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Isolation and Identification of Chemical Compounds from *Garcinia fruticosa* Lauterb Stem Bark Extract

Novia Delita^{1,2}, Berna Elya^{3,*}, Muhammad Hanafi^{4,5}

Novia Delita^{1,2}, Berna Elya^{3,*},
Muhammad Hanafi^{4,5}

¹Graduate Programme of Biology
Pharmacy, Faculty of Pharmacy, Universitas
Indonesia, Depok, INDONESIA.

²Department of Pharmacognosy-
Phytochemistry, Faculty of Pharmacy and
Science, Universitas Muhammadiyah Prof.
Dr. Hamka, 1340 Jakarta, INDONESIA.

³Department of Pharmacognosy-
Phytochemistry, Faculty of Pharmacy,
Universitas Indonesia, Depok, INDONESIA.

⁴Research Center for Chemistry, Indonesian
Institute of Sciences, Serpong, INDONESIA.

⁵Faculty of Pharmacy, Universitas Pancasila,
Jakarta, INDONESIA.

Correspondence

Prof. Dr. Berna Elya, M.Si., Apt.

Department of Pharmacognosy-
Phytochemistry, Faculty of Pharmacy,
Universitas Indonesia, Depok, 16424, West
Java, INDONESIA.

Phone no: +62 81314161497

E-mail: berna.elya@gmail.com

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ABSTRACT

Background: *Garcinia* is a tropical plant that grows in Indonesia. *Garcinia* has many health benefits for the body. *Garcinia* contains many phenolic compounds and their derivatives, such as xanthone, flavonoids, benzophenone, lactone, and phenolic acids. *Garcinia fruticosa* Lauterb. comes from the family Clusiaceae. The results of the phytochemical examination showed that *G. fruticosa* bark extract contained alkaloids, flavonoids, glycosides, tannins, and saponins. **Objective:** This study aims to isolate and identify chemical compounds from the ethyl acetate extract of *G. fruticosa* Lauterb stem bark. **Method:** *G. fruticosa* Lauterb bark, dried, milled, and extracted with Step Gradient Polarity/SGP maceration using n-hexane, ethyl acetate, and methanol. Isolation was done by column chromatography and identified by thin layer chromatography and IR spectroscopy, LC-MS/MS, ¹H-NMR, ¹³C-NMR, 2D-NMR (HSQC, HMBC). **Results:** Compound D7a has a molecular weight 168.0496. The IR spectrum shows the presence of a group -OH appears on 3483 cm⁻¹, aromatic presence in 1609 cm⁻¹. The ¹H-NMR spectrum shows the presence of aromatic signals on 6.96 (d, 8 Hz), 6.96 (d, 2 Hz) and 7.70 (dd, 8; 2 Hz). The ¹³C-NMR spectrum shows the presence of a carboxylic-COOH group appearing at 166.57 ppm, the presence of 2 x C-OH appearing at 147.18 and 151.18. In the HMBC spectrum, the -OCH₃ position is located at C-3 with a correlation between the 3.79 (s) signal and the C signal at the chemical shift 147.18. **Conclusions:** Structural elucidation shows that compound D7a is a 4-hydroxy-3-methoxy benzoate acid (Vanylic Acid) and isolate I-1 is an impure compound namely β-Sitosterol and Stigmasterol.

Key words: Isolation, structural elucidation, *Garcinia fruticosa*, 4-hydroxy-3-methoxy benzoic acid, β-Sitosterol, Stigmasterol.

INTRODUCTION

Garcinia is a tropical plant that grows in Indonesia. *Garcinia* is known to have many health benefits for the body. From several studies that have been conducted on various types of *Garcinia* plants, it is known that *Garcinia* is a source of secondary metabolite compounds that can be medically useful for the treatment of certain diseases, such as antidiabetic and antioxidant.^{1,2}

Garcinia fruticosa Lauterb. comes from the family Clusiaceae.³ In Indonesia, *G. fruticosa* Lauterb grows in Irian Jaya. *Garcinia* itself is widely found in tropical regions, such as Africa and Asia.⁴

Garcinia is a plant that contains many phenolic compounds and their derivatives, such as xanthone, flavonoids, benzophenone, lactone, and phenolic acids.⁵ Research conducted by Shen *et al.* shows that *Garcinia* contains various flavonoid compounds, such as kaempferol, quercetin, and flavanone and flavonoids-O-glycosides.¹ In addition, according to Shen and Yang, *Garcinia* also contain steroid compounds, such as β-sitosterol and stigmasterol.¹

The results of the phytochemical examination showed that the extract of *G. fruticosa* bark

contained alkaloids, flavonoids, glycosides, tannins, and saponins.⁶

MATERIALS AND METHODS

Plant material

Garcinia fruticosa obtained from the Bogor Botanical Gardens Conservation Center and has been determined by the Indonesian Institute of Sciences (LIPI) Biological Research Center, Bogor. This study uses stem bark as a sample.

Chemical material

Glacial acetic acid (Merck, Germany); formic acid; acetone p.a; aqua demineralisata (Brataco Chemika, Indonesia); DMSO; ethyl acetate p.a; technical ethyl acetate; FeCl₃.6H₂O; Concentrated HCl; Chloroform p.a; TLC plates (Merck); methanol p.a; technical methanol; n-hexane p.a; n-hexane technical; para-nitrophenyl-α-D-glucopyranoside (Sigma Aldrich, Switzerland); Sephadex LH 20 (Sweden); silica gel (70-230 mesh, E. Merck).

Tools

Glassware, 100 ml storage bottles, TLC chamber, filter paper, chromatographic columns, refrigerators

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(Panasonic), capillary pipes, drip pipettes, rotary evaporators (IKA, Germany), infrared spectrometers (Shimadzu), magnetic resonance spectrometers core (Jeol, Japan), analytical scales (Accu-Lab), analytical scales, UPLC-LC-MS / MS (Waters), 10 ml vials, Chemdraw (software).

Extraction and fractionation

Ethyl acetate extract of *Garcinia fruticosa* bark was obtained from the Phytochemical Laboratory of the Faculty of Pharmacy, University of Indonesia. Extraction uses the maceration method in sequence with n-hexane, ethyl acetate, and methanol as solvents. A total of 30 grams of ethyl acetate extract was fractionated using column chromatography with polarity gradient n-hexane - ethyl acetate - methanol as the mobile phase and silica gel G60 as the stationary phase. The elution process starts from n-hexane 100; followed by n-hexane: ethyl acetate 95: 5; 90:10; 85:15; 80:20; and so on at intervals 5 to obtain the ethyl acetate fraction 100, then continued with ethyl acetate: methanol 95: 5; 90:10; 85:15; 80:20; and so on at intervals of 5 to get the methanol fraction 100 so that eluents with different polarity gradients are obtained. Each fraction is collected every 100 ml and evaporated and then combined into several fractions based on the thin layer chromatography profile.

Isolation and purification of subfractions D

Subfraction D has brownish yellow deposits. Subfraction D is then refined by recrystallization with n-hexane and ethyl acetate as solvent to obtain pure compounds. From the recrystallization results obtained by a chromatogram profile which is then accommodated in vials. Subfraction was carried out by thin layer chromatography using 3 different eluents (chloroform: acetone: formic acid, 4: 1: 0.25) with a single spot yield. The compound obtained was weighed, and put in a glass vial with a well-closed container. The compounds obtained are identified their compound structure.

