CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The leaves, stem and root of *Ochna integerrima* (Lour.) Merr. were collected from Sakaeraj Environmental Research Station, Nakorn Ratchasima Province, in April 1999 and June 2001. Authentication of the plant materials was done by comparison with herbarium specimens at the Royal Forest Department, Bangkok, Thailand. A voucher specimen (KL 122542) is on deposite at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography (TLC)

2.1.1 Normal phase Thin-Layer Chromatography

Technique : One dimension, ascending

Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate (Aluminium sheet)

Layer thickness : 0.2 mm

Distance : 5 cm

Temperature : room temperature (25-35 ^{o}C)

Detection : 1. Ultraviolet light at 254 and 365 nm

2. 5% H_2SO_4 in EtOH and heated at 110 ^{o}C for 10 min

2.1.2 Normal phase Thin-Layer Chromatography

Technique : One dimension, ascending

Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate (Glass plate)

Layer thickness : 0.25 mm

Distance : 5 cm

Temperature : room temperature (25-35 ^{o}C)

Detection : 1. Ultraviolet light at 254 and 365 nm

2. 5% H_2SO_4 in EtOH and heated at 110 ^{o}C for 10 min

2.1.3 Reverse phase Thin-Layer Chromatography

Technique : One dimension, ascending

Adsorbent : RP_{18} F_{254S} (E. Merck) precoated plate (Glass plate)

Layer thickness : 0.25 mm

Distance : 5 cm

Temperature : room temperature (25-35 ^{o}C)

Detection : 1. Ultraviolet light at 254 and 365 nm

2. 5% H_2SO_4 in EtOH and heated at 110 ^{o}C for 10 min

2.2 Column Chromatography

2.2.1 Column Chromatography

Adsorbent : Silica gel 60 (70-230 mesh); particle size 0.063-0.200 mm (E. Merck) or
Silica gel 60 (230-400 mesh); particle size 0.040-0.063 mm (E. Merck)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small volume of the eluent and gently applied on top of the column.

Detection : Fractions were examined by TLC, observing under UV light at 254 and 365nm, and the TLC plate was then sprayed with 5% H_2SO_4 in EtOH and heated at 110 ^{o}C for 10 min.

2.2.2 Gel Filtration Chromatography

Gel filter : Sephadex LH 20 (Pharmacia)

Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small volume of the eluent and gently applied on top of the column.

Detection : Fractions were examined by TLC, observing under UV light at 254 and 365nm, and the TLC plate was then sprayed with 5% H_2SO_4 in EtOH and heated at 110 ^{o}C for 10 min.

2.3 Medium Pressure Liquid Chromatography (MPLC)

Silica column : Kusano CIG pre-packed column, SiO_2 CPS-HS-221-5 (⌀ 22x100 mm)

ODS column : Kusano CIG pre-packed column, ODS CPO-HS-221-20 (⌀ 22x100 mm)

Flow rate : 2 ml/min

Mobile phase : Chloroform-methanol (Silica column)
Hexane-ethyl acetate (Silica column)
Methanol-water (ODS column)

Sample preparation : The sample was dissolved in a small vclume of solvent and then injected into the column.

Injection volume : 2 ml

Pump : JASCO 875 UV

UV detector : JASCO 880 PU

Temperature : room temperature (25-35 °C)

2.4 High Pressure Liquid Chromatography (HPLC)

Column : Shim-pack ODS Prep

Flow rate : 3 ml/min

Mobile phase : Methanol : H_2O

Sample preparation : The sample was dissolved in a small volume of methanol and filtered through millipore filter paper before injection.

Injection volume : 1 ml

Pump : LC-8A (Shimadzu)

Detector : SPD-10A UV Detector (Shimadzu)

Recorder : C-R6A Chromatopac (Shimadzu)

Temperature : room temperature (25-35 °C)

2.5 Spectroscopy

2.5.1 Ultraviolet (UV) Absorption Spectra

UV spectra (in methanol) were obtained on a Shimadzu UV-160A UV/vis spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University) or a JASCO V 560 spectrophotometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.5.2 Infrared (IR) Absorption spectra

IR spectra were recorded on a Perkin-Elmer FT-IR 1760 X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University) or a JAS FT/IR 230-IR spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.5.3 Mass Spectra

Fast-Atom Bombardment mass spectra (FABMS) and high resolution Fast-Atom Bombardment mass spectra (HRFABMS) were measured on a JEOL JMS-HX-110A spectrometer (The Chemical Analysis Center, Chiba University). Electron impact mass spectra (EIMS) were measured on a JEOL JMS-AM 20 mass spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.5.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectra

^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H-^{1}H COSY, HMQC and HMBC spectra were obtained on a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were obtained with a JEOL ECP 400 spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H-^{1}H COSY, HMQC and HMBC spectra were obtained on a JEOL JNM GSX 500A spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), ^{1}H-^{1}H COSY, NOEs, HMQC and HMBC spectra were obtained on a JEOL ECP 600 spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.6 Physical Properties

2.6.1 Melting Points

Melting points were obtained on a Yanaco MP 53 instrument and were uncorrected (Faculty of Pharmaceutical Sciences, Chiba University).

2.6.2 Optical Rotations

Optical rotations were measured on a JASCO DIP 140 polarimeter (Faculty of Pharmaceutical Sciences, Chiba University) or a Perkin Elmer 341 polarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.6.3 Circular Dichroism spectra

Circular Dichroism spectra were measured on a JASCO CD J-720 W spectrometer (Faculty of Pharmaceutical Sciences, Chiba University).

2.7 Solvents

Throughout this work, commercial grade organic solvents were used and redistilled prior to use.

2.8 Microtiter plate reading

Microtiter plate reading was performed on an Anthos HTL instrument, (Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

3. Extraction and Separation

3.1 Extraction and Separation of the Leaves

3.1.1 Extraction

The dried leaves of *Ochna integerrima* (1.8 kg) were successively extracted successively with petroleum ether (3X20 L), ethyl acetate (3X20 L) and methanol (3X20 L) to give a petroleum ether extract (41 g), an ethyl acetate extract (147 g) and a methanol extract (334 g), respectively.

