data then was compared to other compound of same group of plant which was *Garcinia* from the the DNP 2006. The mass molecule obtained from the calculation (M + H) was confirmed with the mass molecule of the result calculation (M + Na) (Mass molecule Na = 23 gram/mol), (2M + H), and (2M + Na) from other peak(s) at each Retention time (Rt).

RESULTS AND DISCUSSION

The mass molecule resulted at the chromatogram was the mass molecule of a compound which was expected at that peak and H+ ion thus the mass molecule at the chromatogram was [M + H]. Thereby, the mass molecule of a compound was the mass molecule at the chromatogram decreased by 1. The mass molecule that could be obtained from the calculation (M + H) then was confirmed with the mass molecule obtained from the calculation (M + Na) (Mass molecule of Na = 23 gram/mol), (2M + H), and (2M + Na) from other peak(s) at each Retention time (Rt).

Based on the LC-MS analysis result for the etanol extract of ripe fruit (IC), *n*-hexane fraction of half-ripe fruit (IIB), and water fraction of half-ripe fruit (IVB) sesoot (G. *picrorrhiza* Miq.) could be known that the compounds contained inside the extract and the fractions are xanthones, benzophenones and the derivatives, furanones and pyranones which were expected giving cytotoxic effect. The mass molecule resulted at the chromatogram was the mass molecule of a compound which was expected at that peak and H^+ ion thus the mass molecule at the chromatogram was [M + H]. Thereby, the mass molecule of a compound was the mass molecule at the chromatogram decreased by 1. The mass molecule that could be obtained from the calculation (M + H) then was confirmed with the mass molecule obtained from the calculation (M + Na) (Mass molecule of Na = 23 gram/mol), (2M + H), and (2M + Na) from other peak(s) at each Retention time (Rt).

Based on the LC-MS analysis result for the etanol extract of the ripe fruit (IC), the *n*-hexane fraction of half-ripe fruit (IIB), and the water fraction of the half-ripe fruit (IVB) sesoot (G. *picrorrhiza* Miq.) could be known that the compounds contained inside the extract and the fractions were xanthones, benzophenones and the derivatives, furanones and pyranones which were expected giving cytotoxic effect.

Figure 4
The Chromatogram of the Ethanolic Extract Sample of the Ripe Fruit (IC) of Sesoot (G. *picrorrhiza* Miq.)

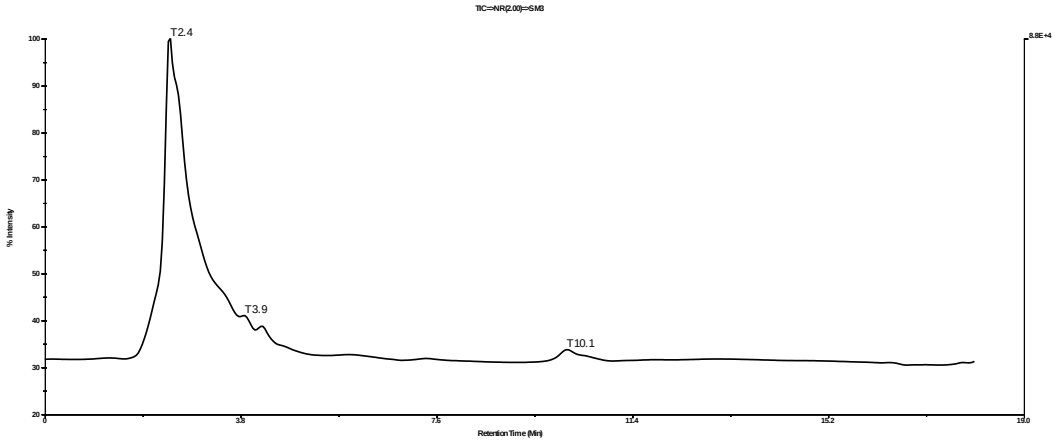


Figure 5
The Chromatogram of the Ethanolic Extract Sample of the Ripe Fruit (IC) of Sesoot (G. *picrorrhiza* Miq.) at Retention time (Rt) 2.4