Isolation and purification of subfraction I

Subfraction I shows white sediment. Subfraction I was then refined using preparative TLC with the mobile phase of n-hexane: ethyl acetate (9: 1). From the preparative TLC results obtained by chromatogram profiles which are then separated and accommodated in vials, and the dominant peak is in the chromatogram pattern number 1 (I-1) in the form of white needle crystals. I-1 subfraction was carried out thin layer chromatography using eluent (n-hexane: ethyl acetate: formic acid, 9: 1: 0.3) with a single spot result, the compound I-1 obtained was weighed, and put in a vial of glass with a well-closed container. The compound I-1 obtained was identified by its compound structure.

Thin layer chromatography (TLC)

1 mg of isolate was dissolved in ethyl acetate and filtered before spotting on the TLC plate. Chamber Saturation time is 15 minutes.

Compound identification

The compounds were identified by analyzing spectroscopic data from IR, MS spectrophotometry, *Nuclear Magnetic Resonance proton* (¹H-NMR) and carbon (¹³C-NMR) spectroscopy, and NMR-2D techniques which included HMQC and HMBC.

a. Infrared Spectrum Check (IR)

1 mg of isolate are crushed with 100 mg KBr until homogeneous. The mixture is pressed with a strength of 10 tons/cm³ to form a thin pellet, then the infrared absorption is measured.

b. Nuclear Magnetic Resonance (NMR) Examination

10 mg of isolate was dissolved in CD₃OD, put into a glass tube placed in the middle of a magnetic field tank containing helium gas and

sealed with liquid nitrogen. From the results of data processing will be obtained the proton core magnetic resonance spectrum (¹H-NMR), carbon (¹³C-NMR), and 2D-NMR techniques which include HMQC and HMBC.

c. Mass Spectrum Examination by HPLC-MS

1 mg of pure isolate was dissolved in methanol. 10 µL sample was taken and injected into LC-MS through the Heliflex AT-5 ms column, 30 m x 0.25 mm x 0.25 µm flow velocity of 0.5 mL/min.

RESULTS AND DISCUSSION

Fractionation of Ethyl Acetate Extract from *G. fruticosa* Lauterb Stem Bark.

Column chromatography results obtained 401 reservoirs. Each storage result was seen by chromatography profile by thin layer chromatography. The beds with the same chromatogram pattern are combined into one so that we get 11 final fractions of ethyl acetate extract, namely A, B, C, D, E, F, G, H, I, J, and K fractions. The weight of each fraction can be seen in Table 1 and 2.

Isolation and Purification of Compounds D

Compound D was obtained by column chromatography which was subsequently recrystallized with n-hexane and ethyl acetate as much as 19.5 mg in the form of a brownish-yellow solid. Chromatogram pattern of compound D with silica gel stationary phase and chloroform: acetone: ac. format (4: 1: 0.25), R_f = 0.625; can be seen in Figure 1.

IR Spectrophotometry Measurement Results Data of Compound D

The IR spectrum shows the presence of a hydroxy group (-OH) from a carboxylic group (-COOH) and phenol appears in the region of wave number 3483 cm⁻¹, aromatic presence at 1609 cm⁻¹, can be seen in Figure 2.

Table 1: Results of ethyl acetate extract fractionation.

Fraction	Storage bottles	Weight (g)
A	1-28	0,487
B	29-48	0,941
C	49-70	1,448
D	71-96	5,048
E	97-126	1,052
F	127-166	1,379
G	167-192	0,948
H	193-234	1,546
I	235-306	4,381
J	307-360	0,752
K	361-401	1,009

Table 2: NMR Results Data of Isolate D7a compared to Vanylic Acid.9,10

NO Position C	Vanylic Acid ^{9,10}		Isolate D7	
	δC	δH (Σ H, mult, J(Hz))	δC	δH (Σ H, mult, J(Hz))
1	122.01	-	122.06	-
2	115.44	7.45 (1H, s)	112.57	7.58 (1H, d)
3	147.63	-	147.63	-
4	150.51	-	151.18	-
5	113.10	6.85 (1H, d, J = 7.8 Hz)	114.64	6.92 (1H, d, J = 8,0 Hz)
6	123.90	7.44 (1H, s)	123.95	7.60 (1H, dd)
COOH	167.64	-	166.57	-
-O-CH ₃	55.95	3.81 (3H,s)	55.41	3.92 (3H,s)

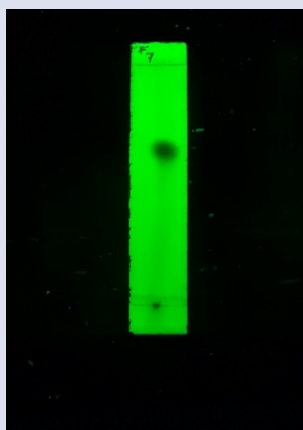


Figure 1: Chromatogram pattern compound D.

Note: Observation of Compound D under UV light 254, Silent phase: Silica gel GF254; Phases of motion: Chloroform: acetone: as. format (4: 1: 0.25).

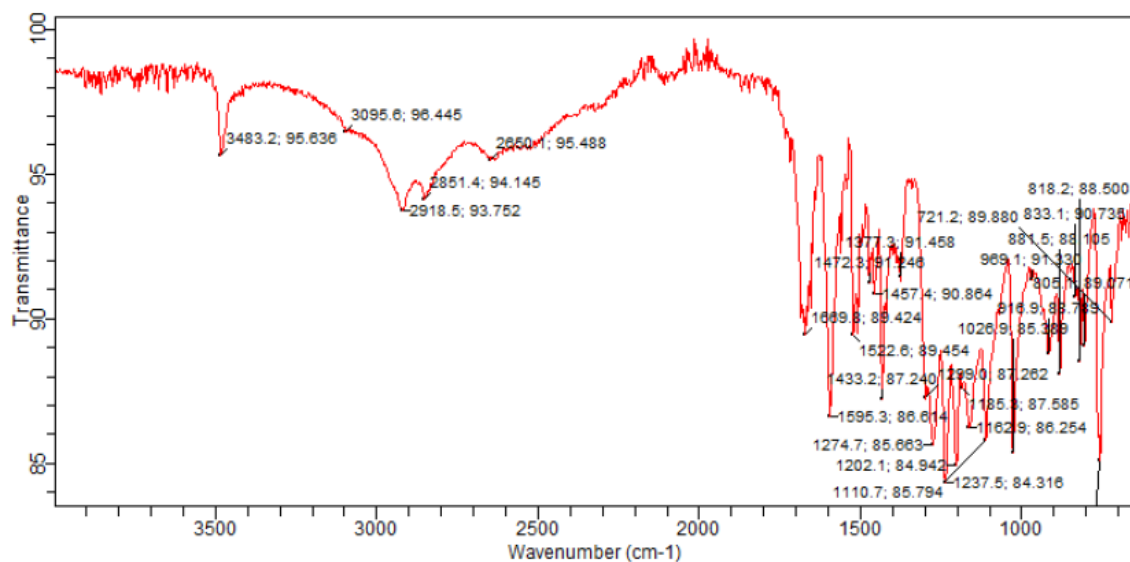


Figure 2: Results of IR Spectrophotometric Measurements.

LC-MS/MS Measurement Results Data of Compound D

Compound D has a molecular weight (BM) of 168.0496, in the presence of an ion peak ($M + H$) 169.0596, with the molecular formula $C_8H_8O_4$, can be seen in Figure 3.