3.1.2 Separation

3.1.2.1 Initial Separation

The ethyl acetate extract (147 g) was divided into three portions. Each portion was then fractionated by vacuum liquid chromatography using a sintered glass filter column of silica gel 60 (800 g). Elution was performed in a polarity gradient manner with ethyl acetate in hexane (500 ml each, 0%, 10%, 20%, 40%, 50%, 60%, 80%, 100%), and the column was then washed with 100% methanol. Then, fractions with similar chromatographic pattern were combined to give 15 fractions, as shown in Table 11.

Table 11 Chromatographic fractions of the ethyl acetate extract of leaves of *O. integerrima*

Fraction	Total weight (g)
P-I	1.82
P-II	1.21
P-III	0.81
P-IV	0.31
P-V	1.18
P-VI	1.16
P-VII	0.70
PVIII	1.05
P-IX	1.98
P-X	1.35
P-XI	8.79
P-XII	8.37
P-XIII	41.14
P-XIV	34.38
P-XV	29.18

3.1.2.2 Isolation of Compound 47

Fractions PI and PII were combined and dried, and then fractionated on a column of silica gel 60. Elution (20 ml each) was performed using mixtures of methanol in chloroform to give fractions OA-1 to OA-9. Fraction OA-3 was further purified, using 1%, 2%, 3% and 6% of methanol in chloroform (20 ml each), to give compound **47** (60 mg, $3.33X10^{-3}$ % based on dried weight of the leaves). The pure compound showed Rf value 0.35, using SiO_2 TLC ($CHCl_3$-MeOH 95:5) and detected under UV 254 nm. This compound was identified as 7″-*O*-methyl ochnaflavone (**47**).

3.1.2.3 Isolation of Compounds 4 and 170

Fractions OA-8 and OA-9, showing similar TLC patterns, were pooled, dried and then subjected to column chromatography on silica gel 60, using methanol-chloroform with polarity gradient as the eluent to give fractions OB-1 to OB-5. Fraction OB-4 was fractionated on a column of silica gel 60, and elution was performed using 1%, 2%, 3%, 6%, 10% and 100% methanol in chloroform (20 ml each) to give fractions OC-1 to OC-6. Fraction OC-5, after drying, gave compound **4** as a yellow powder. Compound **4** was identified as ochnaflavone (5 mg, $2.7X10^{-5}$ % based on dried weight of the leaves). This compound (**4**) showed Rf value 0.28, using SiO_2 TLC ($CHCl_3$-MeOH 95:5) and detected under UV 254 nm. Fraction OC-4 was separated on a sephadex LH 20 column, using MeOH as eluent to give compound **170** (30 mg, $1.6X10^{-4}$ % based on dried weight of the leaves). This compound was characterized as 3,3′,4′,5,7-pentahydroxy-6-prenylflavanone (**170**) and showed Rf value 0.31, using SiO_2 TLC ($CHCl_3$-MeOH 95:5) and detected under UV 254 nm.

3.2 Extraction and Separation of the Stem Bark

3.2.1 Extraction

The dried and powdered stem bark of *O. integerrima* (2.5 kg) was extracted with hexane (4x20 L) and methanol (4x20 L) to give a hexane extract and a methanol extract (5 and 80 g, respectively). Each extract was tested with DPPH radical reagent (as described in Section 4). The extract with a positive result was further studied.

3.2.2 Separation

The methanol extract, which was active against the DPPH radical, was divided into two portions : I (30 g) and II (50 g). Each portion was fractionated by column chromatography on silica gel 60. Elution was performed in a polarity gradient manner with chloroform and methanol as the eluents (200 ml each) (Table 12). Fractions with similar TLC patterns (silica gel, $CHCl_3$-MeOH 8:2 and 1:1) were combined as shown in Table 13.

Table 12 Chromatographic separation of the MeOH extract of the stem bark of *O. integerrima*

Portion I	
Number of eluate	**$CHCl_3$: MeOH**
1-2	100 : 0
3-4	98 : 2
5-6	96 : 4
7-8	94 : 6
9-12	92 : 8
13-16	90 : 10
17-20	88 : 12

Portion II	
Number of eluate	**$CHCl_3$: MeOH**
1-4	100 : 0
5-8	98 : 2
9-16	96 : 4
17-24	94 : 6
25-32	92 : 8
33-40	90 : 10
41-48	88 : 12

Table 12 Chromatographic separation of the MeOH extract of the stem bark of *O. integerrima* (continued)

Portion I	
Number of eluate	**$CHCl_3$: MeOH**
21-24	86 : 14
25-28	84 : 16
29-32	82 : 18
33-36	80 : 20
37-38	70 : 30
39-40	75 : 25
41-42	70 : 30
43-44	50 : 50
45-46	100

Portion II	
Number of eluate	**$CHCl_3$: MeOH**
49-56	86 : 14
57-64	84 : 16
65-72	82 : 18
73-80	80 : 20
81-84	70 : 30
85-88	75 : 25
89-92	70 : 30
93-94	50 : 50
95-96	100

Table 13 Combination of fractions in Table 12

Number of eluate		Fraction	Weight (g)
Portion I	**Portion II**		
1 – 2	1 – 3	B-1	0.20
3 – 4	4 – 5	B-2	0.91
	6 – 12	B-3	0.05
5 – 7	13 – 16	B-4	0.12
8 – 9	17 – 23	B-5	0.20
10 – 12	24 – 33	B-6	0.14
13 – 16	34 – 40	B-7	0.20
17 – 21	41 – 47	B-8	0.25
22 – 24	48 – 56	B-9	0.37

Table 13 Combination of fractions in Table 12 (continued)

Number of eluate		Fraction	Weight (g)
Portion I	Portion II		
25 – 29	57 – 64	B-10	0.23
30 – 32	65 – 72	B-11	0.25
33 – 36	73 – 80	B-12	0.41
37 – 38	81 – 84	B-13	0.13
39 – 40	85 – 88	B-14	0.64
41 – 42	89 – 93	B-15	1.10
43 - 46	94 - 96	B-16	7.69

3.2.2.1 Isolation of Compound 1

Fraction B–5 (0.20 g) was further separated on a silica gel 60 column, using 5% methanol in chloroform as the eluent. Fractions (10 ml each) were collected and combined after examination with TLC (silica gel, $CHCl_3$-MeOH 95:5). The pure compound showed Rf value 0.40 in this system. After removal of the solvent, compound **1** was obtained as a yellow solid, (2 mg, $8X10^{-5}$ % based on dry weight). This compound was identified as lophirone C (**1**).

