



Two tetrahydroxyterpenoids and a flavonoid from *Xylocarpus moluccensis* M.Roem. and their α -glucosidase inhibitory and antioxidant capacity

[Dos tetrahidroxiterpenoides y un flavonoide de *Xylocarpus moluccensis* M.Roem. y su capacidad inhibidora y antioxidante de la α -glucosidasa]

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Abstract

Context: Scientific verification of the anti-hyperglycemic potential of Kayu sarampa (*Xylocarpus moluccensis*), which is popularly used for diabetic medication in North Sulawesi, Indonesia, is strongly important to conduct to avoid inappropriate medication that might cause worse conditions.

Aims: To investigate the anti-hyperglycemic potential of *X. moluccensis* stem bark ethyl acetate extract and isolated compounds through the α -glucosidase enzyme inhibitory and antioxidant capacity determination.

Methods: The active compound was isolated from ethyl acetate extract of *X. moluccensis* stem bark. The structures of those compounds were elucidated by infrared spectroscopy, liquid chromatography-mass spectrometry, and nuclear magnetic resonance spectroscopies. To discover the anti-hyperglycemic potency, α -glucosidase enzyme inhibition assay, antioxidant assay (DPPH and FRAP), and *in silico* study were performed.

Results: Three isolated compounds were obtained and characterized as two tetrahydroxyterpenoids compounds, namely xylocensin E (**1**) and ruageanin D (**2**), and one flavonoid compound, namely 3,5,7,3',4'-pentahydroxyflavan (catechin) (**3**). Compounds **1** and **3** inhibited the α -glucosidase enzyme with IC₅₀ values of 118.60 and 55.19 μ g/mL, respectively, with a competitive inhibition mechanism. This is also in line with *in silico* findings, and both compounds showed good binding affinity of α -glucosidase protein, which indicated their anti-hyperglycemic activity potential by inhibiting α -glucosidase enzyme. Moreover, compounds **1** and **3** showed antioxidant capacity through the DPPH method with an IC₅₀ value of 54.69 and 2.87 μ g/mL. In addition, 100 μ g/mL of compounds **1** and **3** also exhibited antioxidant capacity through the FRAP method with values of 66.35 and 213.82 μ mol Fe²⁺/g, respectively.

Conclusions: The *X. moluccensis* stem bark ethyl acetate extract, and isolated compounds exhibit α -glucosidase enzyme inhibitory activity and antioxidant capacity, which confirms its potency as an alternative medication for hyperglycemia issues.

Keywords: anti-hyperglycemia; α -glucosidase; antioxidant; natural products.

Resumen

Contexto: La verificación científica del potencial anti-hiperglucémico de Kayu sarampa (*Xylocarpus moluccensis*), que se utiliza popularmente para la medicación de los diabéticos en el norte de Sulawesi, Indonesia, es muy importante para evitar una medicación inadecuada que podría causar peores condiciones.

Objetivos: Investigar el potencial anti-hiperglucémico del extracto de acetato de etilo de la corteza del tallo de *X. moluccensis* y de los compuestos aislados mediante la determinación de la capacidad antioxidante e inhibidora de la enzima α -glucosidasa.

Métodos: Se aislaron los compuestos activos del extracto de acetato de etilo de la corteza del tallo de *X. moluccensis*. Las estructuras de esos compuestos se elucidaron mediante espectroscopía infrarroja, cromatografía líquida-espectrometría de masas y espectroscopía de resonancia magnética nuclear. Para descubrir la potencia anti-hiperglucémica, fueron realizados un ensayo de inhibición de la enzima α -glucosidasa, un ensayo antioxidante (DPPH y FRAP) y un estudio *in silico*.

Resultados: Se obtuvieron y caracterizaron tres compuestos aislados: dos compuestos tetrahidroxiterpenoides, a saber, xiloccensina E (**1**) y ruageanina D (**2**), y un compuesto flavonoide, a saber, 3,5,7,3',4'-pentahidroxiflavano (catequina) (**3**). Los compuestos **1** y **3** inhibieron la enzima α -glucosidasa con valores de IC₅₀ de 118,60 y 55,19 μ g/mL, respectivamente, con un mecanismo de inhibición competitiva. Esto también coincide con los hallazgos *in silico*, y ambos compuestos mostraron una buena afinidad de unión con la proteína α -glucosidasa, lo que indica su potencial actividad anti-hiperglucémica mediante la inhibición de la enzima α -glucosidasa. Además, los compuestos **1** y **3** mostraron capacidad antioxidante mediante el método DPPH con un valor IC₅₀ de 54,69 y 2,87 μ g/mL. Además, 100 μ g/mL de los compuestos **1** y **3** también mostraron capacidad antioxidante mediante el método FRAP con valores de 66,35 y 213,82 μ mol Fe²⁺/g, respectivamente.

Conclusiones: El extracto de acetato de etilo de corteza de tallo de *X. moluccensis* y los compuestos aislados exhiben actividad inhibidora de la enzima α -glucosidasa y capacidad antioxidante, lo que confirma su potencia como medicamento alternativo para problemas de hiperglucemia.

Palabras Clave: anti-hiperglucemia; antioxidante; α -glucosidasa; productos naturales.

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INTRODUCTION

Ethnomedicine, particularly natural products derived from medicinal plants, is widely utilized to cure health problems, particularly in developing countries (Mahapatra et al., 2019). For instance, coastal people who live near the mangrove forest utilize mangroves as traditional medicine. The pharmacological properties exhibited by mangrove plants arise from the secondary metabolites that are naturally synthesized to protect against the undesirable environment (Simlai and Roy, 2013). Kayu Sarampa (*Xylocarpus moluccensis* M.Roem., family *Meliaceae*) is a mangrove plant that can be found along the coasts of several Asian countries. It also exhibits wide pharmacological properties such as antibacterial, antioxidant, antitumor, antidiarrheal, and antidepressant. Thus, it has traditionally been used to cure some health issues like malaria, fever, joint pain, and headaches (Uddin et al., 2005; Raja and Ravindranadh, 2014). Several recent investigations have investigated the bioactivity of *X. moluccensis*, such as bark, fruit, leave, and seed (Islam et al., 2020).

People in Ratahan, North Sulawesi, Indonesia, use the *X. moluccensis* plant as an antidiabetic for handling hyperglycemic issues. Many people in developing countries believe that traditional medicine remedies provide free side effects. Moreover, the limited access to sufficient drugs forces them to utilize herbal medicine, which is convenient to find as an alternative medicine. Conversely, the lack of standardization and quality control in the application of traditional medicine might raise problems, including incorrect prescription, which can introduce serious side effects (Ekor, 2014). Hence, a comprehensive scientific study should be conducted to prove the potential of traditional medicine. Thus, the effect of irrelevant medication can be avoided. This effort also contributes to discovering the biodiversity potential, particularly for medical applications.

In terms of suppressing hyperglycemia issues, the utilization of α -glucosidase inhibitors, which have competitive action to reduce carbohydrate absorption, has been approved by the Food and Drug Administration (FDA) for the treatment of type 2 diabetes mellitus (Gao et al., 2018). However, it has gastrointestinal disturbances effects and induces worsened hypoglycemia. Moreover, a strict restriction was also applied for α -glucosidase inhibitors administration for individuals with gastrointestinal disease, renal impairment, and hepatic dysfunction (Rodbard et al., 2009). According to those issues, the application of herbal medication, which can also be administrated as a supplement diet, will strongly pose a promising

solution for the α -glucosidase inhibitors alternative with lower side effects.

Furthermore, hyperglycemia induces the formation of free radicals through auto-oxidation of glucose. The high free radical formation, which endogenous antioxidant defenses cannot handle, leads to diabetic complications, including macro- and microvascular dysfunction, neuropathy, nephropathy, retinopathy, and stroke (Asfandiyarova et al., 2007; Phillips et al., 2004). Several antioxidants have been reported to be effective in reducing diabetic complications. Additionally, traditional medicine, particularly derived from plants, has been well known to contain a wide range of antioxidant compounds (Xu et al., 2017). Therefore, the herbal medicine can be utilized as an antioxidant supplement.