NMR Measurement Results Data of Compound D

The results of measurements of H-NMR (acetone- d_6 , 500 MHz) and C-NMR (125 MHz) reinforce the presence of an aromatic, -OH and -COOH. The spectrum shows aromatic signals in the 6-7 ppm region, which is an aromatic trisubstitution. The signal at the chemical shift 6.92 (d, 8 Hz) which has an ortho position towards the signal at 7.60 (dd, 8; 2 Hz) and the presence of a signal at 7.58 (d, 2 Hz) is a signal with a meta position against the 7.60 signal (dd; 8; 2 Hz). Signal 3 aromatic protons have an ABX system, and the compounds are hydroxy groups at positions 3 and 4, as shown in Figures 4-10.

The form of the above signal is still unclear because the signal is overlapping and has not been expanded. H-NMR measurement results

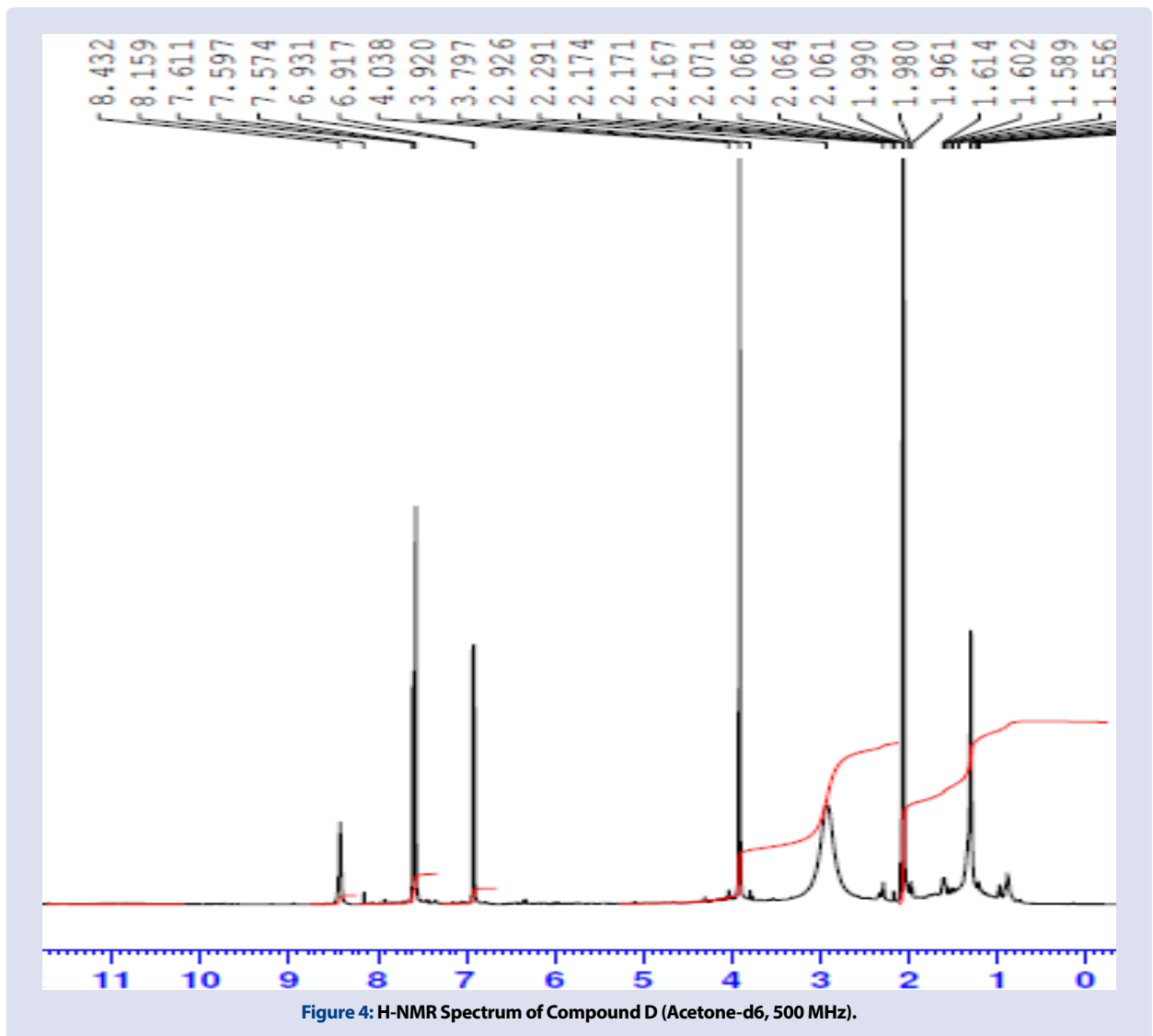
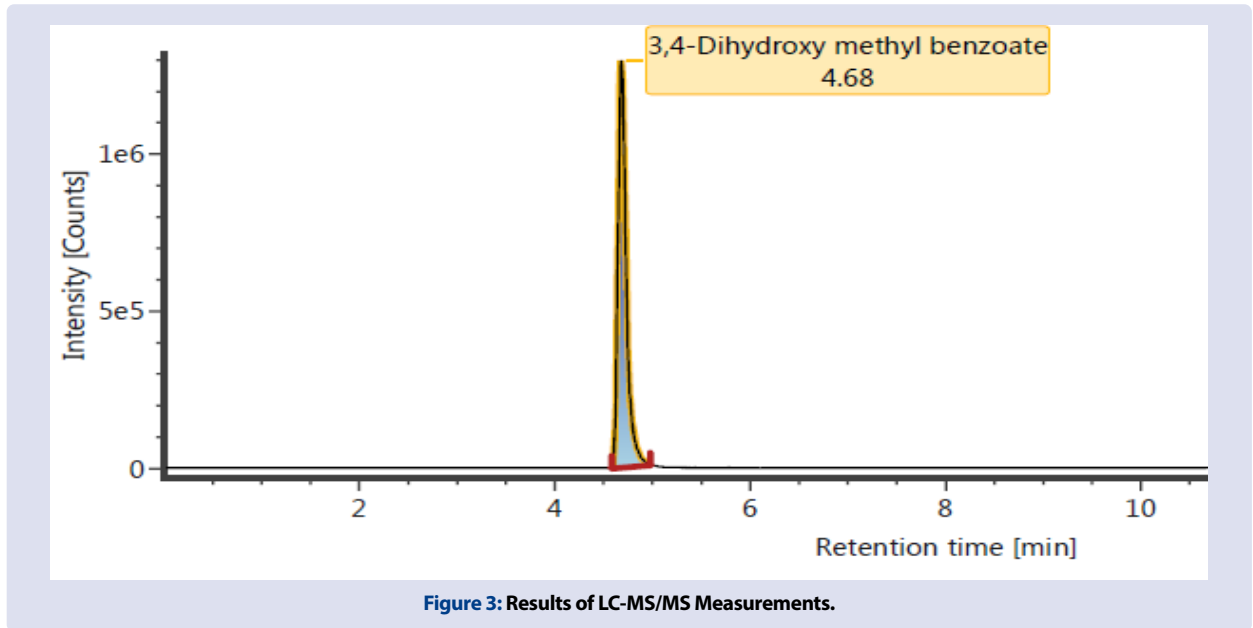
($CDCl_3$, 500 MHz) show clearer signals where at 6.96 (d, 8 Hz), 6.96 (d, 2 Hz) and 7.70 (dd, 8; 2 Hz).