3.2.2.2 Isolation of Compound 171

Fraction B–6 (0.14 g) was separated on a silica gel 60 column. The eluent was 7% methanol in chloroform. Fractions 25-32 were combined and further purified on a sephadex LH 20 column (MeOH) to give compound **171** (5 mg, $2X10^{-4}$ % based on dry weight). The pure compound showed Rf value 0.28, using SiO_2 TLC ($CHCl_3$-MeOH 93:7) and detected under UV 254 nm. This compound was identified as 3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzo-furan-7-yl 2-(4-hydroxyphenyl) ethenyl ketone (**171**).

3.2.2.3 Isolation of Compound 172

Fraction B–7 (0.20 g) was fractionated on a silica gel 60 column (7% methanol in chloroform). The target fractions were combined and re-separated on a sephadex LH 20 column (MeOH), and then purified by MPLC (ODS column, 35% water in methanol) to give compound **172** (5 mg, $2X10^{-4}$ % based on dry weight). Compound **172** showed Rf value 0.2, using RP_{18} TLC (35% water in methanol) and detected under UV 254 nm. This compound was identified as 3-(2,4-dihydroxybenzoyl)-2,3-dihydro-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone (**172**).

3.2.2.4 Isolation of Compound 173

Fraction B–8 (0.25 g) was fractionated on a silica gel 60 column (7% methanol in chloroform). Then, the wanted fractions were combined and further separated on a sephadex LH 20 column (MeOH), and then purified using MPLC on an ODS column (30% water in methanol). Compound **173** showed Rf value 0.25, using RP_{18} TLC (30% water in methanol) and detected under UV 254 nm. The compound was identified as 6‴-hydroxylophirone B (**173**) (20 mg, $8X10^{-4}$ % based on dry weight).

3.2.2.5 Isolation of Compound 27

Fraction B–9 (0.37 g) was subjected to column chromatography on a silica gel 60 column (10% methanol in chloroform). The fractions of interest were combined and separated on a sephadex LH 20 column (MeOH) and purified by MPLC on an ODS column (40% water in methanol) to give compound **27** (50 mg, $2X10^{-3}$ % based on dry weight). Compound **27** showed Rf value 0.21, using RP_{18} TLC (40% water in methanol) and detected under UV 254 nm. This compound was identified as lophirone A (**27**).

3.2.2.6 Isolation of Compound 21

Fraction B–10 (0.23 g) was seperated on a silica gel 60 column (7% methanol in chloroform). The fractions of interest were combined and further separated on a sephadex LH 20 column (MeOH) to give compound **21** (6 mg, $2.4X10^{-4}$ % based on dry weight). Compound **21** showed Rf value 0.34, using SiO_2 TLC (35% water in methanol) and detected under UV 254 nm. Compound **21** was identified as calodenone (**21**).

3.2.2.7 Isolation of Compound 174

Fraction B–14 (0.64 g) was separated on a sephadex LH 20 (MeOH) column, and further purified on an ODS MPLC column, using 50% water in methanol as eluent. Compound **174** (2 mg, $8X10^{-5}$ % based on dry weight) was obtained and showed Rf value 0.2 when developed on RP_{18} TLC (50% water in methanol) then detected under UV 254 nm. This compound was identified as 6‴-hydroxylophirone B 4‴-*O*-β-glucoside (**174**).

3.3 Extraction and Separation of the Stem Wood

3.3.1 Extraction

The dried and milled stem wood of *O. integerrima* (0.6 kg) was macerated with hexane (4x4 L) and then methanol (4x4 L) to yield a hexane extract (2 g) and a methanol extract (40 g). Each extract was tested with DPPH radical reagent (as described in Section 4). The extract with a positive result was further studied.

3.3.2 Separation

The methanol extract, giving a positive result with the DPPH radical test, was chromatographed on a silica gel 60 column. Elution was performed in a polarity gradient manner with chloroform and methanol. The eluates (200 ml each, Table 14)

were examined by TLC (silica gel, 30% ethyl acetate in hexane or 70% ethyl acetate in hexane). Fractions with similar chromatographic patterns were combined. (Table 15).

Table 14 Chromatographic fractions of the MeOH extract of the stem wood of *O. integerrima*

Number of eluate	Chloroform : Methanol
1 – 2	100 : 0
3 – 4	92 : 8
5 – 6	95 : 5
7 – 12	90 : 10
13 – 16	85 : 15
17 – 19	80 : 20
20 – 21	70 : 30
22 – 24	60 : 40
25 – 26	50 : 50
27 – 34	0 : 100

Table 15 Combination of fractions in Table 14

Number of eluate	Fraction	Weight (g)
1 - 2	ST – 1	0.20
3	ST – 2	0.14
4 – 5	ST – 3	0.06
6 – 7	ST – 4	0.04
8	ST – 5	0.37
9 – 10	ST – 6	0.15
11 – 14	ST – 7	0.22

Table 15 Combination of fractions in Table 14 (continued)

Number of eluate	Fraction	Weight (g)
15 –18	ST – 8	0.32
19 – 21	ST – 9	0.52
22 - 23	ST – 10	1.04
24 – 26	ST – 11	1.07
27 - 34	ST – 12	1.44

3.3.2.1 Isolation of Compound 26

Fraction ST-4 (0.04 g) was rechromatographed on a SiO_2 MPLC column (3% MeOH in chloroform). Compound **26** was obtained as a white solid (0.6 mg, $1X10^{-4}$ % based on dry weight) and identified as 5-hydroxy-4′-methoxy-6,7-methylenedioxy isoflavone (**26**). This compound showed Rf value 0.2 when developed on SiO_2 TLC (3% MeOH in chloroform) and detected under UV 254 nm.