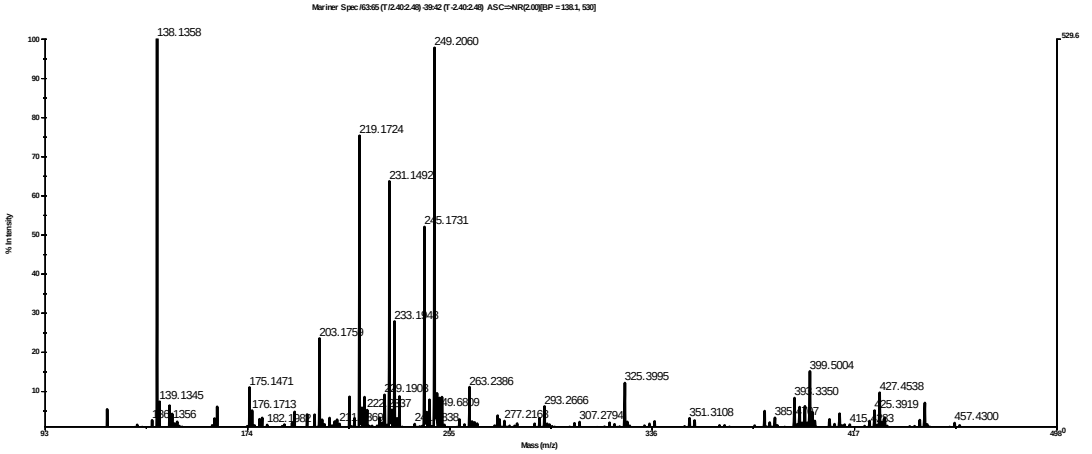


Figure 6
The Chromatogram of the Ethanolic Extract Sample of the Ripe Fruit (IC) of Sesoot (G. *picrorrhiza* Miq.) at Retention time (Rt) 3.9

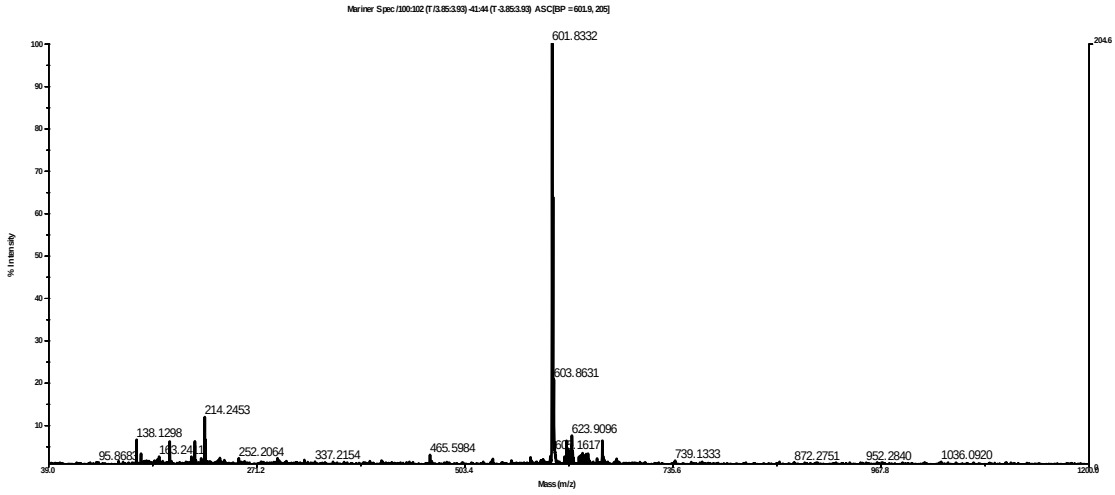


Figure 7
The Chromatogram of the Ethanolic Extract Sample of the Ripe Fruit (IC) of Sesoot (G. *picrorrhiza* Miq.) at Retention time (Rt) 10.1

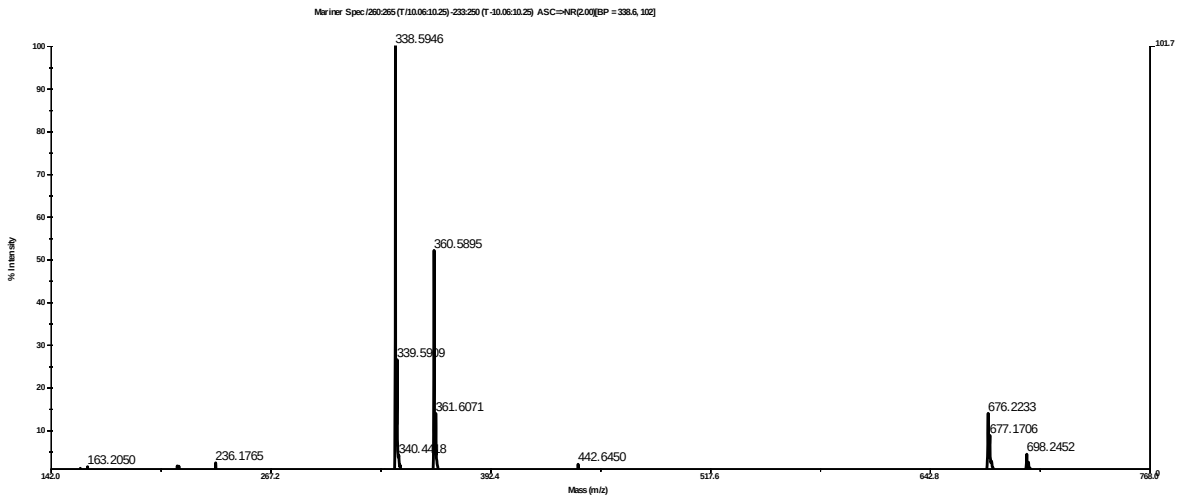


Figure 8

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.)