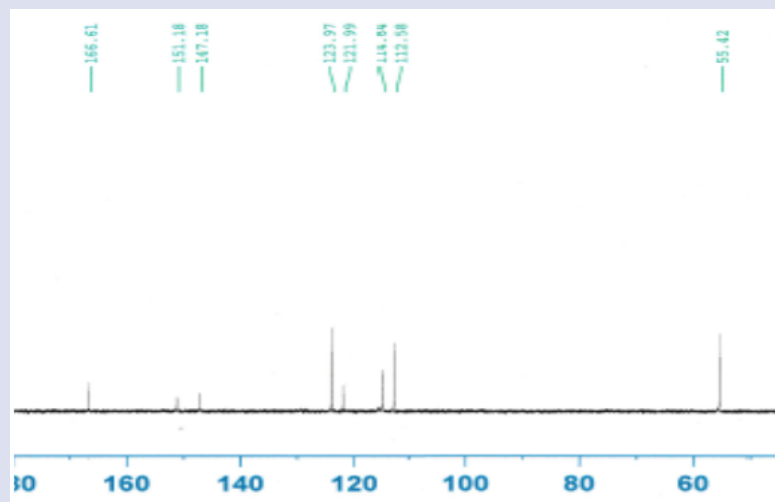
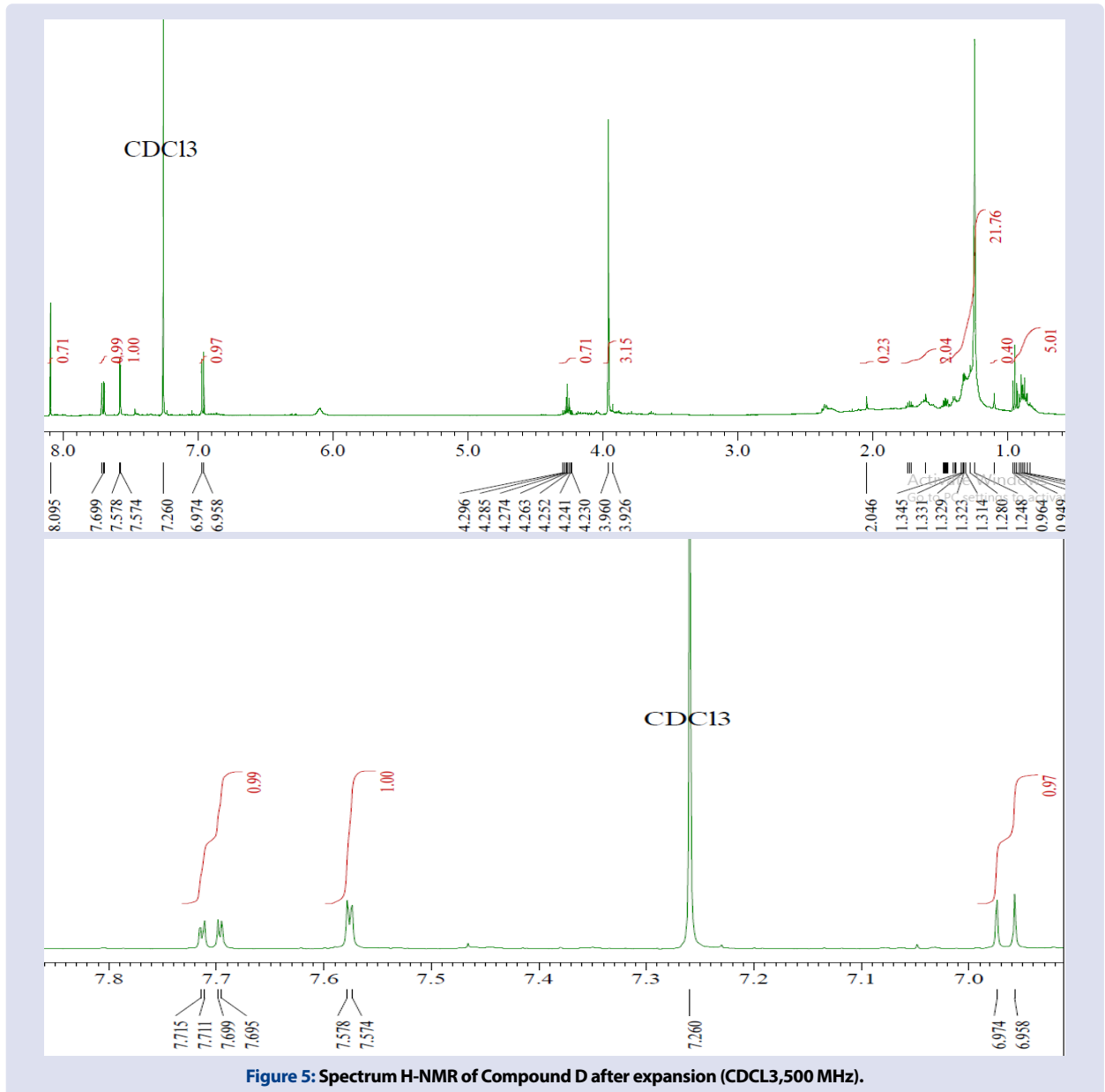
The above assumption is reinforced by the results of the C-NMR measurement, namely the presence of a carboxylic-COOH group appearing at 166.57 ppm, the presence of 2 x C-OH appearing at 147.18 and 151.18.