3.3.2.2 Isolation of Compound 58

Fraction ST–5 (0.32 g) was separated by silica 60 column chromatography (20%, 21%, 22%, 23% 24% and 30% ethyl acetate in hexane, respectively). Fraction 7 was further purified on a SiO_2 MPLC column, using 10% hexane in chloroform as eluent to give compound **58** (1 mg, $1.6X10^{-4}$ % based on dry weight). This compound was identified as squarrosin (**58**). This compound showed Rf value 0.28 when developed on SiO_2 TLC (10% hexane in chloroform) and detected under UV 254 nm.

3.3.2.3 Isolation of Compounds 175 and 60

Fraction ST–6 (0.15 g) was rechromatographed on a silica 60 column, using 40% ethyl acetate in hexane as eluent. Fractions 3 and 4 were

combined and purified on a SiO_2 MPLC column, using 40% ethyl acetate in hexane as eluent to give compound **175** (3 mg, $5X10^{-4}$ % based on dry weight) and compound **60** (3 mg, $5X10^{-4}$ % based on dry weight). Compound **175** was identified as 5,4′-dimethoxy-6,7-methylenedioxy isoflavone (**175**) and compound **60** was identified as 5,3′,4′-trimethoxy-6,7-methylenedioxy isoflavone (**60**). These compounds showed Rf values 0.25 and 0.20, using SiO_2 TLC (40% ethyl acetate in hexane) and detected under UV 254 nm, respectively.

3.3.2.4 Isolation of Compound 176

Fraction ST–7 (0.22 g) was rechromatographed on a silica gel 60 column and eluted with 40%, 60%, 80% and 100% ethyl acetate in hexane, respectively. Fraction 10 was purified by SiO_2 MPLC, (20% ethyl acetate in hexane) to yield compound **176** (1 mg, $1.6X10^{-4}$ % based on dry weight). Fraction ST–8 (0.3198 g) was similarly separated to give compound **176** (3 mg, $5X10^{-4}$ % based on dry weight). Compound **176** was identified as gerontoisoflavone A (**176**). This compound showed Rf value 0.20 when developed on SiO_2 TLC (20% ethyl acetate in hexane) and detected under UV 254 nm.

3.3.2.5 Isolation of Compound 177

Fractions ST–9 (0.52 g) and ST–10 (1.04 g) were combined, dried and then separated by silica gel 60 column chromatography (40%, 60%, 80% and 100% ethyl acetate in hexane). Fraction 8 was further purified by SiO_2 MPLC (10% petroleum in ethyl acetate) to give compound **177** (3 mg, $5X10^{-4}$ % based on dry weight). This compound was identified as 4′,7-dihydroxy 5-methoxy isoflavone (**177**). This compound showed Rf value 0.32 when developed on SiO_2 TLC (10% petroleum ether in ethyl acetate) and detected under UV 254 nm.

3.4 Extraction and Separation of the Root Bark

3.4.1 Extraction

The dried and milled root bark of *O. integerrima* (1.2 kg) was extracted with hexane (4x10 L) and methanol (4x10 L) to give a hexane extract (3 g) and a methanol crude extract (45.02 g). Each extract was analyzed with DPPH radical reagent (as described in Section 4). The extract with a positive result was further studied.

3.4.2 Separation

The methanol extract, which gave a positive result with the DPPH radical test, was chromatographed on a silica gel column. Elution was performed in a polarity gradient manner (chloroform and methanol). The eluates (200 ml each, Table 16) were examined (SiO_2-TLC 20% methanol in chloroform or RP_{18}-TLC 50% water in methanol). Fractions with similar chromatographic pattern were combined. (Table 17).

Table 16 Chromatographic fractions of the MeOH extract of the root bark of *O. integerrima*

Number of eluate	Chloroform : Methanol
1 – 2	100 : 0
3 – 4	97 : 3
5 – 6	95 : 5
7 – 8	93 : 7
9 – 12	90 : 10
13 – 16	87 : 13
17 – 20	85 : 15
21 – 24	83 : 17

Table 16 Chromatographic fractions of the MeOH extract of the root bark of *O. integerrima* (continued)

Number of eluate	Chloroform : Methanol
25 – 28	80 : 20
29 - 32	75 : 25
33 – 36	70 : 30
37 – 38	60 : 40
39 – 40	50 : 50
41 - 42	0 : 100

Table 17 Combination of fractions in Table 16

Number of eluate	Fraction	Weight (g)
1 – 2	RB – 1	0.24
3 – 4	RB – 2	0.12
5	RB – 3	0.07
6 – 7	RB – 4	0.13
8 – 10	RB – 5	0.04
11 – 12	RB – 6	0.13
13 – 16	RB – 7	0.12
17 – 20	RB – 8	0.36
21 – 28	RB – 9	0.15
29 – 36	RB – 10	0.09
37 – 40	RB – 11	1.00
41 - 42	RB – 12	2.42

3.4.2.1 Isolation of Compound 178

Fraction RB–2 (0.12 g) was fractionated on a silica gel 60 column (15% ethyl acetate in hexane), and then purified by sephadex LH 20 (MeOH) gel filtration to give compound **178** (3 mg, $2.5X10^{-4}$ % based on dry weight). This compound was identified as *trans* tetracocyl ferulate (**178**). This compound showed Rf value 0.25 when developed on SiO_2 TLC (15% ethyl acetate in hexane) and detected under UV 254 nm.