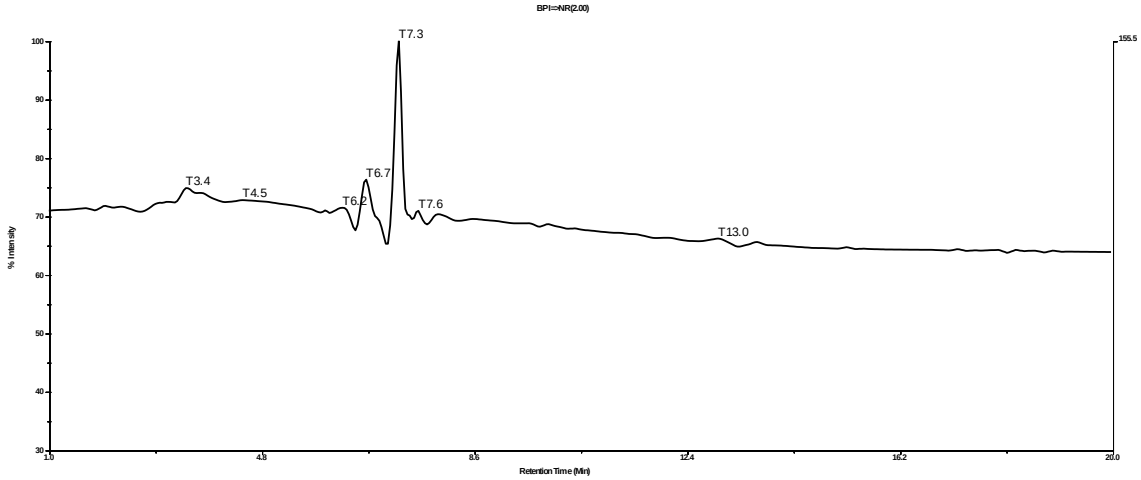


Figure 9

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 3.4

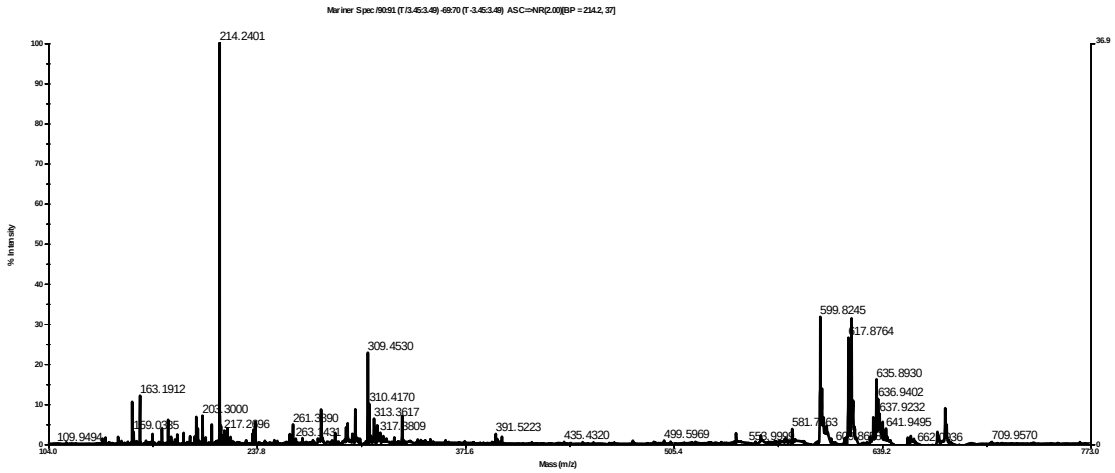


Figure 10

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 6.2

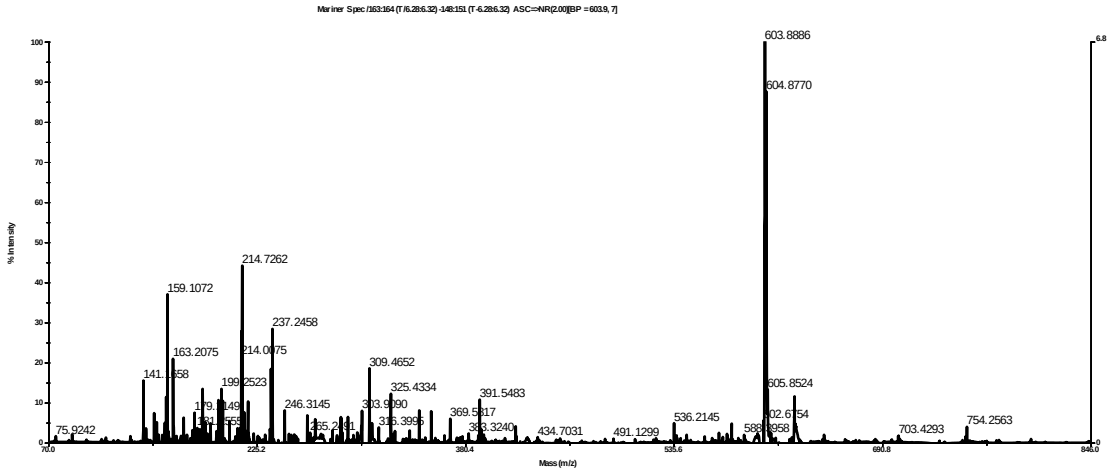


Figure 11

