Fatty Acids and an Ester from the Leaves of *Millettia utilis* Dunn

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Abstract

Two fatty acids, palmitic acid and oleic acid together with an ester, methyl oleate were isolated from dichloromethane crude extract from the leaves of *Millettia utilis* Dunn. The structures of all isolated compounds were elucidated by basic NMR spectroscopy and compared with previous literature. All compounds were tested for their antioxidant activities. Oleic acid revealed weak activity against ABTS assay with IC_{50} = 39.50 μg/mL.

Keywords: *Millettia utilis* Dunn, fatty acids, an ester, ABTS, antioxidant activities

Introduction

The genus *Millettia* (Leguminosae) is represented by over 200 species and distributed in tropical Africa, Asia, and Australia (Chen et al., 2018). The plant *Millettia utilis* Dunn., locally known as " Satorn ", is a tree growing in northeastern and northern regions of Thailand. *Millettia* plants revealed the presence of flavonoids (Sritularak & Likhitwitayawuid, 2006; Pailee, Mahidol, Ruchirawat, & Prachyawarakorn, 2019), isoflavones (Derese et al., 2014), chalcones flavones (Phrutivorapongkul et al., 2003), and rotenoids (Ngandeu et al., 2008); (Chen et al., 2015). Moreover, biological activities of isolated compounds from *Millettia* genus were interesting such as antiviral, anti-inflammatory (Phrutivorapongkul et al., 2003); (Pancharoen, Athipornchai, Panthong, & Taylor, 2008); (Huo et al., 2015), antiplasmodial (Derese et al., 2014), and analgesic activities (Huo et al., 2015). In an earlier report, a triterpenoid and two steroids were isolated from the twigs of *M. utilis* Dunn and to exhibit antioxidant activities (Ruksilp, 2020). In this paper, the isolation and antioxidant activities from dichloromethane crude extract in the leaves of *M. utilis* Dunn have been reported for the first time.

Methods and Materials

General experiment procedures

NMR spectra were recorded in $CDCl_3$ on Bruker spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. PHOMO Microplate reader, Autobio model SPR-960 was obtained for DPPH and ABTS assays. Merck's silica gel 60 No.7734 was used as adsorbents for column chromatography. Analytical thin layer chromatography was performed with Merck's silica gel 60 F_{254}, 0.25 mm precoated TLC aluminium sheets. The detection was visualized under ultraviolet light at the wavelength of 254 and 365 nm. Melting points were obtained on Buchi melting point B-540.

Plant material

The leaves of *M. utilis* Dunn were collected in October, 2018 from Dansai, Loei, Thailand and identified by Dr. Sawai Mattapha. A voucher specimen (LRU No. 001) was deposited in the Herbarium of Loei Rajabhat University, Faculty of Science and Technology, Loei, Thailand.

Extraction and isolation

The air-dried leaves of *M. utilis* Dunn (1.0 kg) were extracted with MeOH (5 L x 3, 1 day for each extraction) at room temperature. The MeOH extract layer was evaporated to obtain crude extract of MeOH (100.5 g). The MeOH crude extract was partitioned with 1.0 L of CH_2Cl_2 : H_2O (1 : 1). The CH_2Cl_2 part was concentrated to afford crude extract of CH_2Cl_2 (15.2 g) and separated on a silica gel column chromatography (CC), using *n*-hexane : CH_2Cl_2(9:1, 4:1, 3:2, 1:1, 3:7, 1:9, 0:10); CH_2Cl_2 : EtOAc (9:1 to 0: 10) and EtOAc : MeOH (9:1 to 0: 10) as the eluant .The 12 fractions (F1-F12) were monitored by TLC analysis. F10 (201.5 mg) was chromatographed over silica gel CC and eluted with a gradient system of *n*-hexane : CH_2Cl_2 (9:1 to 0: 10) and CH_2Cl_2 : EtOAc (9:1 to 0: 10) to give ten subfractions (F10-1 to F10-10). Subfraction F10-6 (105.8 mg) was further seperated over silica gel CC and eluted with *n*-hexane : CH_2Cl_2 (9:1 to 0: 10) and CH_2Cl_2 : EtOAc (9:1 to 0: 10) to afford compound **1** (10.5 mg) and compound **2** (12.2 mg). Subfraction F10-7 (90 mg) was further purified over silica gel CC and eluted with CH_2Cl_2 : EtOAc (9:1 to 0: 10) to provide compound **3** (10.2 mg).

Antioxidant assays

Compounds **1-3** have been tested for their antioxidant activities by DPPH and ABTS assays. PHOMO Microplate reader, Autobio model SPR-960 performed in a 96 well plate was obtained for DPPH and ABTS assays.