3.4.2.2 Isolation of Compounds 175 and 60

Fractions RB–3 (0.07 g) and RB–4 (0.1294 g) were combined and rechromatographed on a silica gel 60 column, eluted with 40% and 50% ethyl acetate in hexane. Fraction 6 from this column was recrystalized from $CHCl_3$ and MeOH (1:1) to give compound **175** (3 mg, $2.5X10^{-4}$ % based on dry weight). Fraction 8 was purified on a sephadex LH 20 (acetone) to give compound **60** (2.5 mg, $2.0X10^{-4}$ % based on dry weight) after drying. Compounds **175** and **60** were identified as 5,4′-dimethoxy-6,7-methylenedioxy isoflavone (**175**) and 5,3′,4′-trimethoxy-6,7-methylenedioxy isoflavone (**60**), respectively.

3.4.2.3 Isolation of Compound 1

Fractions RB–5 (0.04 g) and RB–6 (0.13 g) were combined and rechromatographed on silica gel 60 (7% methanol in chloroform) to give compound **1** (2 mg, $1.6X10^{-4}$ % based on dry weight). This compound was identified as lophirone C (**1**).

3.4.2.4 Isolation of Compound 171

Fraction RB–7 (0.67 g) was separated on sephadex LH 20 (MeOH) to give compound **171** (3 mg, $2.5X10^{-4}$ % based on dry weight). This

compound was identified as 3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl) -1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone (**171**).

3.4.2.5 Isolation of Compounds 27 and 173

Fraction RB–9 (0.15 g) was separated on sephadex LH 20, (MeOH). Fraction 4 from this column was rechromatographed on an ODS-HPLC column (40% acetonitrile in water) to give compound **27** at 49.39 min and compound **173** at 64.96 min. Compounds **27** and **173** were identified as lophirone A (10 mg, $8.3X10^{-4}$ % based on dry weight), (2 mg, $1.6X10^{-5}$ % based on dry weight) and 6‴-hydroxylophirone B (5 mg, $4.0 X10^{-4}$ % based on dry weight), respectively.

3.4.2.6 Isolation of Compounds 21 and 27

Fraction RB–10 (0.09 g) was rechromatographed on a silica gel column (10% MeOH in chloroform). Fraction 4 from this column was purified using sephadex LH 20 (MeOH) to give compound **21** (4 mg, $3.3X10^{-4}$ % based on dry weight). Fraction 6 was purified on an ODS-HPLC column (30% water in methanol) to give compound **27** at 37.54 min (5 mg, $4.0X10^{-4}$ % based on dry weight). Compounds **21** and **27** were identified as calodenone (**21**) and lophirone A (**27**), respectively.

3.4.2.7 Isolation of Compounds 27 and 179

Fraction RB–11 (1.00 g) was separated on a sephadex LH 20 column (MeOH). Fraction 3 from this fraction was purified by ODS-HPLC (40% water in methanol) to give compound **179** (3 mg, $1.6X10^{-4}$ % based on dry weight) at 32.36 min and compound **27** (5 mg, $4X10^{-4}$ % based on dry weight) at 53.44 min. Compounds **179** and **27** were identified as 2,7,4′-trihydroxy isoflavone (**179**) and lophirone A (**27**), respectively. Compound **179** showed Rf value 0.38 when developed on RP_{18} TLC (40% water in methanol) and detected under UV 254 nm.

3.5 Extraction and Separation of the Root Wood

3.5.1 Extraction

The dried and milled root wood of *O. integerrima* (0.47 kg) was extracted with hexane (3x4 L) and MeOH (3X4 L) to yield a hexane extract (1 g) and a methanol extract (30.8 g). Each extract was tested with DPPH radical reagent (as described in Section 4). The extract which exhibited a positive result was further studied.

3.5.2 Separation

The methanol extract, showing a positive result with the DPPH radical test, was chromatographed on a silica gel 60 column. Elution was performed in a polarity gradient manner (chloroform and methanol). The eluates (200 ml each, Table 18) were examined by SiO_2-TLC and RP_{18}-TLC (10% methanol in chloroform and 30% water in methanol, respectively). Fractions with similar chromatographic patterns were combined as shown in Table 19.

Table 18 Chromatographic fractions from the MeOH extract of the root wood of *O. integerrima*

Number of eluate	Chloroform : Methanol
1 – 2	100 : 0
3 – 6	95 : 5
7 – 10	90 : 10
11 – 14	85 : 15
15 – 18	80 : 20
19 – 22	75 25
23 – 26	70 : 30

Table 18 Chromatographic fractions from the MeOH extract of the root wood of *O. integerrima* (continued)

Number of eluate	Chloroform : Methanol
27 – 30	65 : 35
31 - 34	60 : 40
35 – 36	50 : 50
37 – 38	0 : 100

Table 19 Combination of fractions in Table 18

Number of eluate	Fraction	Weight (g)
1 – 2	RST – 1	0.16
3	RST – 2	0.01
4	RST – 3	0.12
5 – 6	RST – 4	0.06
7	RST – 5	0.09
9 – 12	RST – 6	0.25
13 – 15	RST – 7	0.13
16 –19	RST – 8	0.17
20 – 27	RST – 9	0.02
28-35	RST – 10	0.20
36-38	RST – 11	2.54

3.5.2.1 Isolation of Compounds 175 and 60

Fraction RST–4 (0.06 g) was fractionated on a silica gel 60 column (15% ethyl acetate in hexane). Fractions 4-6 from this column were combined, dried and washed with a small volume of methanol to give compound **175** (3

mg, $6.3X10^{-4}$ % based on dry weight). This compound was identified as 5,4′-dimethoxy-6,7-methylenedioxy isoflavone (**175**). Fractions 7-9 were combined and washed with a small volume of methanol to give compound **60** (3 mg, $6.3X10^{-4}$ % based on dry weight). This compound was identified as 5,3′,4′-trimethoxy-6,7-methylenedioxy isoflavone (**60**).

