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Fatty Acid and Alkenil Glycoside from the Fruits of Mengkudu (*Morinda citrifolia* Linn)

Ferlinahayati[1]*, Rizdha Apriyaty[1] and Eliza[1]

[1)]*Chemistry Deparment, Mathematics and Natural Sciences Faculty, Sriwijaya University*
Jalan Raya Palembang-Prabumulih km32 Inderalaya
[*]*Corresponding Author: etihayati74@yahoo.com*

Abstract

Two compounds were isolated from methanol extract of mengkudu fruit (*Morinda.citrifolia* Linn). The extraction was conducted by maceration, while separation and purification using several chromatographic techniques. The compound structures were determined by spectral data including IR, ^{1}H-NMR and GC-MS. Compound **1** was a mixture of 7 fatty acids with hexadecanoic acid as a primarily, meanwhile compound **2** was a mixture of an alkenyl glycoside with an aromatic.

Keywords: Morinda citrifolia, asam lemak, alkenil glikosida

Abstrak (Indonesian)

Dua senyawa telah diisolasi dari ekstrak metanol buah mengkudu (*Morinda citrifolia Linn*). Ekstraksi dilakukan dengan cara maserasi yang dilanjutkan dengan pemisahan dan pemurnian dengan menggunakan berbagai teknik kromatografi. Struktur senyawa ditentukan berdasarkan data-data spektroskopi yaitu IR, ^{1}H-NMR dan GC-MS. Senyawa 1 merupakan campuran dari tujuh asam lemak dengan kandungan utama asam heksadekanoat sedangkan senyawa 2 merupakan campuran alkenil glikosida dengan senyawa aromatik.

Kata kunci: Morinda citrifolia, asam lemak, alkenil glikosida

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INTRODUCTION

Mengkudu is a locally name for *Morinda citrifolia* Linn belonging to Rubiaceae family. It's a tropical plant typically found in Southern Pacific and Southeast Asia [1]. Traditionally, all part of this plant including fruits, roots, leaves and barks have been used to treat a wide range of diseases such as hypertension, diabetic and cancer [2]. In addition, this plant is also used as cosmetics and food [3].

Phytochemical studies showed that anthraquinone derivatives are the major compounds of mengkudu roots besides of benzophenone [4]). Anthraquinones, iridoids, flavonoid glycosides, coumarins and terpenoids have been reported from the leaves of mengkudu [5]. Meanwhile, the fruit of mengkudu contains of lignans, neolignans, phenylpropanoids and anthraquinones [6-8]. Some isolated compounds have been reported their biologically activities such as lipoxygenase inhibitory [6], antioxidant [8], larvacidal [9] and cytotoxic activity [4]. As a part of our investigation on Indonesian medicinal plant, we report the isolation and structure elucidation of two isolated compound from methanol extract of mengkudu fruits.

EXPERIMENTAL SECTION

General experimental procedures

Infrared spectra were recorded in KBr pellets on a Perkin Elmer Spectrum One spectrometer. ^{1}H-NMR (500 MHz) spectra were obtained using Agilent DD2 spectrometer with TMS as internal standard or using residual solvent peaks as reference standards. GC-MS were obtained from a Shimadzu-QP 5050 spectrometer. Vacuum liquid chromatography (VLC) was performed on silica gel 60 (Merck) while radial

chromatography was performed on silica gel 60 PF_{254}. Precoated silica plates 0.25 mm (Merck) were used for TLC analysis.

Plant material

The pale yellow fruits of *M. citrifolia* were collected from Palembang, South Sumatera in August 2014. The plant was identified at the Biology Department, Faculty of Mathematics and Natural Science, Sriwijaya University, South Sumatera, Indonesia.

Extraction and isolation

The dried fruit powder of *M. citrifolia* was extracted by maceration with methanol as the solvent for a day (two times) to give 96,7 g of methanol extract after evaporation the solvent under reduced pressure. The methanol extract was then dissolved in acetone to afford 13.8 g of acetone extract. The acetone extract was fractionated on silica gel by VLC with a gradient of *n*-hexane-ethyl acetate, ethyl acetate-methanol and methanol to give ten fractions (A-J). Purification of fraction D (123 mg) with radial chromatography and eluted with *n*-hexane-ethyl acetate (8:2, 7:3 and 6:4) yield compound **1** (16 mg). Fraction E (430 mg) was further separated with radial chromatography and eluted with n-hexane-ethyl acetate (7:3, 6:4, 1:1, 4:6, 3:7 and 2:8) to give nine fractions. The seventh fraction was purified by column chromatography with *n*-hexane-acetone (7:3) as eluent to obtain compound **2** (12.5 mg).

RESULT AND DISCUSSION

The acetone soluble fraction of methanol extract of mengkudu fruits was separated by several chromatography techniques to obtain compound **1** and **2**. Compound **1** was identified by ^{1}H-NMR (in $CDCl_3$) and GC-MS, while compound **2** was identified by IR and ^{1}H-NMR (in acetone-d_6).