Srivastava et al. (2014) reported that the fruit extract of *X. moluccensis* exhibited bioactivity as an α -glucosidase enzyme inhibitor. Furthermore, a previous study (Budiarso et al., 2020) found that a 100 μ g/mL ethyl acetate extract of *X. moluccensis* stem bark inhibited the α -glucosidase enzyme more effectively than the control acarbose, with values of 49.7% and 29.32%, respectively. It also demonstrated antioxidant capacity, as evidenced by a DPPH IC_{50} of 34.02 μ g/mL and a FRAP antioxidant power of 131.84 mol Fe^{2+} /g. This finding suggests that the ethyl acetate extract of *X. moluccensis* stem bark has antidiabetic potential. As a result, this study conducted extension research to assess bioassay-guided fractionation of *X. moluccensis* stem bark extract with α -glucosidase enzyme inhibitory activity. In addition to elucidating more information about the active compound on potential fraction, we also conducted compound isolation and structure characterization. Moreover, to strengthen our hypothesis, we also performed the α -glucosidase enzyme inhibition assay, antioxidant capacity assay, and *in silico* molecular docking of the isolated compounds from the potential fraction of ethyl acetate extract of *X. moluccensis* stem bark.

MATERIAL AND METHODS

General experimental procedures

IRPrestige-21 Shimadzu was employed to analyze the infrared spectroscopy (FTIR) spectrum. JEOL JNM-ECZ500R/S1 instrument was used to record the 1H and ^{13}C nuclear magnetic resonance (NMR) spectrum at 500 MHz. Waters liquid chromatography-mass spectrometry (UPLC-MS) XEVO G2-XS QT instrument, a Versamac microplate reader, and a Bio-Tek EL800 microplate reader were used for additional analyses.

Plant material

Xylocarpus moluccensis stem bark was collected from Ratatotok Subdistrict, North Sulawesi (GPS coordinate in -6.494370233786004, 106.85126425433128), and identified as specimen number 967/IPH.1.01/If.07 in Herbarium Bogoriensis, Indonesian Institute of Sciences. Herbarium Bogoriense is one of the renowned herbariums in Southeast Asia, with about one million specimens of vascular plants, ferns, fungi, mosses, lichens, and macroalgae. The Herbarium is located in Bogor, Indonesia.

Extraction and isolation

Reflux equipment with gradient solvents (n-hexane and EtOAc) was applied to extract 2000 g of air-dried powdered stem bark from *X. moluccensis* to produce the various extracts, which were subsequently evaporated using a rotary evaporator and vacuum oven. Following partitioning on silica gel chromatography with a gradient of hexane-ethyl acetate and ethyl acetate-methanol systems, the concentrated ethyl acetate extract (25 g) produced 10 fractions (A-J). All fractions were submitted for α -glucosidase inhibition and antioxidant activities. Furthermore, isolation from the fraction was carried out by considering the fraction with the biggest weight (D fraction) as well as the fraction with the highest α -glucosidase inhibition and antioxidant activities (F fraction). D fraction was recrystallized to obtain two isolated compounds, **1** (45 mg) and **2** (25.27 mg). Meanwhile, the F fraction was fractionated again using Sephadex LH-20 and chloroform-methanol (1:1) to obtain isolated compound **3** (91.20 mg).

Evaluation of α -glucosidase inhibitory activity

Using an *in vitro* assay, all fractions and isolated compounds were evaluated for their potential as the α -glucosidase inhibitor. The procedure refers to Kim et al. (2009). Samples were made into five variant concentrations (from 5 to 100 μ g/mL) in a volumetric flask, and then 30 μ L of each concentration was mixed with 36 μ L phosphate buffer pH 6.8 and 17 μ L p-nitrophenil- α -D-glucopyranoside 5 mM. Additionally, the combined solution was incubated at 37°C for 5 min before being added to 17 μ L of α -glucosidase 0.15 unit/mL. Then, the incubation was continued for 15 minutes at 37°C. To terminate the enzymatic reaction, 100 μ L of Na₂CO₃ 200 mM was added to the solution once the second incubation was complete. Furthermore, solution absorbance was calculated at 405 nm using a microplate reader. In this study, acarbose was employed as a standard. Three replicates of the experiment were carried out. The IC₅₀ was established as the concentration that inhibits 50% of α -glucosidase activity.

Kinetics of α -glucosidase inhibitor

The isolated compounds with high α -glucosidase inhibition activity were selected and determined for their kinetics in inhibiting α -glucosidase activity by measuring the p-nitrophenil- α -D-glucopyranoside concentration. Furthermore, the Lineweaver-Burk plots were employed to determine the type of inhibition and to calculate the Michaelis-Menten kinetic constant (Elya et al., 2012).

Antioxidant capacity assay with DPPH method

The DPPH assay was conducted using modified methods (Burda and Oleszek, 2001). Fractions and isolated compounds (5 mg) were dissolved in 5.0 mL of methanol to obtain a 1000 μ g/mL concentration. Then, each test solution was prepared in graded concentrations of 20, 40, 60, 80, and 100 μ g/mL. The experiment was conducted in triplicate. Next, 0.5 mL of test solution and 1.5 mL of 150 mol/mL DPPH were mixed, shaken until homogeneous, and incubated at room temperature in a dark room for 30 minutes. Then, the absorbance was determined at 517 nm. In addition, ascorbic acid served as the positive control. The antioxidant capacity was determined as in [1]:

$$AC (\%) = \frac{A_c - A_s}{A_c} \times 100\% \quad [1]$$

Where: AC: Antioxidant capacity; A_c: Control absorbance; A_s: Sample absorbance

Furthermore, the IC₅₀ of the antioxidant capacity was determined using a linear regression equation.

Antioxidant capacity assay with FRAP method

The FRAP assay was carried out following the procedures outlined by Bhagat et al. (2019). 100 μ L of each fraction and the isolated compounds were reacted with 3 mL of FRAP reagent, then the absorbance was determined at 593 nm. FeSO₄·7H₂O methanol solutions with 100 to 500 μ M concentrations were prepared and utilized to create a Fe²⁺ concentration calibration curve. The equivalent concentration parameter was identified as the concentration of antioxidants with the ability to reduce iron TPTZ equivalent to 1 mM FeSO₄·7H₂O.

Molecular docking experiment

The *in silico* study aims to predict the potential of isolated compounds to inhibit the α -glucosidase enzyme as additional data. The ligands used in the study were xylocensin E and catechin (positive control) obtained from PubChem (<http://pubchem.ncbi.nlm.nih.gov/>), and castanospermine (as a native ligand for α -glucosidase with PDB ID: 5IED). The docking procedure has been de-

scribed previously by Gani et al. (2021). Briefly, all ligand structures were designed and optimized using ChemDraw version 15.0.0.106 and MMFF94 Minimization in Chem3D version 15.0.0.106, then saved in MOL2 format. The protein for docking used in this study was α -glucosidase II (PDB ID: 5IED), obtained from the Protein Data Bank (<https://www.rcsb.org/>). Before running the molecular docking, the protein was prepared by removing water molecules, introducing a Kolman charge, and repairing the missing atoms, then saved in PDBQT format in AutoDockTools version 1.5.6.

The molecular docking procedure was processed using Inter (R) Celeron (R) 2955U @ 1.40 GHZ processor, RAM 2.00 GB system type 64-bit Operating System. The grid box used for docking was 40 Å × 40 Å × 40 Å centered at 4.601, 13.459, and 6.89. The docking procedure was validated by calculating the root mean square deviation (RMSD) of the protein with its co-crystalline ligand using PyMOL software version 2.3.4 (Schrödinger LLC). Finally, the interactions between protein and ligand were visualized using Discovery Studio Visualizer v17.2.016349 (Gani et al., 2021).

Statistical analysis

The data were obtained by three replications. Hence it was represented as mean $\pm$ standard deviation (SD). All the statistical analysis were processed using Microsoft Excel. Moreover, to compare the means of two independent groups, we conducted a two-sample t-test using the TTEST function in Microsoft Excel, with a significance level of 0.05 ($p < 0.05$). In addition, to analysis the inhibition kinetics mechanism, the Michaelis-Menten kinetic constant and Lineweaver-Burk plot were also calculated and arranged using Microsoft Excel.

RESULTS

α -Glucosidase inhibition and antioxidant capacity of *Xylocarpus moluccensis* stem bark ethyl acetate extract fractions

Using chromatographic techniques, 25 g of concentrated *X. moluccensis* stem bark ethyl acetate extract was divided into ten fractions (A-J). In addition, the fractions' weight after solvent evaporation and the α -glucosidase inhibition activity of each fraction are shown in Table 1. The result shows that the F fraction had the highest percent inhibition of 72.58%, followed by the I fraction at 61.86% inhibition, the D fraction at 53.78%, and the control acarbose at 48.33%.