3.5.2.2 Isolation of Compound 1

Fraction RST–5 (0.09 g) was fractionated on a silica gel 60 column (7% methanol in chloroform) to give compound **1** (6 mg, $1.2X10^{-3}$ % based on dry weight). This compound was identified as lophirone C (**1**).

3.5.2.3 Isolation of Compound 171

Fraction RST–7 (0.12 g) was separated on a sephadex LH 20 column (methanol) as eluent. Fraction 3 from this column was further purified using ODS-HPLC (15% water in methanol). Compound **171** was obtained at 69.67 min (2 mg, $4.2X10^{-4}$ % based on dry weight). This compound was identified as 3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)1-benzofuran-7-yl 2-(4-hydroxy-phenyl) ethenyl ketone (**171**).

3.5.2.4 Isolation of Compounds 172 and 173

Fraction RST–8 (0.17 g) was subjected to sephadex LH 20 (MeOH) column chromatography. Fractions 5-7 were combined, dried and purified by ODS-HPLC (20% water in methanol) to give compound **173** (2 mg, $4.2X10^{-4}$ % based on dry weight) at 38.22 min and compound **172** (0.8 mg, $1.7X10^{-4}$ % based on dry weight) at 45.34 min. Compounds **173** and **172** were identified as 6‴-hydroxylophirone B (**173**) and 3-(2,4-dihydroxybenzoyl)-2,3-dihydro-4,6-dihydroxy-2-(4-hydroxyphenyl) 1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone (**172**), respectively.

3.5.2.5 Isolation of Compound 177

Fraction RST–10 (0.21 g) was separated on a sephadex LH 20 column. Fraction 3 was purified on an ODS-HPLC column, (25% water in methanol) to give compound **177** (1 mg, $2.1X10^{-4}$ % based on dry weight) at 8.32 min. Compound **177** was identified as 4′,7-dihydroxy 5-methoxy isoflavone (**177**).

3.5.2.6 Isolation of Compounds 21, 27 and 180

Fraction RST–11 (2.54 g) was separated on a sephadex LH 20 (MeOH) column. Fraction 3 from this column was further purified on an ODS-HPLC column (25% water in methanol) to give compound **27** (10 mg, $2.1X10^{-3}$ % based on dry weight) at 44.15 min, compound **180** (2 mg, $4.2X10^{-4}$ % based on dry weight) at 59.18 min and compound **21** (4 mg, $8.4X10^{-4}$ % based on dry weight) at 76.83 min. Compounds **27**, **180** and **21** were identified as lophirone A (**27**), protocatechuic acid (**180**) and calodenone (**21**), respectively. Compound **180** showed Rf value 0.2 when developed on RP_{18} (25% water in methanol) and detected under UV 254 nm.

4. DPPH Free Radical Scavenging Activity Assay of Pure Compounds

4.1 Preliminary Screening

DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as source of free radicals. Samples were spotted on a TLC plate and then developed with appropriate solvent. After drying, the TLC plate was sprayed with a solution of 0.2% DPPH in ethanol, and left for 30 min before examination. Active compounds, which reduced DPPH radical, appeared as yellow spots against a purple background (Scheme 1). Quercetin (**83**) was used as a positive control (Cuendent, Hostettmann and Potterat, 1997).

4.2 IC_{50} Determination

A solution of 100 μM DPPH radical in ethanol was prepared by dissolving 2 mg of DPPH radical in ethanol (100 ml). The reaction was carried out in a 96-well microtiter plate. The test sample was prepared by dissolving the extract or compound (1 mg) in ethanol or dimethyl sulfoxide (3 ml). Each well contained 20 μl of test sample and 180 μl of DPPH solution and was incubated at 37 °C for 30 min. The absorbance was measured at 510 nm. Percentage of radical scavenging activity of the test sample was determined by comparison with a DPPH solution as blank. Test samples with >50% inhibitory activity were selected for further analysis.

A series of solutions of the active sample was prepared by two-fold dilution to give 7 test solutions of different concentrations (~100-0.1 mM). Each solution was tested as previously described. An IC_{50} value which denotes the concentration of sample which is required to scavenge 50% DPPH free radicals (Ur-Rahman, *et al.*, 2001) was then determined. Quercetin (**83**) was used as a positive control.

DPPH·
(Purple)

DPPH-H
(Pale yellow)

DPPH· = 2,2-diphenyl-1-picrylhydrazyl

AH = antioxidant

Scheme 1 Antioxidant as DPPH free radical scavenger

5. Physical and Spectral data of Isolated Compounds

5.1 Characterization of Compound 47

Compound **47** was obtained as a yellow solid, soluble in DMSO.

FABMS : *m/z* (% relative intensity) 553 $C_{31}H_{21}O_{10}$ ($[M+H]^+$, 1), 307 (22), 289 (2), 154 (100), 136 (93), 107 (23), 89 (20), 39 (10)

UV : λ_{max} nm, in methanol 332, 287, 270, 246, 211

^{1}H NMR : δ ppm, 600 MHz in DMSO-d_6, see Table 21 page 76

^{13}C NMR : δ ppm, 150 MHz in DMSO- d_6, see Table 21 page 76

5.2 Characterization of Compound 4

Compound **4** was obtained as a yellow solid, soluble in DMSO.

FABMS : *m/z* (% relative intensity) 539 $C_{30}H_{19}O_{10}$ ($[M+H]^+$, 2), 307 (11), 289 (11), 273 (1), 242 (2), 197 (2), 154 (100), 107 (15)

UV : λ_{max} nm, in methanol 332, 284, 270, 246, 211

^{1}H NMR : δ ppm, 500 MHz in DMSO-d_6, see Table 22 page 78

^{13}C NMR : δ ppm, 125 MHz in DMSO-d_6, see Table 22 page 78

5.3 Characterization of Compound 170

Compound **170** was obtained as a yellow solid, soluble in methanol.