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 6.6

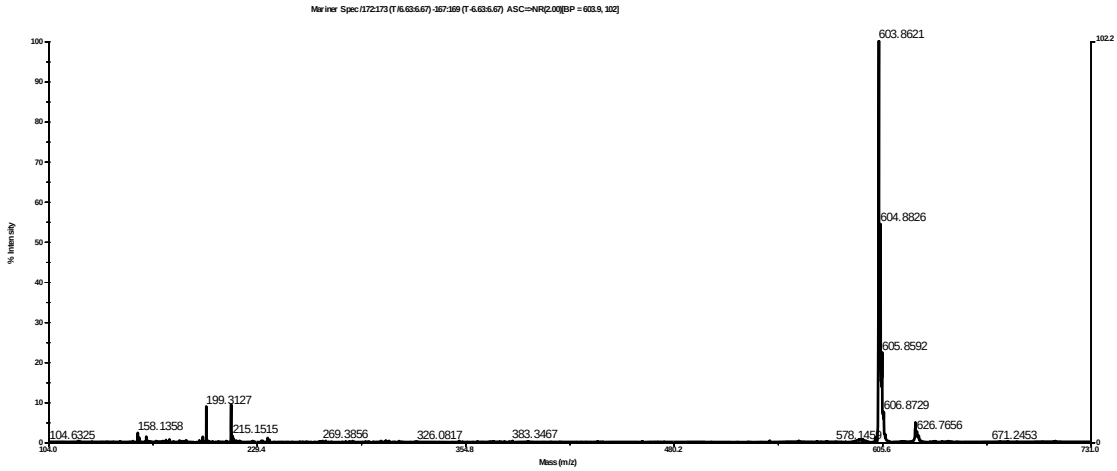


Figure 12

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 7.3

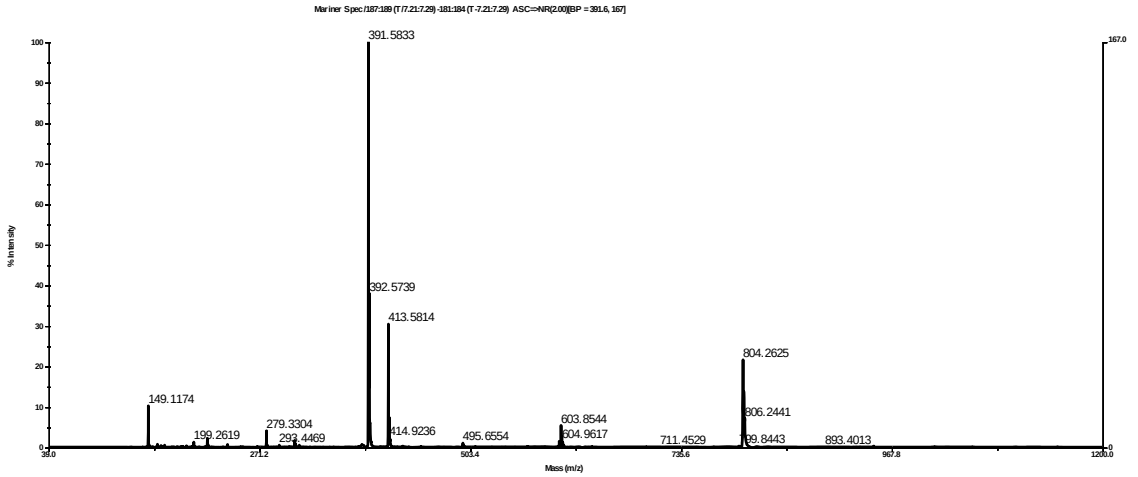


Figure 13

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 7.3

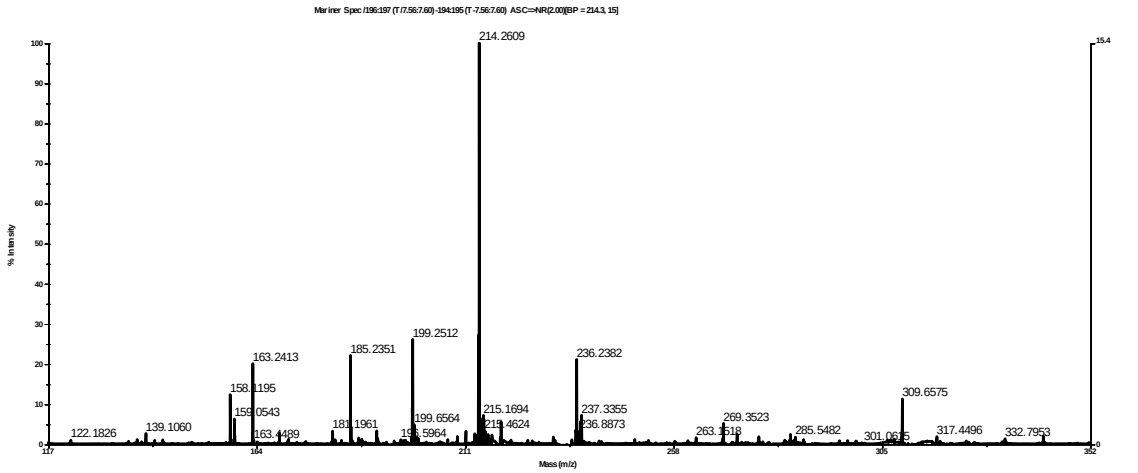