In addition, the antioxidant capacity test was carried out at a fraction concentration of 100 μ g/mL using two methods, consisting of the DPPH and FRAP methods. The findings (Table 2) exhibit that the % antioxidant capacity of the 10 fractions (A-J) ranging from 1.77% to 81.15%, and the antioxidant power of the fractions ranged from 27.70 to 301.63 μ mol Fe²⁺/g. The results of both DPPH and FRAP methods demonstrated that the F fraction showed the best antioxidant capacity among all fractions, since it had antioxidant activities of 81.15% and 301.63 μ mol Fe²⁺/g, respectively. In addition, the antioxidant capacity of the F fraction is slightly lower compared to the control ascorbic acid.

Structure characterization of isolated compounds

Due to its higher α -glucosidase inhibition and antioxidant activities compared to other fractions, the F fraction was further separated using column chromatography to isolate the active compound. Then, compound **3** was isolated from the F fraction. In addition, the D fraction was also recrystallized because it had the biggest fraction weight. From the D fraction, compounds **1** and **2** were obtained. The structure of three isolated compounds is shown in the Fig. 1.

Compound **1** (Fig. 2), characterized as xylococcins E (**1**), was characterized as white needles, mp 216–218°C. In the LC-MS-MS spectrum, a molecular ion peak was present at m/z 687.69 [M+H]⁺ (calc. for 686.69), calculated masses for C₃₅H₄₂O₁₄. IR spectrum revealed CO-group (1750.1 cm⁻¹) and furan ring (870.1 cm⁻¹).

The ¹H-NMR spectrum of compound **1** revealed signals for four methyl groups singlet at δ H = 0.88, 1.06, 1.14 and 1.67 ppm; three acetyl groups at δ H = 1.96, 1.63, and 2.68 ppm; methyl ester group at δ H = 3.69 (s); and the presence of protons attached to a double bond in the furan ring at δ H = 6.64 (d; J = 1.5 Hz), 7.52 (s), 7.41 (t, J = 1.5 Hz). The ¹³C-NMR and DEPT spectrums exhibited the existence of 35 carbons, including four methyl groups at δ C = 14.7, 16.7, 19.7, and 21.18 ppm; three acetyl groups at δ C = 21.4, 21.73, and 21.89 ppm; methyl ester group at δ C = 52.28 ppm; the carbonyl group of acetyl and ester at δ C = 168.73, 170.54, 170.58 and 172.82 ppm; a lactone group at δ C = 170.40 ppm; five methylene groups at δ C = 25.5, 26.8, 29.2, 33.4 and 40.2 ppm; eight methine groups at δ C = 35.5, 43.2, 69.4, 78.7, 81.1 ppm and δ C = 109.8, 140.8, 143.2 ppm located on the furan ring having oxygen atom; and nine quaternary carbons at δ C = 31.12, 46.3, 45.83, 85.33, 85.39, 86.01, 86.9, 119.1 and 121.1 ppm.

Table 1. Fraction weight and α-glucosidase inhibition activity of *X. moluccensis* stem bark ethyl acetate extract fractions and acarbose.

Sample	Fraction weight (g)	Inhibition (%)
Control (acarbose)	-	48.33
A	1.68	19.51
B	2.25	23.73
C	3.06	23.37
D	5.48	53.78
E	1.71	8.44
F	3.62	72.58
G	1.65	41.30
H	1.20	12.30
I	1.54	61.86
J	2.20	2.81

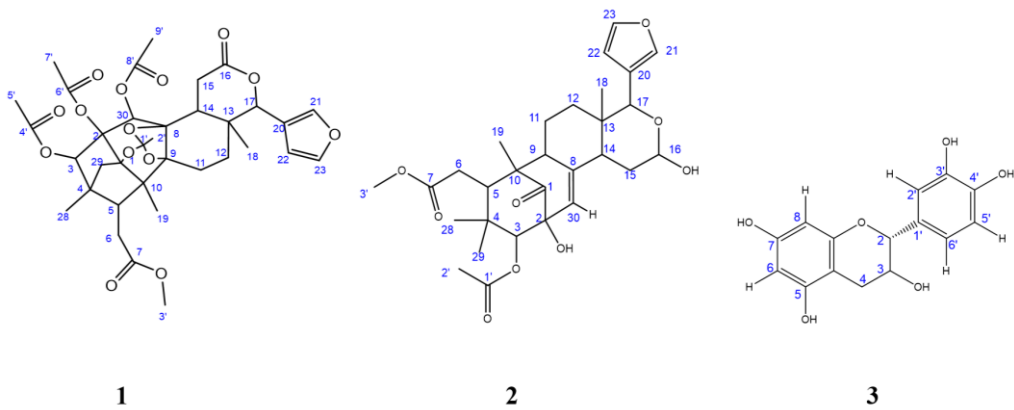
X. moluccensis stem bark ethyl acetate extract fractions and acarbose were tested at a concentration of 100 µg/mL.

Tabel 2. Antioxidant capacity of *X. moluccensis* stem bark ethyl acetate extract fractions and ascorbic acid.

Fraction	Antioxidant capacity with DPPH method (%)	Antioxidant power with FRAP method (µmol Fe ²⁺ /g)
Control (ascorbic acid)	85.64 ± 0.07	340.41 ± 0.39
A	6.42 ± 0.37	29.38 ± 2.65
B	9.75 ± 0.45	34.57 ± 2.06
C	8.07 ± 3.19	43.26 ± 1.84
D	49.71 ± 0.68	126.78 ± 4.49
E	1.77 ± 0.50	27.70 ± 1.62
F	81.15 ± 0.50	301.63 ± 3.40
G	12.92 ± 0.26	58.96 ± 3.40
H	71.44 ± 0.19	264.53 ± 1.70
I	69.84 ± 0.36	252.47 ± 2.43
J	11.28 ± 0.31	44.95 ± 0.81

X. moluccensis stem bark ethyl acetate extract fractions and ascorbic acid were tested at a concentration of 100 µg/mL.

Figure 1. Structures of compounds **1**, **2** and **3**.



lar formula of C₁₅H₁₄O₆. IR spectrum revealed the hydroxyl (-OH) group at Vmax 3295.0 dan 1290.1 cm⁻¹ (proton from alcohol) and the presence -COC- group at Vmax 1210.5 cm⁻¹.

Table 3. The comparison of ¹H-NMR and ¹³C-NMR data of compound **1** and xylocensin E (Sarigaputri et al., 2010).

No.	Compound 1		Xylocensin E		DEPT
	C NMR	H-NMR	C-NMR	H-NMR	
C-1	86.9		86.8		C quaternary
C-2	19.75	1.06 (s)	21.0		CH ₃
C-3	81.1	5.10 (s)	81.1	5.10 (s)	CH
C-4	46.3		46.2		C quaternary
C-5	35.5	2.98 (d, 8 Hz)	35.5	2.96 (d, 8.5 Hz)	CH
C-6	33.4	2.27 2.17	33.3	2.47 (m) 2.24 (m)	CH ₂
C-7	172.8		172.7		R-COOCH ₃
C-8	86.0		85.6		C quaternary
C-9	85.3		85.3		C quaternary
C-10	45.8		45.7		C quaternary
C-11	25.5	1.66 1.41	25.4	2.07 (m) 1.65 (m)	CH ₂
C-12	34.4	1.56 1.31		1.54 (m) 1.30 (m)	CH ₂
C-13	31.12		34.3		C quaternary
C-14	43.24	2.36	43.1		CH
C-15	34.47	2.48 2.23			CH ₂
C-16	170.4		170.4		C=O
C-17	78.7	5.55 (s)	78.6	5.54 (s)	CH
C-18	21.1	1.63 (s)			CH ₃
C-19	16.7	1.14 (s)	16.5	1.14 (s)	CH ₃
C-20	121.1		121.1		C quaternary
C-21	140.9	7.52 (s)	140.8	7.51 (d)	CH
C-22	109.8	6.44 (d, 1.5 Hz)	109.7	6.44 (s)	CH
C-23	143.2	7.41 (t, 1.5 Hz)	143.0	7.40 (s)	CH
C-28	14.73	0.88 (s)	14.6	0.89 (s)	CH ₃
C-29	40.29	1.65 1.40	40.2	1.98 (m) 1.67 (m)	CH ₂
C-1'	119.1		119.0		C quaternary
C-2'	21.2	1.27 (d)	21.1	2.25(s)	CH ₃
C-3'	52.8	3.69 (s)		3.69(s)	-OCH ₃
C-4'	170.54			170.2	C=O
C-5'	21.18	2.26 (s)	21.1	2.25	CH ₃ -CO
C-6'	170.58			172.7	CO
C-7'	22.1	2.16 (s)	21.0	1.66	CH ₃ -CO
C-8'	168.73			170.2	C=O
C-9'	21.4	2.17 (s)	21.6	1.94	CH ₃ -CO

Table 4. The comparison of ¹H-NMR and ¹³C-NMR data of compound **2** and ruageanin D (Mootoo et al., 1996).