FABMS : *m/z* (% relative intensity) 373 $C_{20}H_{21}O_7$ ($[M+H]^+$, 25), 317 (27), 307 (15), 154 (100), 136 (70), 107 (20), 89 (25), 77 (15)

IR : ν cm^{-1}, KBr disc 3337, 1632, 1112

UV : λ_{max} nm, in methanol 295, 245, 204

^{1}H NMR : δ ppm, 600 MHz in MeOH-d_4, see Table 23 page 80

^{13}C NMR : δ ppm, 150 MHz in MeOH-d_4, see Table 23 page 80

5.4 Characterization of Compound 1

Compound **1** was obtained as a yellow solid, soluble in acetone.

$[\alpha]^{20}_{D}$: 0.24 (*c* 0.109, in MeOH 100 ml)

FABMS : *m/z* (% relative intensity) 511 $C_{30}H_{23}O_8$ ($[M+H]^+$, 1), 391 (1), 375 (1), 329 (1), 307(40), 154 (100), 107 (20), 89 (15)

UV : λ_{max} nm, in methanol 370, 391, 286, 254, 203

^{1}H NMR : δ ppm, 600 MHz in acetone-d_6, see Table 24 page 82

^{13}C NMR : δ ppm, 150 MHz in acetone-d_6, see Table 24 page 82

5.5 Characterization of Compound 171

Compound **171** was obtained as an orange solid, soluble in acetone.

FABMS : *m/z* (% relative intensity) 525 $C_{30}H_{21}O_9$ ($[M+H]^+$, 5), 277 (6), 241 (4), 185 (90), 149 (10), 93 (100), 75 (35), 57 (35), 45 (20)

IR : ν cm^{-1}, KBr disc 2926, 1741, 1441, 1241, 1172, 807

UV : λ_{max} nm, in methanol 359, 342, 299, 259, 201

^{1}H NMR : δ ppm, 400 MHz in acetone-d_6, see Table 25 pages 84-85

^{13}C NMR : δ ppm, 100 MHz in acetone-d_6, see Table 25 pages 84-85

5.6 Characterization of Compound 172

Compound **172** was obtained as a yellow solid, soluble in acetone.

$[\alpha]^{20}_{D}$: 0.72 (*c* 0.097, MeOH)

FABMS : *m/z* (% relative intensity) 527 $C_{30}H_{23}O_9$ ($[M+H]^+$, 4), 277 (6), 241 (6), 185 (100), 149 (25), 93 (100), 75 (45), 57 (25), 45 (19)

IR : ν cm^{-1}, KBr disc 3647, 3110, 2358, 2330, 1625, 1169, 979

UV : λ_{max} nm, in methanol 368, 300, 285, 261, 202

^{1}H NMR : δ ppm, 600 MHz in acetone-d_6, see Table 26 page 87

^{13}C NMR : δ ppm, 150 MHz in acetone-d_6, see Table 26 page 87

5.7 Characterization of Compound 173

Compound **173** was obtained as a yellow solid, soluble in acetone.

$[\alpha]^{24}_{D}$: -55.2 ° (*c* 0.33, MeOH)

Melting point : 180-182 °C (uncorrected)

CD : $[\theta]_{nm}$, in MeOH $[\theta]_{378.6\ nm}$ $-5.4x10^3$, $[\theta]_{323.2\ nm}$ $+5.5x10^3$, $[\theta]_{288.2\ nm}$ $-3.7x10^4$, $[\theta]_{251.8\ nm}$ $+6.1x10^3$, $[\theta]_{215.4\ nm}$ $+8.6x10^4$

HRFABMS : *m/z* 527.1362 $C_{30}H_{23}O_9$ $[M+H]^+$

FABMS : *m/z* (% relative intensity) 527 ($[M+H]^+$, 3), 277 (7), 241 (4), 185 (80), 149 (11), 93 (100), 75 (300), 57 (23), 45 (14), 19 (5)

IR : ν cm^{-1}, KBr disc 3300, 1628, 1225, 1161, 825

UV : λ_{max} nm (log ε), in methanol 374 (5.08), 292 (4.93), 203 (5.31)

^{1}H NMR : δ ppm, 600 MHz, in acetone-d_6, see Table 27 page 90

^{13}C NMR : δ ppm, 150 MHz , in acetone-d_6, see Table 27 page 90

5.8 Characterization of Compound 27

Compound **27** was obtained as a white solid, soluble in acetone.

$[\alpha]^{20}_{D}$: -1.58 (*c* 0.10, MeOH)

FABMS : *m/z* (% relative intensity) 511 $C_{30}H_{23}O_8$ ($[M+H]^+$, 2), 277 (13), 241 (11), 185 (100), 149 (24), 117 (5), 93 (100), 75 (65), 57 (36), 45 (28), 19 (10)

IR : ν cm^{-1}, KBr disc 3170, 1627, 1199, 1139, 974, 788

UV : λ_{max} nm, in methanol 286, 263, 234, 227, 202

^{1}H NMR : δ ppm, 400 MHz, in acetone-d_6, see Table 28 pages 93-94

^{13}C NMR : δ ppm, 100 MHz, in acetone-d_6, see Table 28 pages 93-94

5.9 Characterization of Compound 21

Compound **21** was obtained as a white solid, soluble in acetone.

$[\alpha]^{20}_{D}$: -4.96 (*c* 0.11, MeOH)

FABMS : *m/z* (% relative intensity) 525 $C_{31}H_{25}O_8$ ($[M+H]^+$, 5), 327 (6), 277 (8), 257 (7), 185 (100), 149 (18), 93 (100), 75 (50), 57 (30), 45 (22), 19 (9)

IR : ν cm^{-1}, KBr disc 3141, 2922, 2852, 2359, 1628, 1132, 956

UV : λ_{max} nm, in methanol 284, 262, 233, 227, 202

^{1}H NMR : δ ppm, 400 MHz, in acetone-d_6, see Table 29 pages 96-97

^{13}C NMR : δ ppm, 100 MHz, in acetone-d_6, see Table 29 pages 96-97

5.10 Characterization of Compound 174

Compound **174** was obtained as a yellow solid, soluble in acetone.