The DPPH assay was modified from the method of Kim, Lee, Lee, and Lee (2002). The DPPH reagent was weighed 8 mg, dissolved in 100 mL EtOH for a solution concentration of 80 μg/mL. To determine the scavenging activity, 100 μL DPPH reagent was mixed with 100 μL of sample in a 96-well plate and allowed to stand in the dark at room temperature for 30 min. The absorbance was measured at 515 nm using PHOMO microplate reader. Vitamin E was used as standard and 95 % ethanol was used as a control.

The ABTS assay was modified from the method of Arnao, Cano, and Acosta (2001). Stock solution of ABTS (2,2′-azobis-(3-ethylbenzothiazoline-6-sulfonic acid)) was prepared by dissolving 0.0036 g ABTS in deionized water 1.0 mL. Potassium persulfate 0.00067 g was mixed to the solution and allowed to stand in the dark for 16 hours to obtain the radical cation $ABTS^{\bullet+}$. The assay was initiated by the addition of sample 50 μL to $ABTS^{\bullet+}$ solution 100 μL to a final volume of 150 μL in 96 well plate, and allowed to stand for 15 min. The absorbance at 734 nm was monitored, using vitamin E as a standard compound.

Results and Discussion

Three compounds (**1-3**) were isolated from the leaves of *M. utilis* Dunn. They were classified into two groups. Two fatty acids (compounds **1-2**) and one methyl ester of fatty acid (compound **3**), (Figure 1).

Palmitic acid (**1**) White powder, m.p. 63-64 ^{0}C (m.p. lit. 62.9^{0}C); (Joshi et al., 2009), ^{1}H NMR and ^{13}C NMR are revealed in Table 1.

Oleic acid (**2**) White powder, m.p. 13-14^{0}C (m.p. lit.13-14^{0}C); (ABE et al., 2009), ^{1}H NMR and ^{13}C NMR are revealed in Table 1.

Methyl oleate (**3**) White oil, ^{1}H NMR and ^{13}C NMR were revealed in Table 1, (Wineburg & Swern, 1972)

Table 1 ^{1}H NMR and ^{13}C NMR spectral data of compounds 1-3, palmitic acid, oleic acid and methyl oleate

Position	Compound 1		Palmitic acid		Compound 2		Oleic acid		Compound 3		Methyl oleate	
	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)
1		174.0		173.70		180.0		179.7		174.0		173.9
2	2.28,*t*, *J*=8.0	34.0	2.30	34.0	2.30,*t*, *J*=8.0	34.0	2.35, *t*, *J*=7.5	34.0	2.30	34.1	2.30	34.1
3	1.60	24.5		24.5	1.60,m	24.7	1.63,m	24.7	1.60	25.08	1.60	25.08
4						29.8		29.8		29.24		29.24
5						29.6		29.7		29.25		29.25
6						29.4		29.5		29.29		29.29
7						29.3		29.3		29.82		29.82
8					2.00,m	27.2	2.01,m	27.2	1.95	29.9	1.90	29.9
9					5.30,m	130.0	5.34,m	130.0	5.25	130.0	5.30	130.0
10					5.30,m	129.7	5.34,m	129.7	5.25	129.7	5.30	129.7
11					2.00,m	27.1	2.01,m	27.1	1.95	29.8	1.90	29.8
12						29.2		29.14		29.95		29.95
13						29.1		29.06		29.5		29.5
14		32.02		31.94		29.0		29.03		32.1		32.1
15		22.70		22.71						29.4		29.4
16	0.80 (m, 3H)	14.10	0.84 (m, 3H)	14.13		31.9		31.9		29.80		29.80
17						22.7		22.7		22.8		22.8
18					0.80	14.1	0.88,t, J=7.0	14.1	0.80	14.2	0.85	14.16
OCH_3									3.60,	51.3,	3.50,	51.3,

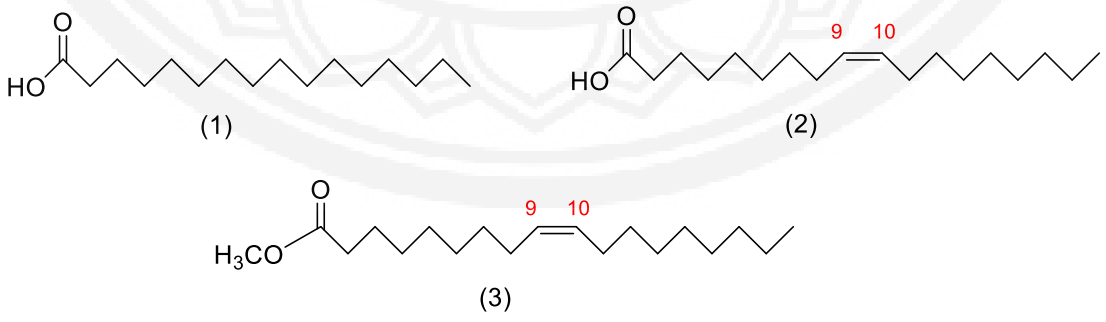

Figure 1 Palmitic acid (1), Oleic acid (2) and Methyl oleate (3) isolated from the leaves of *M. utilis* Dunn