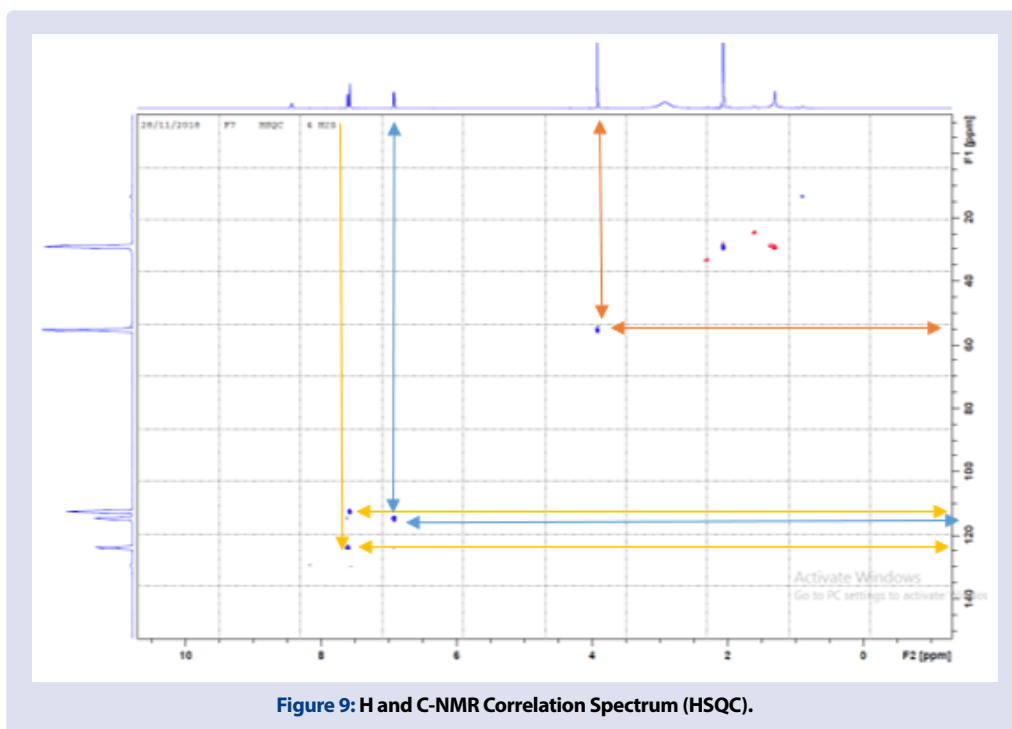
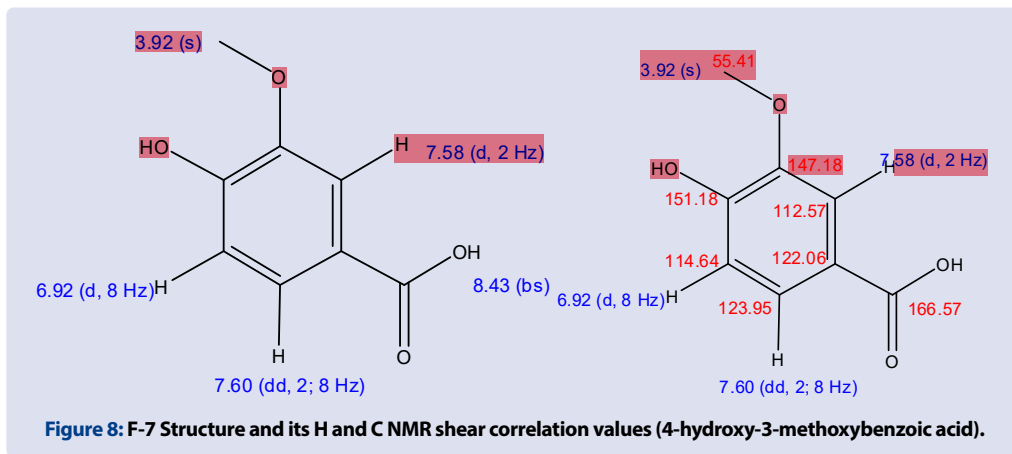
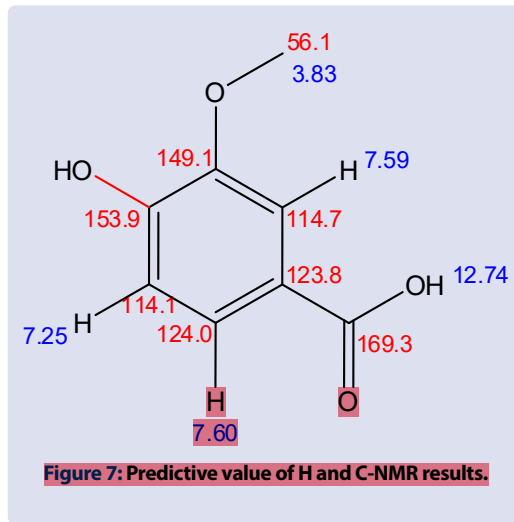
And by predicting H and C-NMR values, the correlation of H and C can be determined and also supported by the 2D-NMR spectrum (HSQC).

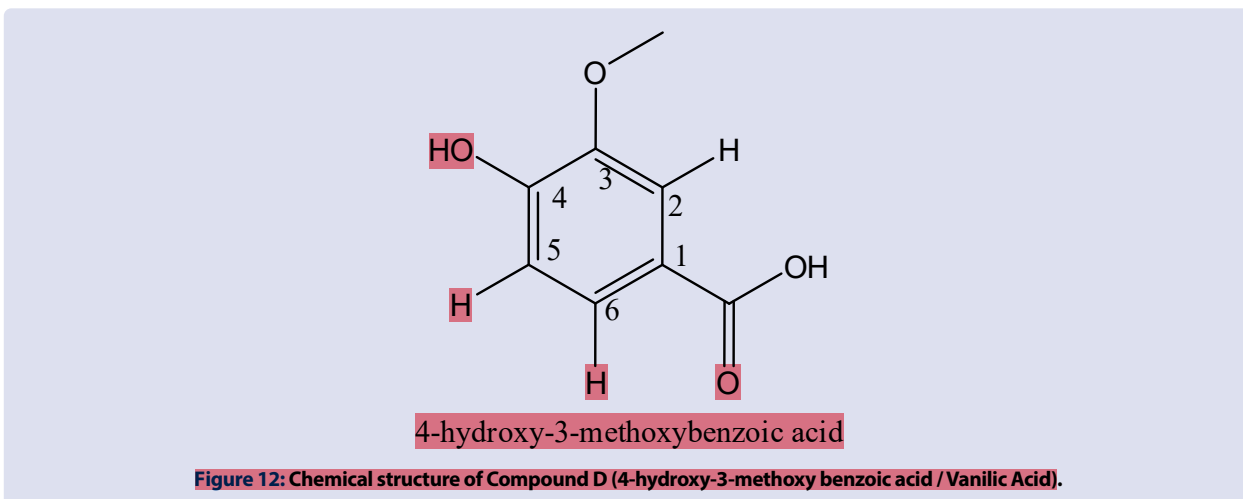
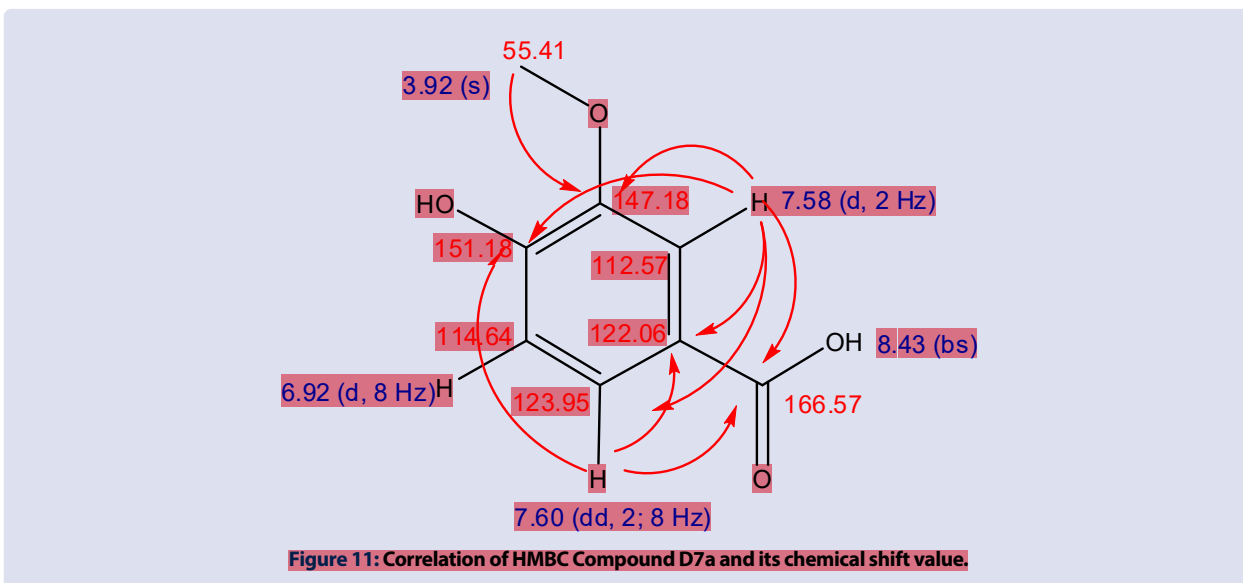
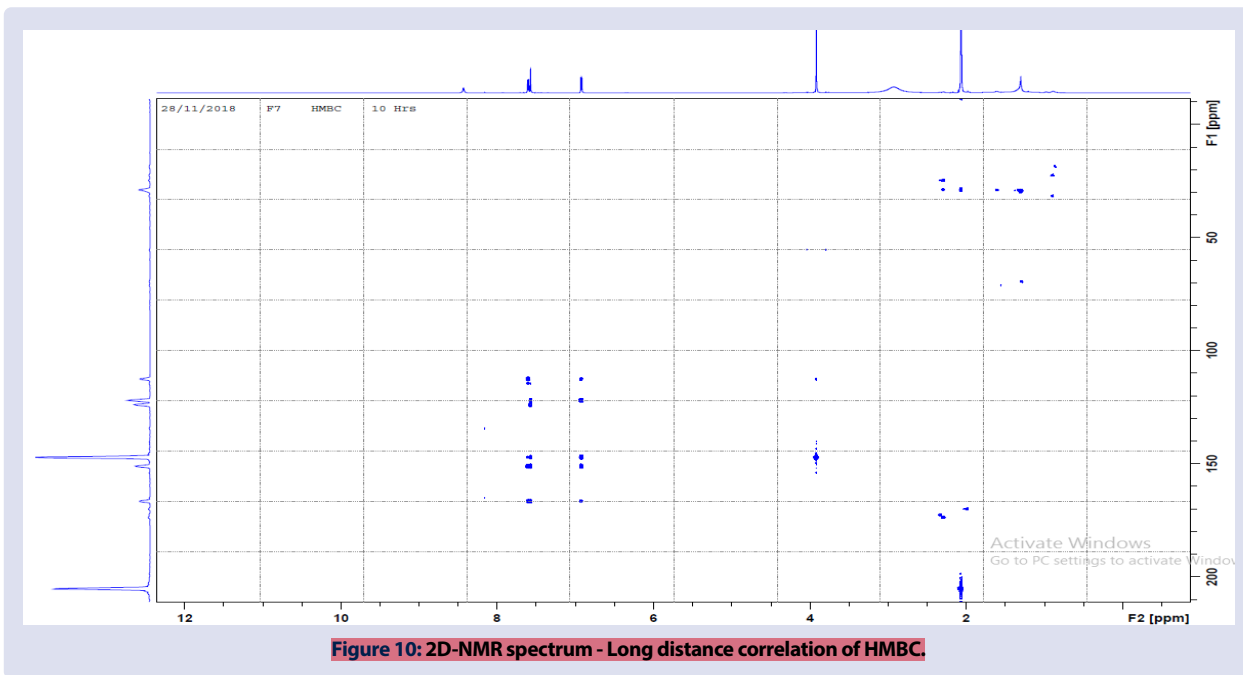
To determine the position of methoxy ($-OCH_3$) whether in C-3 or C-4, it can be ascertained by measurements of 2D-NMR - HMBC, long distance correlation (2-3 bonds).