Figure 14

The Chromatogram of the *n*-Hexane Fraction Sample of the Half-Ripe Fruit (IIB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 8

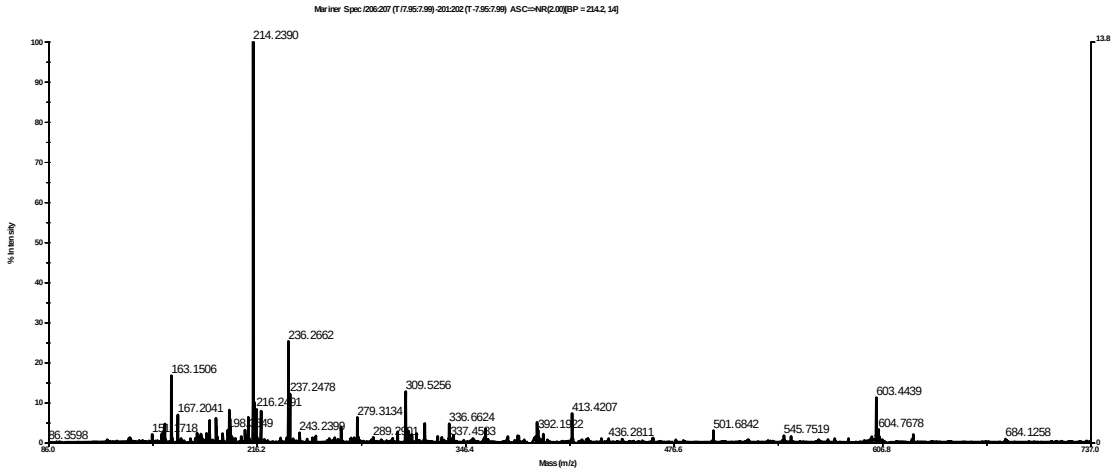


Figure 15

The Chromatogram of the Water Fraction Sample of the Half Ripe Fruit (IVB) of Sesoot (G. *picrorrhiza* Miq.)