No.	Compound 2		Ruageanin D		DEPT
	C-NMR	H NMR	C-NMR	H-NMR	
C-1	215.1		213.0		C=O
C-2	77.0		78.3		C-
C-3	85.0	4.85 (s)	84.7	5.14 (s)	CH
C-4	39.1		40.1		C quaternary
C-5	41.7	3.27	42.3	3.24	CH
C-6	32.8	2.35 (bd. 10 Hz)	32.9	2.34 (m)	CH ₂
C-7	174.0		173.8		C, COOCH ₃
C-8	136.6		139.7		C quaternary
C-9	56.4	2.25	55.1	1.90 (m)	CH
C-10	49.6		49.1		C
C-11	20.3	1.65 (m) 1.44 (m)	19.4	2.07 (m) 1.90 (m)	CH ₂
C-12	34.3	1.67 (m) 1.43 (m)	33.2	1.22 (m) 1.97 (m)	CH ₂
C-13	36.8		34.3		C quaternary
C-14	44.9	1.71	45.2	1.61 (13; 6 Hz)	CH
C-15	30.1	2.88 (m. 2 Hz) 2.86	33.4	2.83 (16; 6Hz) 3.50 (16; 13 Hz)	CH ₂
C-16	169.5		171.2		C=O
C-17	77.3	5.68 (s)	78.8	5.18 (s)	CH
C-18	22.1	1.08 (s)	26.2		CH ₃
C-19	15.9	1.22 (s)	16.1	1.00 (s)	CH ₃
C-20	120.7		120.1		C quaternary
C-21	142.0	7.79 (s)	140.8	7.49 (1.8 Hz, 0.8 Hz)	CH
C-22	109.8	6.45 (d, 1 Hz)	110.1	6.44 (1.8 Hz, 0.8 Hz)	CH
C-23	143.1	7.41 (t, 2Hz)	143,1	7.43 (1.8 Hz)	CH
C-28	20.6	0.75(s)	20,5	0.81 (s)	CH ₃
C-29	19.8	0.79 (s)	22,0	0.79 (s)	CH ₃
C-30	129.1	5.37 (t)	67.3	3.51 (s)	CH
C-1'	171.0		166.92		CO
C-2'	22.0	2.12 (s)	12.6	1.97	CH ₃
C-3'	52.4	3.71 (s)	52.3	3. 74 (s)	-OCH ₃

The ¹H-NMR of compound **3** exhibited one methylene group at δH = 2.86 (dd, 6; 16 Hz) and 2.53 (dd, 9; 16 Hz). Other specific characteristic spectra included the presence of an aromatic compound group (benzene ring) with one methine group revealed at δH = 5.92 (d, 2Hz) (δC = 96.24 ppm), 5.85 (d, 2 Hz) (95.56 ppm), 6.77 (d, 8.5 Hz); 6.72 (dd); 6.83 (d, 2Hz).

Corresponding ¹³C-NMR and the DEPT spectrum of compound **3** showed the existence of 15 carbon resonances. It showed the availability of a methyl carbon at δC = 28.65 ppm, aromatic compound group (benzene ring) with five methine groups at δC = 96.34, 95.56, 115.34, 116.16, and 120.14 ppm, hydroxyl (-OH) at δC = 157.02, 157.93, 146.35 and 157.02 and -

OH at $\delta_C = 68.90$ on the C ring, and quaternary carbon atoms at $\delta_C = 100.89, 132.28,$ and 157.69 ppm (Table 5).

Based on the HMQC and HMBC spectra of compound **3**, the position of the hydroxyl ring and two aromatic rings was determined to be the basic framework of flavonoids. Moreover, the spectrum revealed

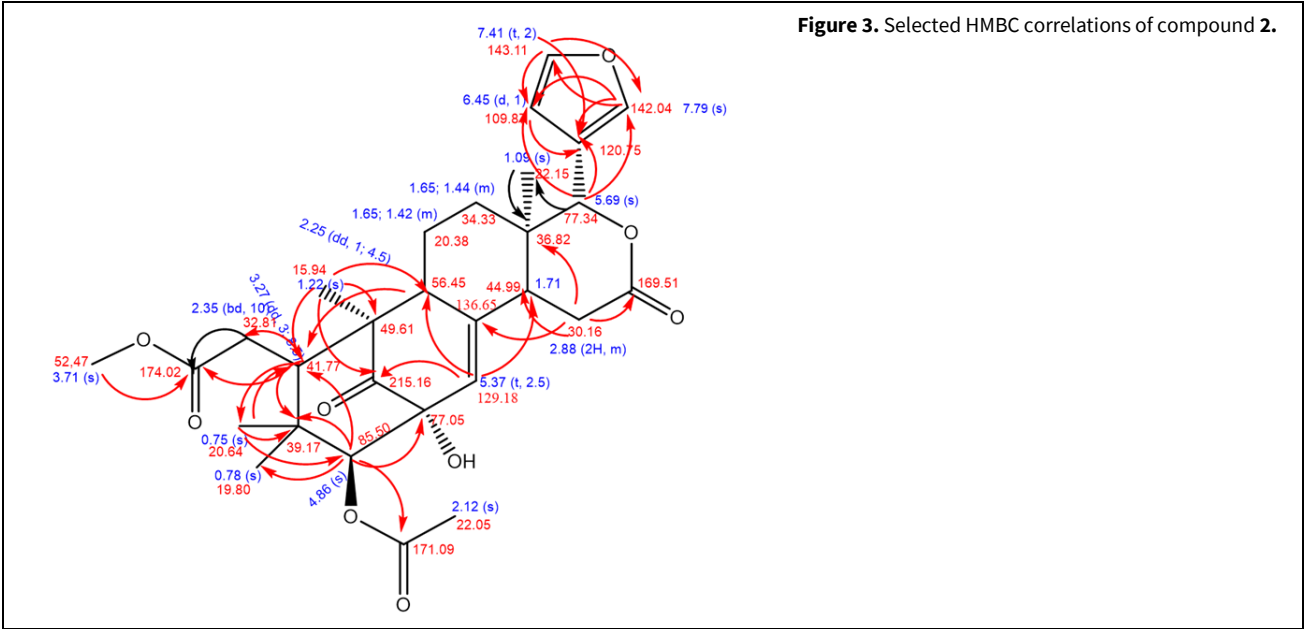


Figure 3. Selected HMBC correlations of compound **2**.

Table 5. The comparison of ^1H -NMR and ^{13}C -NMR data of compound **3** and catechin (Sarian et al., 2017).

No.	Compound 3		Catechin		DEPT
	C NMR	H NMR	C-NMR	H-NMR	
C-2	82.95	4.55 (d; 7,5 Hz)	81.78	3.20 (s)	CH
C-3	68.90	3.97 (q, 7,5 Hz)	67.44	2.91 (dd)	CH
C-4	28.65	2.53 (dd, 9; 16 Hz) 28.6 (dd, 6;16 Hz)	27.96	2.55 (dd)	CH ₂
C-5	157.02		156.36		OH
C-6	95.56	5.85 (d, 2 Hz)	95.30	4.58 (d)	CH
C-7	157.93		156.8		OH
C-8	96.34	5.92 (d, 2Hz)	94.48	4.01 (m)	CH
C-9	157.69		155.95		C quaternary
C-10	100.89		99.69	5.89 (d)	C quaternary
C-1'	132.28		131.16	6.90 (d, 1 Hz)	C quaternary
C-2'	115.34	6.83 (d, 2Hz)	114.37	6.75 (dd)	CH
C-3'	146.35		144.81	7.98 (d, OH)	OH
C-4'	157.02		144.87	8.29 (d, OH)	OH
C-5'	116.16	6.77 (d, 8.5 Hz)	114.80		C H
C-6'	120.14	6.72 (dd, 2; 8.5 Hz)	119.12		C H