On the HMBC spectrum, it is shown that the $-OCH_3$ position is located at C-3 due to the correlation between signal 3.79 (s) and signal C at the chemical friction of 147.18 and not with the chemical friction at 166.57 (-COOH). The signals at 6.92 (d) and 7.60 (dd) are close to -COOH with the HMBC correlation of 166.57 (Figures 11,12).









These results confirm that this compound is 4-hydroxy-3-methoxy benzoic acid, known as Vanillic acid (Fig. 12). This compound has never been isolated from *Garcinia* but has been isolated from the *Bistorta manshuriensis* plant⁷ and *Delonix regia* (Boj.) Raf roots (Family Fabaceae).⁸

Determination of Molecular Structure of Compounds I-1

Compound I-1 was obtained by preparative TLC by solvent n-hexane and ethyl acetate (9: 1), as much as 4 mg in the form of needle crystals. Chromatogram pattern of compound I-1 with stationary silica gel and eluent as mobile phase n-hexane: ethyl acetate: formic acid (9: 1: 0.3), R_f = 0.95; can be seen in Figures 13-15.

The H-NMR spectrum shows the specific signal of a β -sitosterol as the main component and stigmasterol as a minor component (3: 1), seen in the integrase ratio of 1: 0.73, of which 0.73 should be 2 H, so the ratio of the two compounds is 3: 1.

The specific signal at 5.34 (1H, d, 5 Hz) is a double bond signal at C-5, while the specific signal at 5.16 (dd, 15.5; 8.5 Hz) and 5.00 (dd; 8.5; 15.5

Hz) which is double bond H-C = C-H of C22-C23 but the integral value is only 0.73, so this is considered a minor component.

The signal at 3.52 (m) is an H-C-OH signal at C-3. For other characteristics that this compound is β -sitosterol, it can be seen in more detail the signal in the region of 1 ppm, where there are several methyl groups (s, d and t). Signals at 0.68 (3H, s), 0.81 (t, 6.5 Hz), 0.82 (d, 6.5 Hz), 0.84 (d, 6.5 Hz), 1.01 (s) and 0.88 (d, 6.5 Hz).

The C-NMR spectrum shows the characteristic of a mixed steroid of β -Sitosterol and Stigmasterol, where the double bonds at C-5-C6 appear at chemical fractions of 140.95 (= C =) and 121.92 (C-5, = CH), respectively. smaller than C22-C23 appeared at chemical fractions 138.51 (= CH) and 129.68 (= CH), while the hydroxy (-OH) group at C-3 appeared at 72.02.

Based on these results and compared with literature (Saputra, Handayani, & Wartono, 2016), that compound I-1 has the same chemical shear value with β -Sitosterol and Stigmasterol, which can be seen in Table 3. The structure of β -Sitosterol and Stigmasterol can be seen in Figure 16.

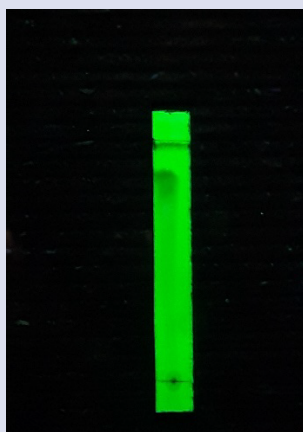


Figure 13: Pattern of chromatogram of compounds I-1.
Note: Observation of Compound I-1 under UV light 254, stationary phase: Silica gel GF254; Mobile phase: n-hexane: ethyl acetate: 9: 1: 0.3 formic acid

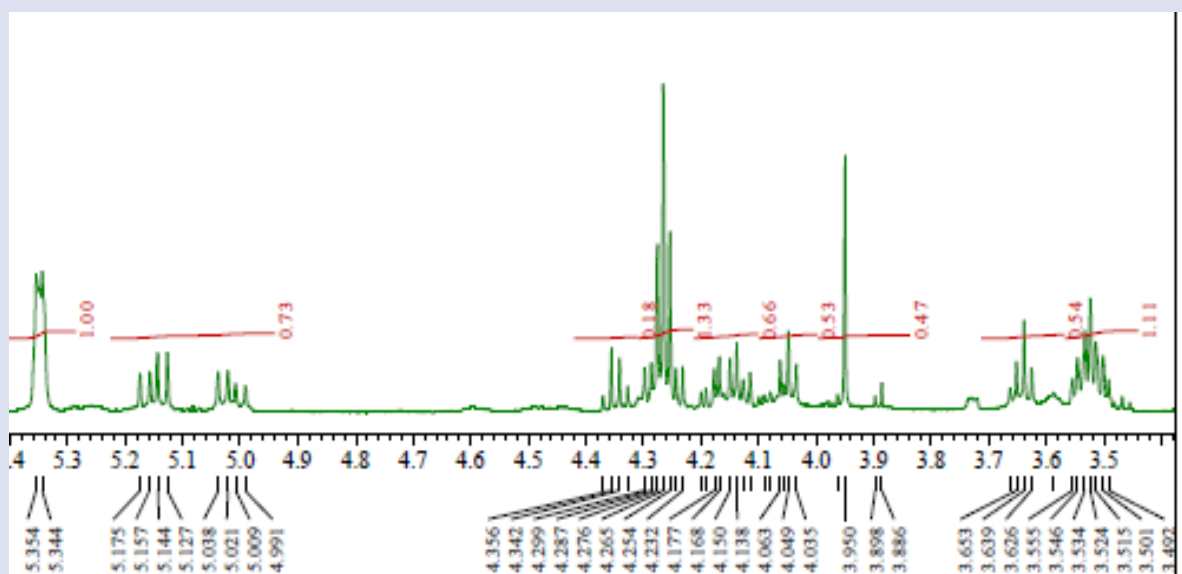


Figure 14: Spectrum H-NMR of Compound I-1.