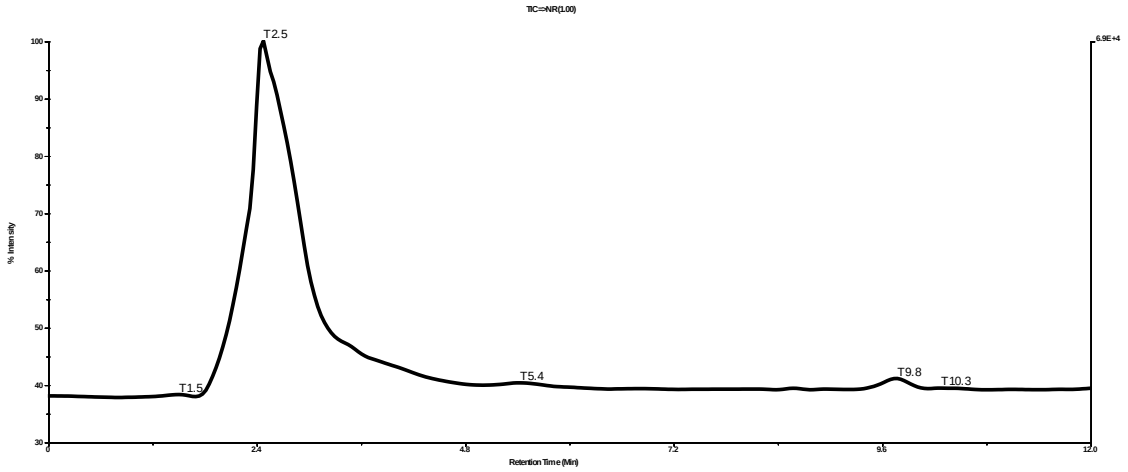


Figure 16

The Chromatogram of the Water Fraction Sample of the Half-Ripe Fruit (IVB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 2,5

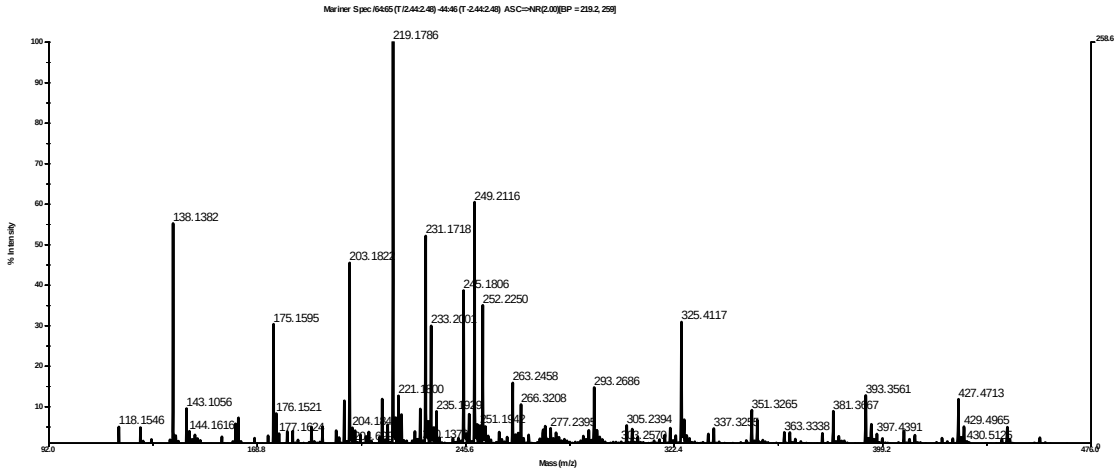


Figure 17

The Chromatogram of the Water Fraction Sample of the Half-Ripe Fruit (IVB) of Sesoot (G. *picrorrhiza* Miq.) at Rt 5,4