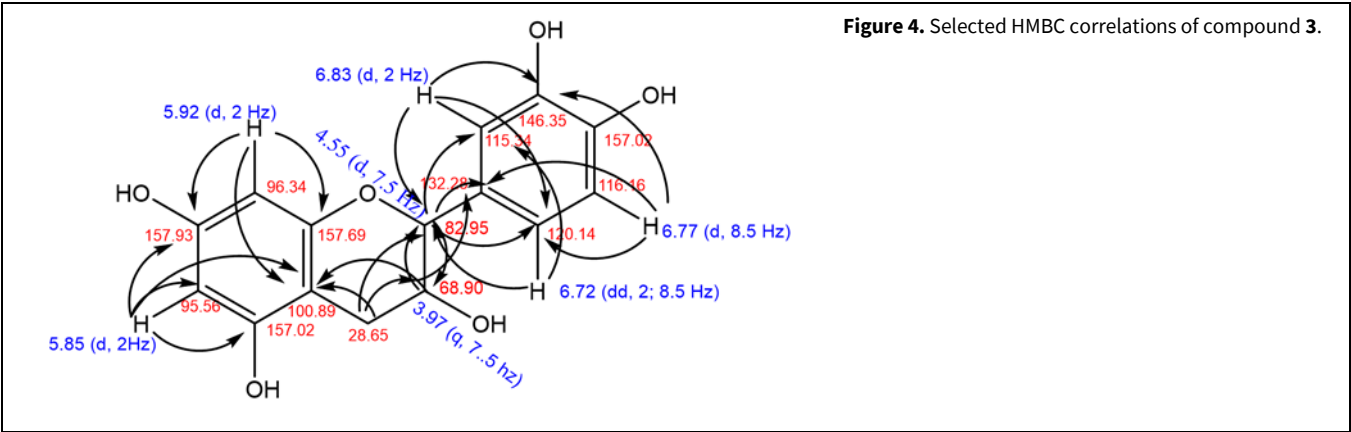


Figure 4. Selected HMBC correlations of compound **3**.

Table 6. Regression and IC₅₀ of alpha-glucosidase inhibition of compounds **1**, **2**, **3**, and acarbose.

Compound	Regression equation	R ²	IC ₅₀ (µg/mL)
Control (acarbose)	y = 0.2156x + 28.79	0.9886	98.36 ± 0.53
1	y = 0.3281x + 11.072	0.9813	118.60 ± 3.93
2	y = 0.2087x + 10.671	0.9228	188.44 ± 38.63
3	y = 0.1986x + 39.04	0.9611	55.19 ± 5.00***

***P<0.001 respect to the control group. IC₅₀ values are represented as mean ± SD (n = 3).

the presence of an aromatic group (benzene ring) with five methin groups at δH = 5.92 (d, 2Hz) (δC = 96.24 ppm), δH = 5.85 (d, 2Hz) (95.56 ppm) on the A ring, δH = 6.77 (d, 8.5 Hz) (δC = 116.16 ppm), δH = 6.72 (dd) (δC = 120.14 ppm), δH = 6.83 (d, 2Hz) (δC = 115.34 ppm) on the C ring; hydroxyl (-OH) at δC = 157.93 and 157.02 on the A ring and δC = 146.35, 157.02 on the C ring, and -OH at δC = 68.90 on the B ring; quaternary carbon atoms at δC = 100.89, 132.28, 157.69 ppm; and methyl groups at δH = 2.53 (dd, 9, 16 Hz) and 2.86 (dd, 6, 16 Hz).

From the comparison results of the chemical shift spectrum of ¹H-NMR and ¹³C-NMR, and 2D NMR, compound **3** was 3,5,7,3',4'-pentahydroxyflavan (catechin) (Fig. 4).

α-Glucosidase enzyme inhibition activity of isolated compounds

Compounds **1**, **2**, and **3** were tested for their potential to inhibit α-glucosidase at 20 to 100 µg/mL graded concentrations. The percent inhibition at each concentration was used to formulate the linear regression equation, which was then utilized to evaluate the IC₅₀. The IC₅₀ value of compounds represents their capability to block 50% of the α-glucosidase enzyme. The linear regression equation and the IC₅₀ of α-glucosidase inhibition activity of the compounds are served in Table 6. The results exhibited that the IC₅₀ of compound **3** had the highest activity to block the α-glucosidase enzyme compared to the acarbose. The

IC₅₀ of compound **1** (xylocensin E), compound **2** (runganenin D), and compound **3** (2',3,5,5',7 pentahydroxyflavan) are 118.6, 188.44, and 55.19 µg/mL; meanwhile, the control acarbose showed IC₅₀ = 98.36 µg/mL.

Inhibition kinetics mechanism

The kinetics examination was conducted to determine the inhibition type of an enzyme, whether the inhibition was competitive, non-competitive, or a combination of both. The inhibition type was performed using Lineweaver-Burk plots to obtain the Michaelis-Menten kinetic constant (Elya et al., 2012). The Michaelis-Menten kinetic constant was calculated using the regression equation y = a + b x, 1/[S] as the x-axis and 1/[V] as the y-axis (Murray et al., 2012).

Fig. 5 shows that there is an intersection between the sample (with inhibitor) and blank (without inhibitor) on the y-axis on the Lineweaver-Burk graph of both compounds **1** and **3**. This graph led to a competitive inhibition profile. It indicated that compounds **1** and **3** have similar working properties as substrates towards the action of the α-glucosidase enzyme.

Molecular docking

Different ligand binding energies of compounds were observed in the range of -6.3 to -8.4 kcal/mol (Table 7). The binding energy of acarbose (standard) was calculated as -7.0 kcal/mol. From this bioinformatics study, it was observed that compounds **1** (xy-

loccensin E) and **3** (catechin) showed strong binding affinity with α -glucosidase protein as evidenced by their low binding energies (-7.3 and -6.90 kcal/mol) with specific interaction as present in Fig. 6.

Antioxidant capacity of isolated compounds

Testing of antioxidant capacity using DPPH and FRAP was also carried out on compounds **1**, **2**, and **3**. The linear regression equation and the IC₅₀ of the DPPH assay of the compounds are presented in Table

8. Compound **3** (IC₅₀ = 2.88 μ g/mL) showed the highest antioxidant capacity compared to compounds **1** (IC₅₀ = 35.85 μ g/mL) and **2** (IC₅₀ = 2,348.08 μ g/mL). In addition, the result of the FRAP and DPPH assay of 100 μ g/mL isolated compound, which is represented in Table 9, also showed that compound **3** has the greatest antioxidant capacity compared to the other compounds. The antioxidant capacity of compound **3** is slightly lower than control ascorbic acid.

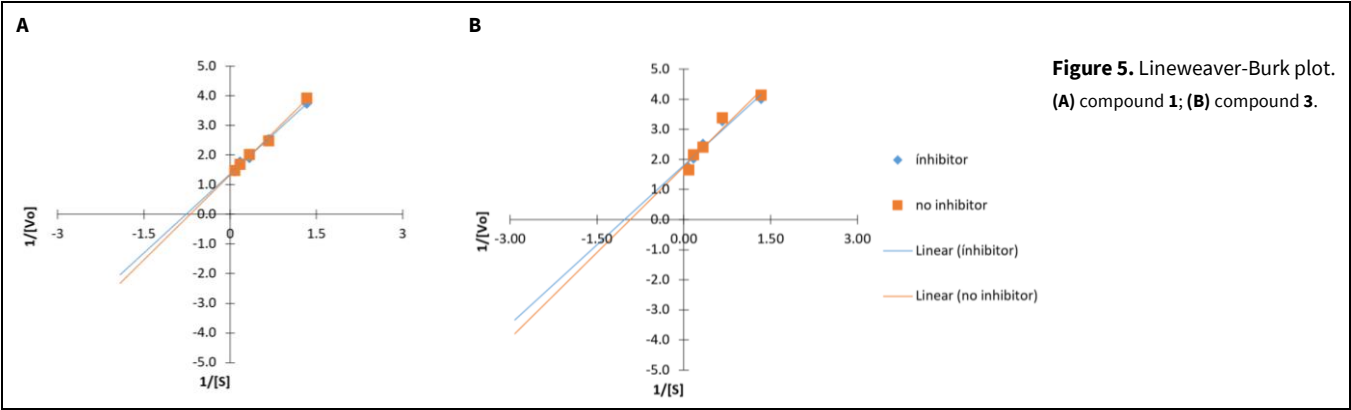


Table 7. Binding affinity of potential α -glucosidase inhibitors.

Ligand	Binding affinity (kcal/mol ⁻¹)
Xylocensin E	-7.3
Catechin	-8.4
Acarbose	-7.0
Castanospermine ^a	-6.3

^aCo-crystalline ligand for PDB ID: 5IED. Data was processed in AutoDockTools version 1.5.6.