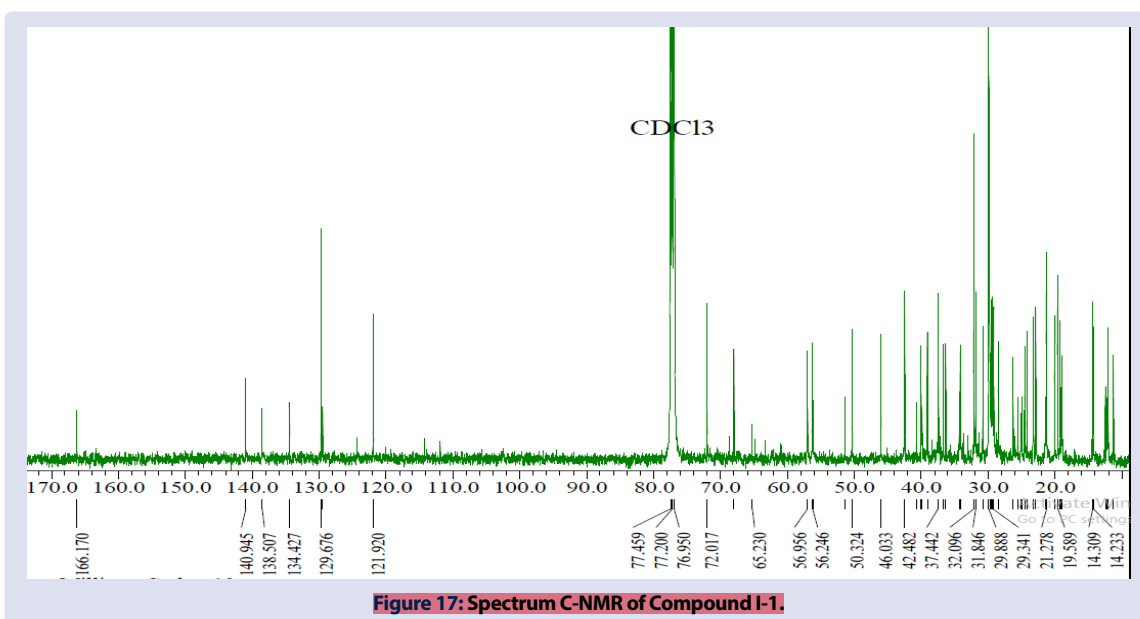
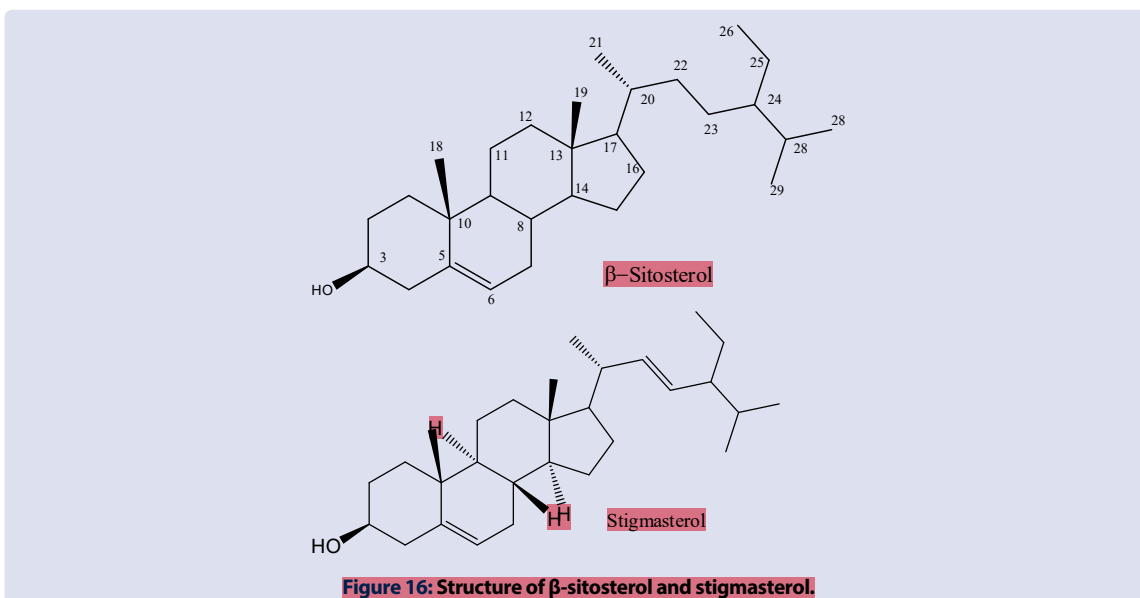
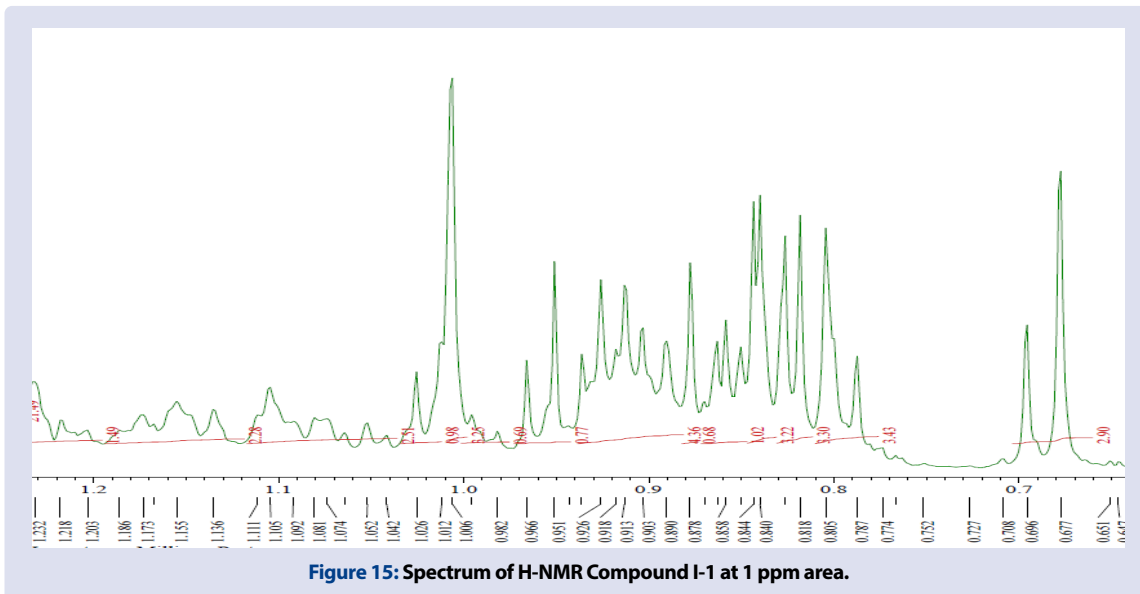