Table 1. Fatty acids composition of compound **1** detected by GC-MS

No	Rt (min)	Name	Concentration (%)	Similarity (%)	Base peak (*m/z*)
1	3.65	Hexanoic acid	15.13	97	74
2	5.21	Butanedioic acid	3.25	94	115
3	6.69	Octanoic acid	21.20	97	74
4	18.72	Hexadecanoic acid	37.61	96	74
5	20.77	9,12-Octadecadienoic acid	3.36	90	81
6	20.82	9-Octadecenoic acid	7.19	95	55
7	21.10	Octadecanoic acid	12.26	96	74

Compound **1** was isolated as oily yellow. ^{1}H-NMR spectra (Figure 1) revealed the characteristic of fatty acid due to the signal at δ_H 1.15-1.45 (24H) ppm for methylene chain signal. Another characteristic of fatty acid appears at δ_H 1.63 (2H) and 2.35 (2H) ppm caused by two CH_2 proton, namely β and α to the carboxyl group (C-3 & C-2) respectively. Meanwhile, the terminal methyl appears at δ_H 0.88 (3H) ppm as triplet. All signals are consistent with saturated fatty acid signals (Knothe, 2004). Base on the number of protons in methylene chain (24H) is suggested as hexadecanoic acid (palmitic acid). In order to determine the impurities, the GC-MS analysis was carried out.

Table 2. MS data of saturated FAME of compound **1**

Rt (min)	Name	M^+ (m/z)	$[M\text{-}OCH_3]^+$ (m/z)	McLafferty rearrangement (m/z)	$[M\text{-}C_3H_7]^+$ (m/z)	$[CH_3\text{-}OCO(CH_2)n]^+$ (m/z)
3.65	Methyl Hexanoic	130	99	87, 74*	87	-
6.69	Methyl Octanoic	158	127	87, 74*	115	101, 87
18.72	Methyl Hexadecanoic	270	239	87, 74*	227	213, 199, 185, 171, 157, 143, 129, 115, 101, 87
21.10	Methyl Octadecanoic	298	267	87, 74*	255	241, 227,213, 199, 185, 171, 157, 143, 129, 115, 101, 87

Chromatogram of compound **1** showed seven peaks corresponding to seven fatty acids methyl ester (FAME). Four of them are the main peak which is a saturated FAME (Table 1) containing primarily methyl hexadecanoic (methyl palmitic). Peak 1, 3, 4 and 7 (saturated FAME) showed the similar mass spectra (Table 2), i.e., a base peak at m/z 74 arise from McLafferty rearrangement, fragment with gaps of 14 amu for the loss of the each successive of methylene group from each molecular ion, besides at M-31 (M-CH_3O^+). Another similar peak is at m/z 87. This fragment also appears as McLafferty rearrangement following by transfer of α hydrogen to γ karbon with γ bond cleavage (Figure 2) (Takayama, 1995). Total loss of methylene fragments determines a chain length of fatty acids and supported by comparing their mass fragmentation with those of mass spectra data base (NIST08.LIB). The minor peaks (5 and 6) on chromatogram is an unsaturated fatty acid, namely methyl 9,12-octadecadienoic and methyl 9-octadecenoic which are an impurity that appears on ^{1}H-NMR spectra as well as succinic acid (peak 2). Based on these observations, the structure of compound **1** was established as shown in Figure 3. Previously, acyl glycoside namely *6-O-(β*-D-glucopyranosyl)-1-*O*-

octanoyl-*β*-D-glucopyranose and 6-*O*-(*β*-D-glucopyranosyl)1-*O*-hexanoyl- *β* -D-glucopyranose were reported from Hawaii mengkudu [2]. But, in this research, we were only isolated as fatty acid. We suggest the isolated fatty acid derived from hydrolysis of acyl glycoside.

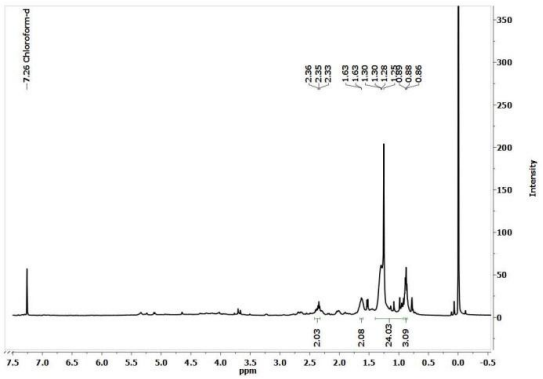

Figure 1. ^{1}H-NMR spectra of compound **1**

Compound **2** was isolated as yellow oil. IR spectra of **2** showed the absorption at 3427 cm-^{1} (*br*) for polyhydroxyl unit, 1755 cm^{-1} for C=O ester, 1452-1622 cm^{-1} for C=C aromatic, 1111 cm^{-1} for C-O and 2961 cm^{-1} for aliphatic C-H. The ^{1}H-NMR spectra clearly showed that compound **2** is a mixture of aromatic and the glycoside compound. The aromatic signal can't be interpreted due to overlapping signal with the high enough integration. ^{1}H-NMR spectra showed signal characteristic for glycoside moiety such as signal at δ_H 3.2 – 4.2 ppm (12H) and two anomeric protons at δ_H 4.30 & 4. 27 ppm. These signal indicated the presence of β-D-glucopyranosil-β-D-glucopyranose moiety [2, 10]. The up field signal for two anomeric protons indicate the sugar moiety linked to an alkyl group not to an acyl group [2]. In addition, signals for sugars, the ^{1}H-NMR spectrum showed the presence of a terminal methylene [δ_H 4.61 (1H) & 4.60 (1H)], a methylene [δ_H 2.35 (2H)] and an oxymethylene [δ_H 3.99 (1H) & 3.65(1H)]. These signals revealed the presence of an alkenyl group which probably attach to a sugar. Based on these data, we suggested the compound **2** is a mixture of an aromatic compound and an alkenyl glycoside compound.

CONCLUSION

The mixture of fatty acid primarily a palmitic acid/hexadecanoic acid and an alkenyl glikoside had been successfully isolated and identified from methanol extract of *M. citrifolia* fruits.

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