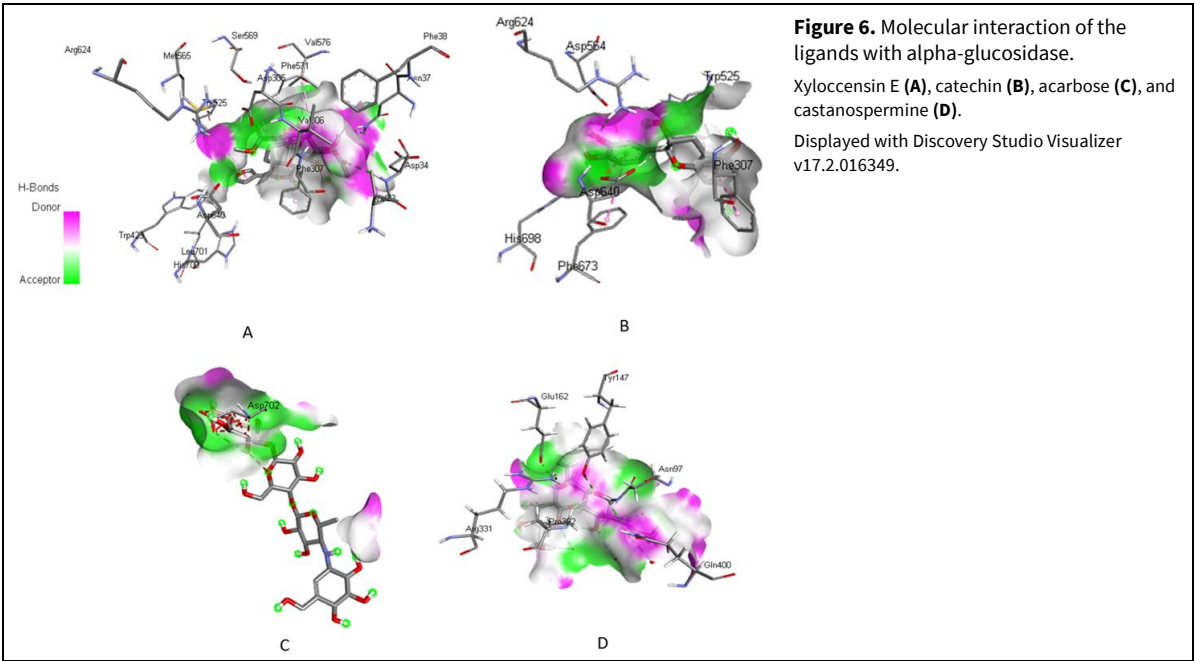


Table 8. Regression equation and IC₅₀ of DPPH assay of compounds **1**, **2**, **3**, and ascorbic acid.

Compound	Regression equation	R ²	IC ₅₀ (µg/mL)
Control (ascorbic acid)	y = 0.3557x + 49.552	0.9872	1.25
1	y = 0.7749x + 22.214	0.8433	35.85 ± 1.26***
2	y = 0.0195x + 4.2123	0.8707	2,348.08 ± 554.85***
3	y = 0.3775x + 48.91	0.9556	2.88 ± 0.77*

***P<0.001, *p<0.05 respect to the control group. IC₅₀ values are represented as mean ± SD (n = 3)

Table 9. Antioxidant capacity of FRAP and DPPH at 100 µg/mL.

Compound	FRAP antioxidant power (µmol Fe ²⁺ /g)	DPPH inhibition activity (%)
Control (ascorbic acid)	400.98 ± 9.34	84.08 ± 0.32
1	66.35 ± 1.91***	63.76 ± 1.43***
2	30.42 ± 1.95***	6.43 ± 0.31***
3	213.82 ± 5.73***	84.37 ± 0.32

***P<0.001 respect to the control group. Values are represented as mean ± SD (n = 3).

DISCUSSION

Indonesia is a country with prodigious biodiversity. Indonesian people have been utilizing it to sustain their way of life. The application of ethnomedicine, which commonly employs living organisms, particularly plants, is still popular among Indonesians. One plant that has been utilized in traditional medicine is Kayu Sarampa (*Xylocarpus moluccensis*). In addition, people in Ratahan, North Sulawesi, Indonesia, traditionally use *X. moluccensis* as an antidiabetic. Nevertheless, scientific evidence of its antidiabetic activity is rarely found. Thus, a scientific study was done to investigate its anti-hyperglycemic and antioxidant capacity, particularly from the stem bark ethyl acetate extract.

The α-glucosidase enzyme is an intestine epithelium enzyme that is essential in carbohydrate digestion by producing absorbable glucose. Indeed, inhibition of the α-glucosidase enzyme helps to delay carbohydrate digestion, lowering blood glucose levels and suppressing postprandial hyperglycemia (Ross et al., 2004). Srivastava et al. (2014) reported that the ethyl acetate fraction of *X. moluccensis* fruit was effective in inhibiting the α-glucosidase enzyme with a percent inhibition of about 80% at a concentration of 100 µg/mL (Srivastava et al., 2014). Furthermore, a previous study has shown that 100 µg/mL ethyl acetate extract of *X. moluccensis* stem bark inhibits 49.7% of α-glucosidase enzyme activity (Budiarso et al., 2020). Interestingly, several fractions of the stem bark ethyl acetate extract exhibit higher inhibition power compared to the crude extract. The F fraction showed the highest inhibition percentage of 72.58%. Although the

stem bark extract fraction has a slightly lower activity than the fruit extract fraction, it still has the capacity to function as an anti-hyperglycemic agent.

Several investigations have determined that hyperglycemic events induce oxidative stress and excessive reactive oxygen species (ROS) production leads to diabetic pathogenesis (Papachristoforou et al., 2020). Furthermore, in diabetes mellitus cases, an antioxidant molecule can reduce the harmful effects of hyperglycemia and increase glucose absorption (Sarian et al., 2017). Thus, the antioxidant capacity of the ethyl acetate stem bark fraction was also carried out to strengthen the evidence of its potential as an antidiabetic. The results of the DPPH and FRAP assay methods demonstrated that the F fraction showed the best antioxidant capacity with 81.15% and 301.63 µmol Fe²⁺/g, respectively. In addition, the antioxidant capacity of the F fraction is slightly lower compared to the control ascorbic acid.

The active compounds from the F and D fractions had been isolated, investigated, and characterized. From the D fraction, two compounds were isolated and characterized as xylocensin E and ruageanin D. Meanwhile, the compound isolated from the F fraction was identified as 2',3,5,5',7-pentahydroxyflavan (catechin). This finding demonstrated that stem bark also contains xylocensin E and seed (Islam et al., 2020; Ravangpai et al., 2011). Interestingly, until it was grown in this study, *X. moluccensis* was not known to contain the ruageanin D compound. Furthermore, Wangenstein et al. (2009) reported that catechin was found in the bark of *X. moluccensis*.

Furthermore, those three isolated compounds were further investigated to determine their α -glucosidase inhibition and antioxidant capacity. This outcome confirms the postulate concerning the high antioxidant capacity of catechin through several mechanisms (Zanwar et al., 2014). Meanwhile, xylococcensin E exhibits antioxidant capacity, although not as high as catechin. Furthermore, the antioxidant capacity of these compounds is linear with their α -glucosidase inhibition activity. Das et al. (2014) investigated the antidiabetic activity of *X. granatum* ethanol bark extract, and they reported that the active compound is xylococcensin L. Hereinafter, the IC₅₀ of xylococcensin L (160 μ g/mL) was lower than xylococcensin E (118 μ g/mL) and three times lower than catechin (55 μ g/mL) (Das et al., 2014). The molecular docking result also strengthens those compounds' anti-hyperglycemic activity by describing the interaction with the α -glucosidase enzyme. Catechin exhibits the strongest binding energy compared to xylococcensin E and control acarbose. Meanwhile, the binding energy of xylococcensin E is slightly lower than control acarbose. Indeed, the phenol moieties of catechin play an important role in the ligand-enzyme bond, wherein it bonds in the active site of the α -glucosidase enzyme. Flavonoids, including catechins, are hydroxylated and galloylated at the OH C-3 position, which significantly impacts their ability to inhibit the α -glucosidase (Xiao et al., 2013). Hereinafter, inhibition kinetic assay results exhibited that catechin performed a competitive inhibition kinetic mechanism against the α -glucosidase enzyme. This result was also confirmed by Fu et al. (2021). Contrary to xylococcensin E and catechin, ruageanin D indicated an inactive profile as an α -glucosidase inhibitor and also showed weak antioxidant capacity. Nevertheless, it has been reported to have neuroprotective activity (Li et al., 2015).