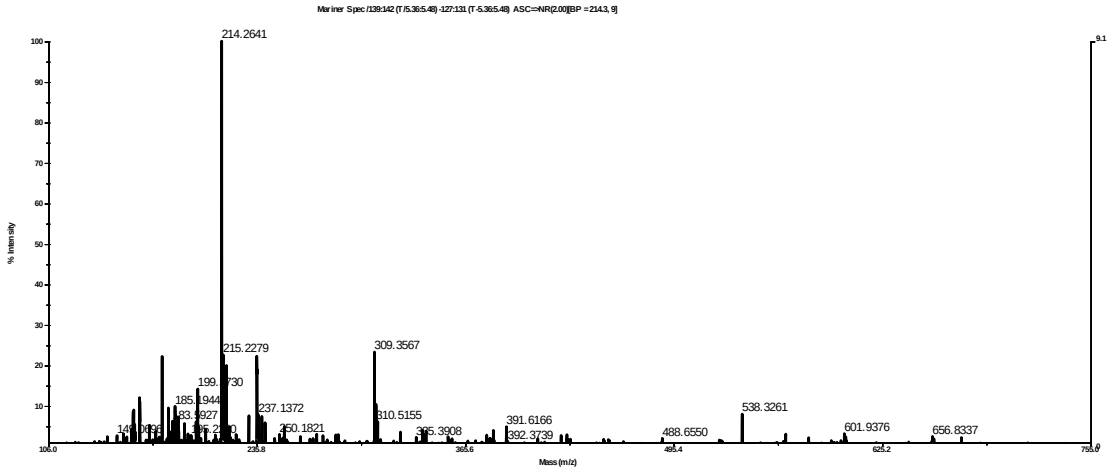
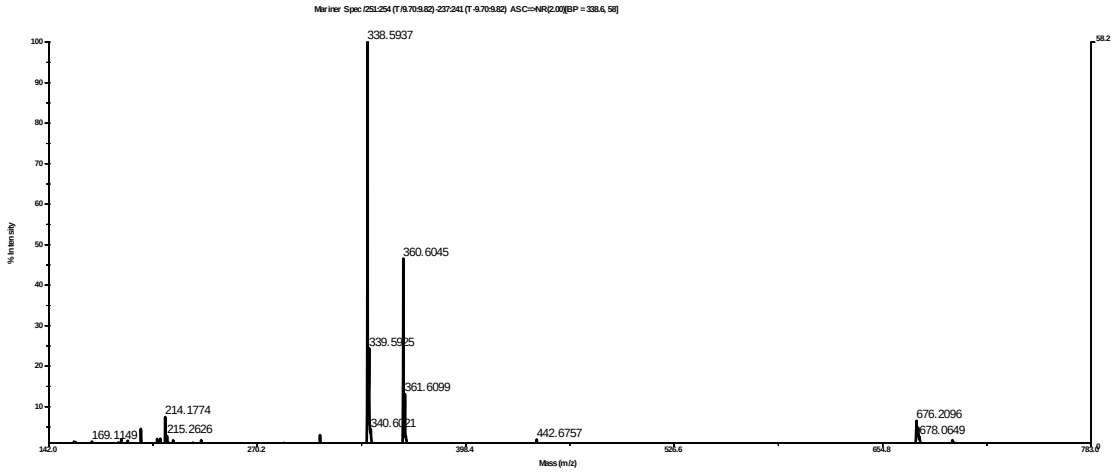


Figure 18
The Chromatogram of the Water Fraction Sample of the Half-Ripe Fruit (IVB) of Sesoot (*G. picrorrhiza* Miq.) at Rt 9,8



The possible compounds contained in the fruit of sesoot (G. *picrorrhiza* Miq.) were based on the result from the separation using LC-MS and they were compared to the DNP literature 2006. Those compounds were obtained from the type of *Garcinia* which had been isolated before. These are the tables of the possible compounds contained in the fruit of G. *picrorrhiza* Miq. based on the LC-MS analysis result obtained from the extract and fractions.

Table 1
The Analysis of the LC-MS Result of the Ethanol Extract of the Ripe Fruit (IC) of Sesoot (*G. picrorrhiza* Miq.)

No.	Mass Molecule	Molecular Formula	Compound Name
1.	228,191	$C_{13}H_8O_4$	1,5-Dihydroxyxanthone
2.	324,4348	$C_{19}H_{16}O_5$	Forbexanthone;Demethoxy, 5-Me ether
3.	338,5909	$C_{21}H_{38}O_3$	5-(8-Heptadecenyl)dihydro-3-hydroxy-2(3*H*) –furanone
4.	392,335	$C_{19}H_{20}O_9$	2,4,6-Trihydroxybenzophenone, 8CI; 2-O -β-D-Glucopyranoside
5.		$C_{24}H_{24}O_5$	Calabaxanthon
6.		$C_{23}H_{20}O_6$	Pyranojacareubin
7.		$C_{23}H_{20}O_6$	Rheediaxanthone A

8.	464,5984	$C_{28}H_{32}O_6$	*Garcinia*none A
9.		$C_{28}H_{32}O_6$	Bractatin
10.		$C_{28}H_{32}O_6$	1-(3,7-Dimethyl-2,6-octadienyl)-2,3,6,8-tetrahydroxy-7-(3-methyl-2-butenyl)xanthone; (E)-form
11.		$C_{28}H_{32}O_6$	4-(3,7-Dimethyl-2,6-octadienyl)-2,3,6,8-tetrahydroxy-1-prenylxanthone; (E)-form
12.		$C_{28}H_{32}O_6$	Dulciol B
13.		$C_{28}H_{32}O_6$	Forbesione
14.	600,8332	$C_{38}H_{48}O_6$	Gambogenin; 29-Deoxo

Table 2

The Analysis of the LC-MS Result of the *n*-Hexane Fraction of the Half-Ripe Fruit (IIB) of Sesoot (*G. picrorrhiza* Miq.)