The ^{1}H NMR spectrum of compound **1** displayed the terminal methyl protons at δ 0.80 (H-16). The signal at δ 2.28, *t*, *J*= 8.0 and 1.60 indicated CH_2 protons adjacent to a carboxyl group, H-2 and H-3 respectively. Further signal at 174.0 was shown carboxyl group(C-1) and 14.10 was the signal of terminal methyl group(C-16). On this basis, the structure of the compound **1** was determined as a Palmitic acid (Joshi et al., 2009).

The spectrum of ^{1}H NMR of compound **2** displayed an olefinic proton multiplet at δ 5.30 (m, H-9, and H-10), and showed terminal CH_3 at δ 0.80 (H-18). The two methylene groups of H-8 and H-11 revealed at δ 2.00. The signal at δ 2.30 and 1.60 were indicated CH_2 protons adjacent to a carboxyl group, H-2 and H-3, respectively. The signal of δ 180.0, 14.1 indicated a carboxyl group(C-1) and a terminal methyl group (C-18). The structure of the compound **2** was revealed as an oleic acid (ABE et al., 2009).

Compound **3** displayed the ^{1}H NMR spectrum at δ 3.60 which could be assigned for 3H of methoxy group. The signal at δ 2.30 and 1.60 indicated CH_2 protons adjacent to a methyl ester of a carboxyl group, H-2 and H-3, respectively. At δ 5.25 revealed the presence of vinylic protons at H-9, and H-10. The spectrum also showed CH_2 protons adjacent to protons at δ 1.95, H-8 and H-11. The signals of δ 174.0, and 14.2 showed carboxyl group(C-1) and a terminal methyl group(C-18). The structure of the compound **3** was revealed as a methyl oleate (Wineburg & Swern, 1972).

Table 2 Antioxidant levels of isolated compounds from the leaves of *M. utilis* Dunn

Compounds	(IC_{50}, μg/mL)			
	DPPH	Antioxidant levels	ABTS	Antioxidant levels
Palmitic acid (1)	$IC_{50} > 50$	inactive	$IC_{50} > 50$	inactive
Oleic acid (2)	$IC_{50} > 50$	inactive	39.50 ± 1.57	weak
Methyl oleate (3)	$IC_{50} > 50$	inactive	$IC_{50} > 50$	inactive
Vitamin E	5.39 ± 0.01	high	2.43 ± 0.01	high

Compounds **1-3** were tested for their antioxidant activities by DPPH and ABTS assays in Table 2. Compound **2** showed weak activity by ABTS assay with IC_{50} value 39.50 ± 1.57 μg/mL. Compound 1 and 3 were weakly active. Palmitic acid was reported to be highly active against the human breast (MCF-7), liver (WRL-68), adherent colon (Caco-2), and suspension colon (Colo-320-DM) cancer cell lines with IC_{50} 0.55, 1.0, 0.75 and 0.35 μg/mL, respectively (Sasena et al., 2007). Oleic acid was weakly active by 5α-reductase with IC_{50} 54.50 ± 3.0 μg/mL (ABE et al., 2009). The branched-chain derivatives (methyl, *n*-butyl, phenyl) of methyl oleate were tested *in vitro* for their antiproliferative activities against two cancer cell lines: MCF-7 (human breast) and HT-29 (human colon). The branched phenyl derivative of oleic acid were shown weakly active, with IC_{50} at 48 ppm against both MCF-7 and HT-29 (Dailey, Wang, Chen, & Huang, 2011).

Conclusion and Suggestions

The leaves of *M. utilis* Dunn were dried and ground into powder and there were extracted with MeOH to afford MeOH crude extract then partitioned with CH_2Cl_2 : H_2O (1 : 1). The dichloromethane part was concentrated to obtain dichloromethane crude extract. The chromatographic seperations and purification of dichloromethane crude extract yielded two fatty acids, palmitic acid (1), oleic acid (2) and one ester of fatty acid, methyl oleate (3). All compounds were tested for their antioxidant activities by DPPH and ABTS assays. Oleic acid (2) showed weak activity with IC_{50} value 39.50 ± 1.57 μg/mL. by ABTS assay.

The other parts of *M. utilis* Dunn such as fruits, roots should be investigated for their constituents, and biological activities since no reports are found.

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