CONCLUSION

The ethyl acetate extract of *Xylocarpus moluccensis* stem bark was fractionated using chromatographic methods, affording 10 fractions. From the D fraction, which had the biggest fraction weight, two tetrahydroxyterpenoids compounds were isolated, namely xylococcensin E (**1**) and ruageanin D (**2**). Meanwhile, a flavonoid compound was isolated from the F fraction, which showed the highest α -glucosidase inhibition and antioxidant activities, namely 3,5,7,3',4'-pentahydroxyflavan (catechin) (**3**). Compounds **1** and **3** showed inhibitory activity of α -glucosidase enzyme with IC₅₀ values of 118.60 and 55.19 μ g/mL, respectively, with a competitive inhibition mechanism. Through an *in silico* molecular docking study, both compounds showed good binding affinity of α -glucosidase protein, indicating their antidiabetic ac-

tivity that also strengthened the α -glucosidase enzyme inhibition assay. Moreover, 100 μ g/mL of compounds **1** and **3** also showed antioxidant capacity through the DPPH and FRAP assay. This result demonstrates that *Xylocarpus moluccensis* stem bark has the potential activity to develop as an alternative therapy for hyperglycemia cases through α -glucosidase inhibition and antioxidant capacity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Elya B	Budiarso FS	Hanafi M	Gani MA	Prasetyaningrum PW
Concepts or ideas	x		x		
Design	x	x	x		
Definition of intellectual content	x		x		
Literature search	x	x		x	x
Experimental studies	x	x		x	
Data acquisition	x	x	x	x	x
Data analysis	x	x	x	x	x
Statistical analysis		x		x	x
Manuscript preparation	x	x			x
Manuscript editing		x			x
Manuscript review	x	x	x	x	x

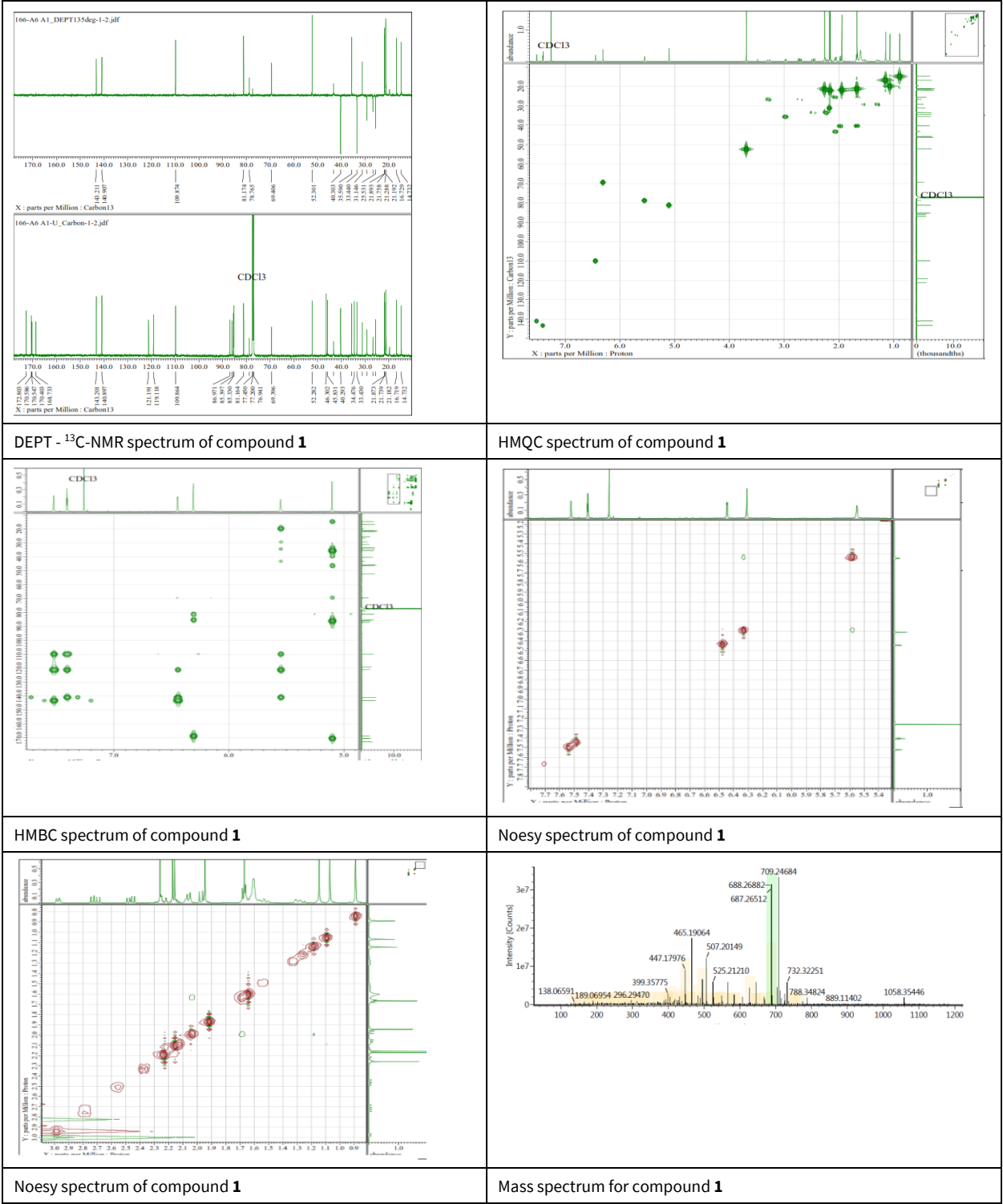
Citation Format: Elya B, Budiarso FS, Hanafi M, Gani MA, Prasetyaningrum PW (2024) Two tetrahydroxyterpenoids and a flavonoid from *Xylocarpus moluccensis* M.Roem. and their α -glucosidase inhibitory and antioxidant capacity. J Pharm Pharmacogn Res 12(3): 453–476.
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Compound 1