No.	Mass Molecule	Molecular Formula	Compound Name
1.	262,152	$C_{13}H_{10}O_6$	2,3',4,4',6-Pentahydroxybenzophenone,8CI
2.		$C_{13}H_{10}O_6$	2,3',4,5',6-Pentahydroxybenzophenone
3.	284,548	$C_{18}H_{20}O_3$	3,4',5-Trihydroxy-4-prenylbiphenyl; 3-Meether
4.		$C_{18}H_{20}O_3$	2,2-Dimethyl-7-phenyl-2H-1-benzopyran-5-ol, 9CI; 4'-Hydroxy, 3,4-dihydro, 5-Me ether
5.	302,909	$C_{14}H_{22}O_7$	1,2-Dihydroxy-1,2,3-propanetricarboxylic acid,8CI; (1*S*, 2*S*)-form, γ-Lactone, dibutyl ester
6.	324,4348	$C_{19}H_{16}O_5$	Forbexanthone;Demethoxy, 5-Me ether
7.	338,5909	$C_{21}H_{38}O_3$	5-(8-Heptadecenyl)dihydro-3-hydroxy-2(3*H*) –furanone
8.	390,524	$C_{25}H_{42}O_3$	4-Hydroxy-3-(3-methyl-2-butenyl)-6-pentadecyl-2H-pyran-2-one, 9CI
	390,9721		

9..	392,335	$C_{19}H_{20}O_9$	2,4,6-Trihydroxybenzophenone, 8CI; 2-O -β-D-Glucopyranoside
10.		$C_{24}H_{24}O_5$	Calabaxanthon
11.		$C_{23}H_{20}O_6$	Pyranojacareubin
12.		$C_{23}H_{20}O_6$	Rheediaxanthone A
13.	464,5984	$C_{28}H_{32}O_6$	*Garcinia*none A
14.		$C_{28}H_{32}O_6$	Bractatin
15.		$C_{28}H_{32}O_6$	1-(3,7-Dimethyl-2,6-octadienyl)-2,3,6,8-tetrahydroxy-7-(3-methyl-2-butenyl)xanthone; (E)-form
16.		$C_{28}H_{32}O_6$	4-(3,7-Dimethyl-2,6-octadienyl)-2,3,6,8-tetrahydroxy-1-prenylxanthone; (E)-form
17.		$C_{28}H_{32}O_6$	Dulciol B
18.		$C_{28}H_{32}O_6$	Forbesione
19.	576,5612	$C_{33}H_{36}O_9$	Morellin; 29-Carboxylicacid, Δ^{18}-isomer, 17-hydroxy
20.		$C_{34}H_{40}O_8$	Moreollin; (E)-Isomer, 10-O -de-Et, 10-O –Me
21.		$C_{34}H_{40}O_8$	Scortechinone A; 18-Oxo
22.	600,8332	$C_{38}H_{48}O_6$	Gambogenin; 29-Deoxo
23.	602,8886	$C_{38}H_{50}O_6$	Guttiferone A
24.		$C_{38}H_{50}O_6$	Guttiferone E; (+)-form

25.		$C_{38}H_{50}O_6$	Guttiferone E; (+)-form, $\Delta^{36,37}$-Isomer
26.		$C_{38}H_{50}O_6$	Guttiferone E; (–)-form
27.		$C_{38}H_{50}O_6$	Isoxanthochymol; (+)-form
28.	604,8592	$C_{38}H_{52}O_6$	Pedunculol
29.		$C_{31}H_{24}O_{13}$	3'',4',4''',5,5'',7,7''-Heptahydroxy-3,8''-biflavanone, 8CI; 3',3'''-Dihydroxy, 4'-Me ether
30.	616,8764	$C_{39}H_{52}O_6$	Guttiferone E; (–)-form,13-Me ether
31.		$C_{39}H_{52}O_6$	Isoxanthochymol; (–)-form, 13-Me ether

Table 3

The Analysis of the LC-MS Result of the Water Fraction of the Half-Ripe Fruit (IVB) of Sesoot (*G. picrorrhiza* Miq.)

No.	Mass Molecule	Molecular Formula	Compound Name
1.	302,909	$C_{14}H_{22}O_7$	1,2-Dihydroxy-1,2,3-propanetricarboxylic acid,8CI; (1*S*, 2*S*)-form, γ-Lactone, dibutyl ester
2.	338,5925	$C_{21}H_{38}O_3$	5-(8-Heptadecenyl)dihydro-3-hydroxy-2(3*H*) –furanone

CONCLUSION

Based on the LC-MS analysis result for the ethanolic extract of ripe fruit (IC), *n*-hexane fraction of half-ripe fruit (IIB), and water fraction of half-ripe fruit (IVB) sesoot (G. *picrorrhiza* Miq.) could be known that the compounds contained inside extract and fractions were xanthones, benzophenones and the derivatives, furanones dan pyranones which were expected giving cytotoxic effect.

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