Table 3: Data of Chemical H and C-NMR Friction Value between Isolate I-1 and β -Sitosterol, Stigmasterol.^{9,10}

No	I-1a		β -Sitosterol		Stigmasterol		I-1 (b)	
	H-NMR	C-NMR	H-NMR	C-NMR	H-NMR	C-NMR	H-NMR	C-NMR
1	-	37.44	-	37.23	-	37.2	-	-
2	-	31.85	-	31.61	-	31.8	-	-
3	3.52 (m)	72.02	3.48 (1H, m)	71.78	3.50 (m)	71.8	3.64 (m)	-
4	-	42.48	-	42.45	-	41.3	-	-
5	-	140.95	-	140.71	-	140.7	-	-
6	5.34 (1H, d, 5 Hz)	121.92	5.37 (1H, m)	121.68	5.30 (1H, d, J = 4.5 Hz)	121.6	-	-
7	-	32.01	-	31.88	-	31.8	-	-
8	-	32.01	-	31.88	-	31.9	-	-
9	-	50.32	-	50.11	-	50.2	-	-
10	-	36.70	-	36.48	-	36.6	-	-
11	-	21.78	-	21.06	-	21.2	-	-
12	-	39.97	-	39.75	-	39.5	-	-
13	-	42.48	-	42.30	-	42.3	-	-
14	-	56.96	-	56.78	-	56.4	-	-
15	-	24.18	-	24.34	-	24.3	-	-
16	-	29.34	-	28.22	-	28.9	-	-
17	-	56.15	-	56.02	-	56.4	-	-
18	1.01 (s)	12.05	0.68 (3H, s)	11.84	0.68 (3H, s)	12.1	-	-
19	0.68 (s)	19.22	1.02 (3H, s)	19.27	-	19.5	-	-
20	-	36.34	-	36.12	-	40.6	-	-
21	0.88 (d, 6.5 Hz)	18.97	0.94 (3H, d, J = 6.6 Hz)	18.76	1.01 (3H, d, J = 7.4 Hz)	21.2	-	-
22	-	34.14	-	33.92	5.13 (1H, m)	138.3	5.00 (dd; 8.5; 15.5 Hz)	128.92
23	-	26.26	-	26.06	4.98 (1H, dd, J = 8.5, 15.2 Hz)	129.3	5.16 (dd, 15.5; 8.5 Hz)	138.51
24	-	46.03	-	45.81	-	51.3	-	-
25	-	29.89	-	29.13	-	31.8	-	-
26	0.82 (d, 6.5 Hz)	20.01	-	19.79	0.83 (3H, d, J = 5.6 Hz)	19.0	-	-
27	0.84 (d, 6.5 Hz)	19.59	-	19.02	0.77 (3H, d, J = 7.2 Hz)	21.3	-	-
28	-	23.26	-	23.05	-	25.5	-	-
29	0.81 (t, 6.5 Hz)	12.05	-	11.98	0.81 (3H, m)	12.2	-	-

CONCLUSION

Structural elucidation showed that isolate D was 4-hydroxy-3-methoxy benzoic acid (vanillic acid) and isolate I-1 was β -Sitosterol and Stigmasterol.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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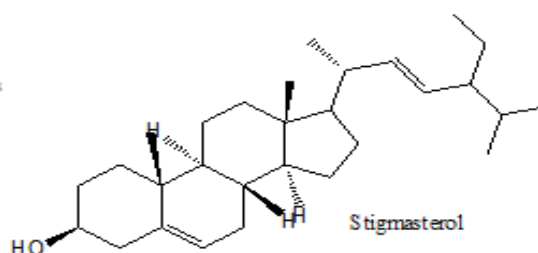
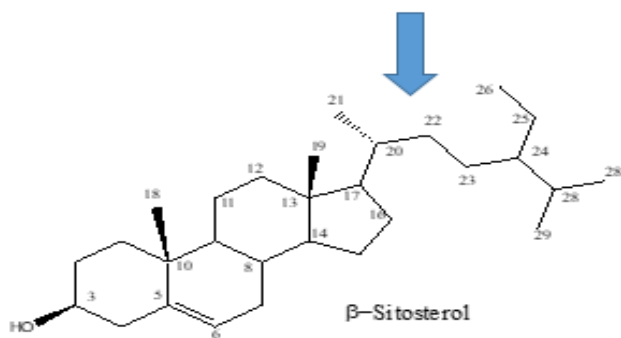
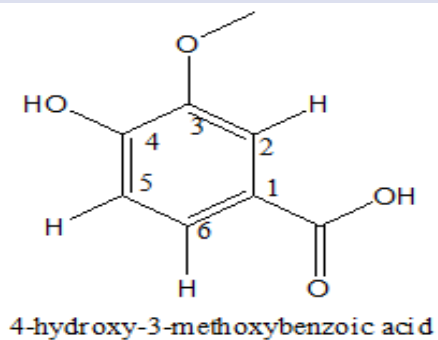
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GRAPHICAL ABSTRACT



Ethyl acetate extract of *Garcinia fruticosa* Lauterb. stem bark, Column chromatography, IR, LC-MS, ¹H-NMR, ¹³C-NMR, HMQC, HMBC.



SUMMARY

Compound D7a has a molecular weight 168.0496. The IR spectrum shows the presence of a group –OH appears on 3483 cm^{-1} , aromatic presence in 1609 cm^{-1} . The H-NMR spectrum shows the presence of aromatic signals on 6.96 (d, 8 Hz), 6.96 (d, 2 Hz) and 7.70 (dd, 8; 2 Hz). The C-NMR spectrum shows the presence of a carboxylic-COOH group appearing at 166.57 ppm, the presence of 2 x C-OH appearing at 147.18 and 151.18. In the HMBC spectrum, the $-\text{OCH}_3$ position is located at C-3 with a correlation between the 3.79 (s) signal and the C signal at the chemical shift 147.18. Structural elucidation shows that compound D7a is a 4-hydroxy-3-methoxy benzoate acid (Vanylic Acid) and isolate I-1 is an impure compound namely β -Sitosterol and Stigmasterol.

ABOUT AUTHORS



Novia Delita, S.Farm., Apt.

Master Student at Biology Pharmacy, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia.

The master research focus on natural medicine development as isolation and identification of chemical compounds from *Garcinia fruticosa* Lauterb stem bark extract.

Lecturer and researcher at Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. Dr. Hamka, Klender, Jakarta 13460, Indonesia.



Prof. Dr. Berna Elya, M.Si., Apt.

Professor and Head of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia (UI) Depok, West Java, Indonesia. She is expert in the area of Pharmacognosy and Phytochemistry, working in drug discovery of herbal plants, extraction technology, structure elucidation, and degenerative disease such as diabetes mellitus.



Prof. Dr. Muhammad Hanafi, MSc.

Professor Research in Research Centre for Chemistry – Indonesian Institute of sciences (LIPI).

And Lecturer in Faculty of Pharmacy, Universitas Pancasila, Jakarta, Indonesia. Expert in Drug Discovery and Development, especially in structure elucidation of active compounds and interest in Medicinal Chemistry.

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