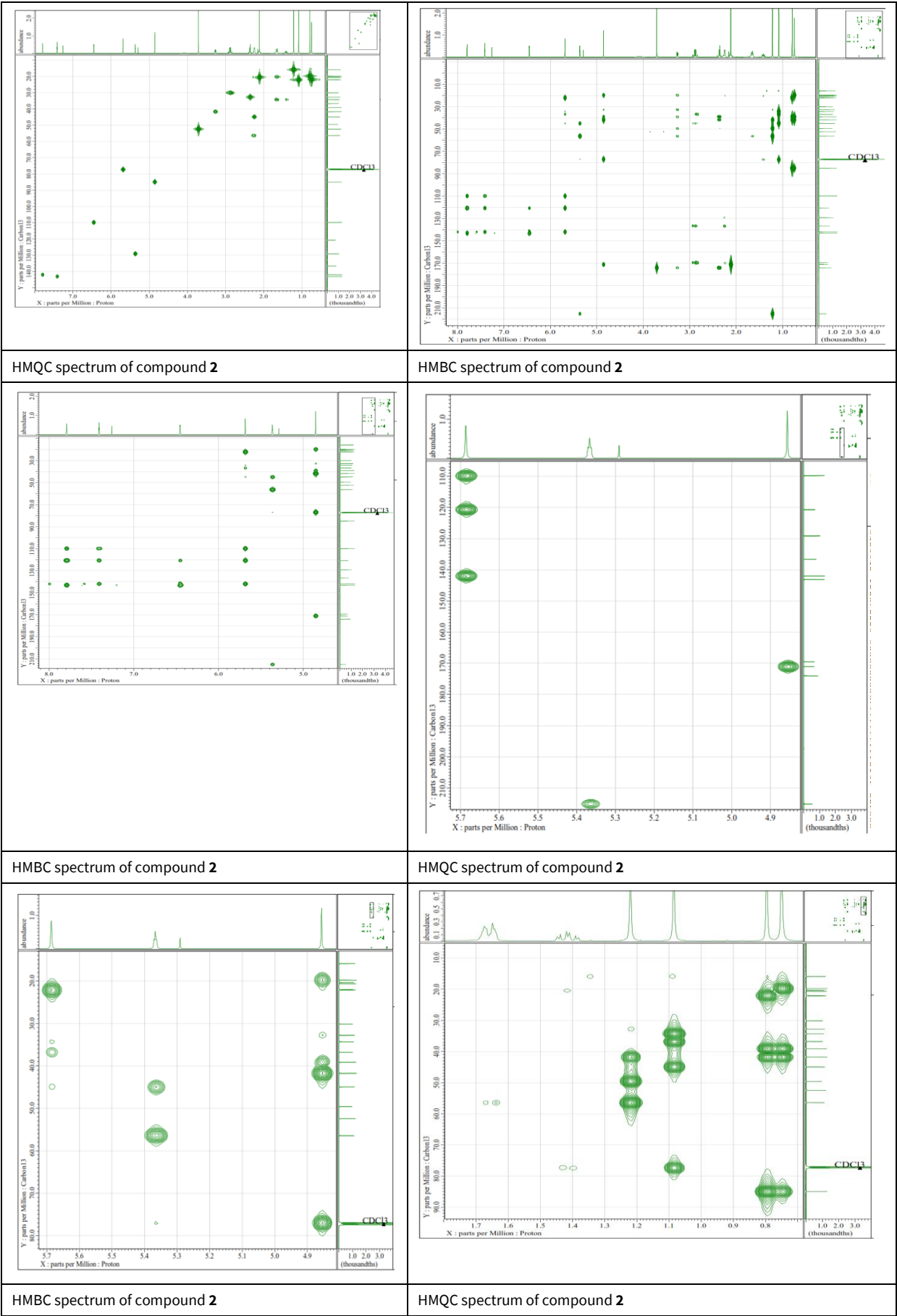


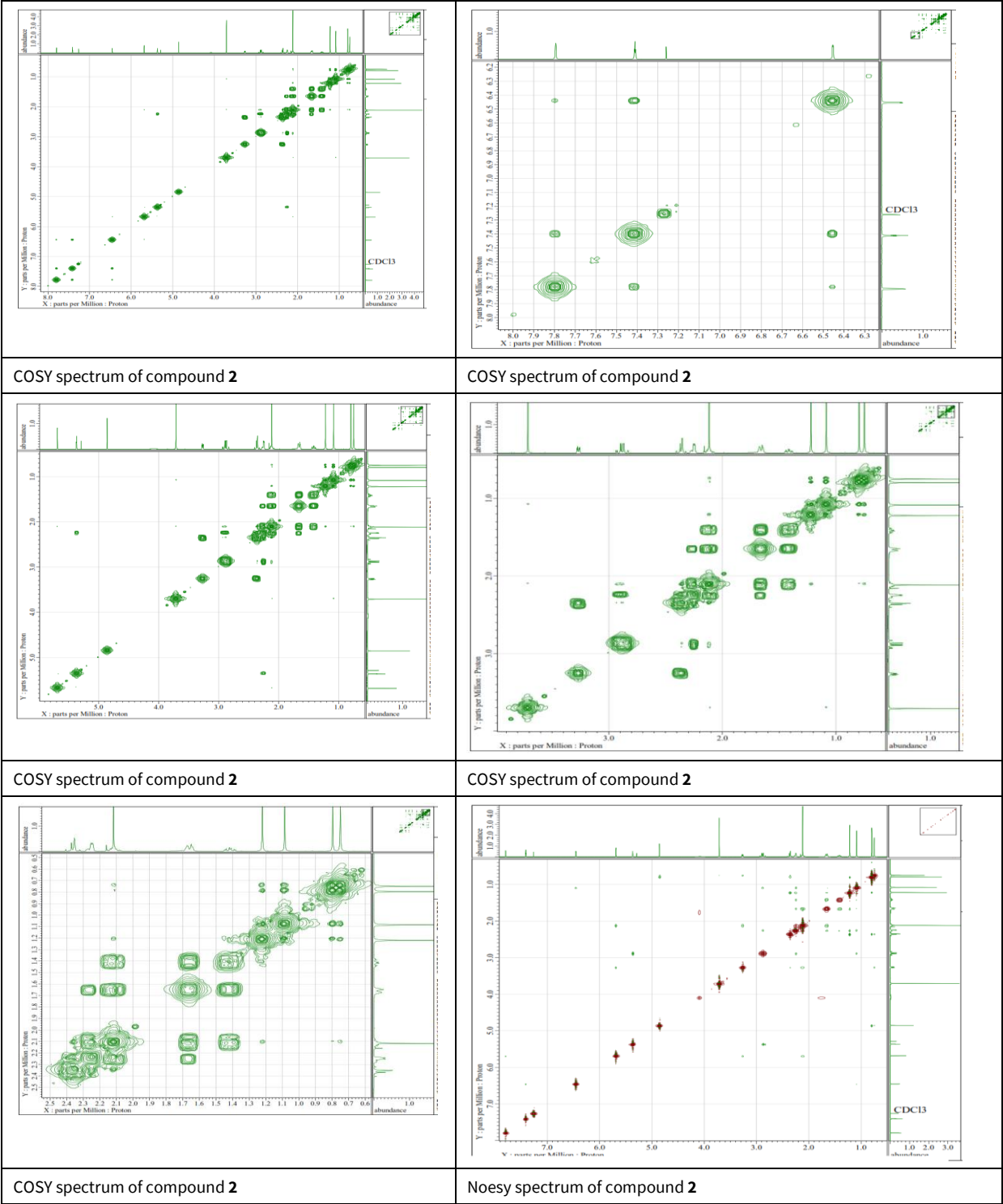
¹H NMR spectrum of compound 2

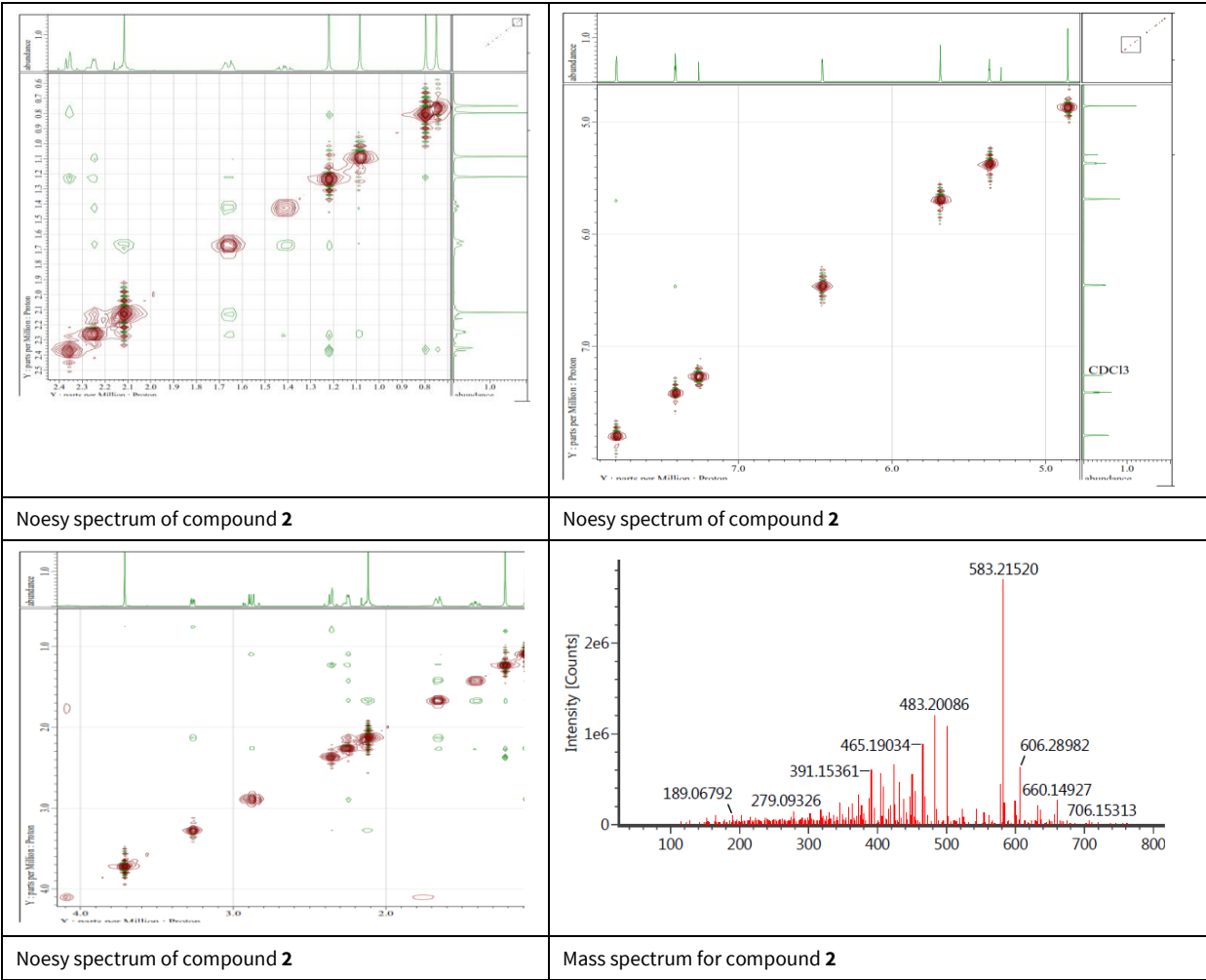
¹H NMR spectrum of compound 2

¹³C NMR spectrum of compound 2

DEPT-¹³C NMR spectrum of compound 2







Compound 3