$[\alpha]^{24}_D$: -0.5 ^{o} (*c* 0.09, MeOH)

Melting point : 263-265 ^{o}C (uncorrected)

CD : $[\theta]_{nm}$, in MeOH $[\theta]_{352.6\,nm}$ $+3.8x10^3$, $[\theta]_{287.0\,nm}$ $-2.0x10^4$, $[\theta]_{246.6\,nm}$ $+3.1x10^3$, $[\theta]_{229.4\,nm}$ $-7.5x10^3$, $[\theta]_{217.6\,nm}$ $+4.9x10^3$, $[\theta]_{208.4\,nm}$ $-1.3x10^{-4}$

HRFABMS : *m/z* 689.1837 $C_{36}H_{33}O_{14}$ $[M+H]^+$

FABMS : *m/z* (%relative intensity) 689 ($[M+H]^+$, 1), 359 (3), 307 (61), 289 (29), 154 (100), 137 (100), 107 (30), 77 (22), 39 (8)

IR : ν cm^{-1}, KBr disc 3343, 2924, 1632, 1228, 1172, 834, 669

UV : λ_{max} nm (log ε), in methanol 369 (4.30), 285 (4.30), 205 (4.70)

^{1}H NMR : δ ppm, 600 MHz, in acetone-d_6, see Table 30 pages 99-100

^{13}C NMR : δ ppm, 150 MHz , in acetone-d_6, see Table 30 pages 99-100

5.11 Characterization of Compound 26

Compound **26** was obtained as a white solid, soluble in chloroform.

FABMS : *m/z* (% relative intensity) 313 $C_{17}H_{13}O_6$ ($[M+H]^+$, 29), 289 (11), 154 (88), 136 (66), 69 (100), 55 (63), 43 (50)

IR : ν cm^{-1}, KBr disc 2930, 1783, 1679, 1185, 942

UV : λ_{max} nm, in methanol 271, 236, 201

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$, see Table 31 page 102

5.12 Characterization of Compound 58

Compound **58** was obtained as a white solid, soluble in chloroform.

FABMS : *m/z* (% relative intensity) 343 $C_{18}H_{15}O_7$ ($[M+H]^+$, 20), 307 (20), 154 (100), 136 (65), 107 (17), 89 (15), 77 (13), 39 (5)

IR : ν cm^{-1}, KBr disc 2970, 2355, 1679, 1100, 930

UV : λ_{max} nm, in methanol 273, 241, 219, 212, 202

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$, see Table 32 page 104

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see Table 32 page 104

5.13 Characterization of Compound 175

Compound **175** was obtained as a yellow solid, soluble in $CHCl_3$.

FABMS : *m/z* (% relative intensity) 327 $C_{18}H_{15}O_6$ ($[M+H]^+$, 100), 307 (20), 289 (11), 154 (97), 136 (65), 107 (17), 89 (15), 77 (14), 39 (6)

UV : λ_{max} nm, in methanol 323, 307, 264, 235, 203

^{1}H NMR : δ ppm, 600 MHz, in acetone-d_6, see Table 33 page 106

^{13}C NMR : δ ppm, 150 MHz, in acetone-d_6, see Table 33 page 106

5.14 Characterization of Compound 60

Compound **60** was obtained as a white solid, soluble in $CHCl_3$.

FABMS : *m/z* (% relative intensity) 357 $C_{19}H_{17}O_7$ ($[M+H]^+$, 44), 307 (27), 289 (12), 154 (97), 136 (65), 107 (17), 89 (15), 77 (14)

UV : λ_{max} nm, in methanol 266, 240, 203

^{1}H NMR : δ ppm, 600 MHz, in $CDCl_3$, see Table 34 page 108

^{13}C NMR : δ ppm, 150 MHz, in $CDCl_3$, see Table 34 page 108

5.15 Characterization of Compound 176

Compound **176** was obtained as a white solid, soluble in DMSO.

FABMS : *m/z* (% relative intensity) 315 $C_{17}H_{15}O_6$ ($[M+H]^+$, 45), 289 (15), 154 (100), 136 (64), 107 (25), 77 (10), 39 (5)

UV : λ_{max} nm, in methanol 257, 232, 203

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6, see Table 35 page 110

^{13}C NMR : δ ppm, 125 MHz, in DMSO-d_6, see Table 35 page 110

5.16 Characterization of Compound 177

Compound **177** was obtained as a white solid, soluble in DMSO.

FABMS : *m/z* (% relative intensity) 285 $C_{16}H_{13}O_5$ ($[M+H]^+$, 30), 107 (15), 89 (12), 77 (10)

UV : λ_{max} nm, in methanol 256, 227, 202

^{1}H NMR : δ ppm, 600 MHz, in DMSO-d_6, see Table 36 page 112

^{13}C NMR : δ ppm, 150 MHz, in DMSO-d_6, see Table 36 page 112

5.17 Characterization of Compound 178

Compound **178** was obtained as a white solid, soluble in $CHCl_3$.

FABMS : *m/z* (% relative intensity), 531 $C_{34}H_{59}O_4$ ($[M+H]^+$, 9), 502 (12), 307 (13), 289 (12), 195 (24), 177 (100), 136 (75), 55 (35), 43 (25)

UV : λ_{max} nm, in methanol 218, 234, 324

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$, see Table 37 page 113

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$, see Table 37 page 113

5.18 Characterization of Compound 179

Compound **179** was obtained as a white solid, soluble in acetone.

$[\alpha]^{20}_D$: -4.29 ^{o} (*c* 0.09, in 100 ml MeOH)

FABMS : *m/z* (% relative intensity) 273 $C_{15}H_{13}O_5$ ($[M+H]^+$, 5), 307 (29), 134 (100), 107 (16), 77 (10)

UV : λ_{max} nm, in methanol 220, 258, 295

^{1}H NMR : δ ppm, 300 MHz, in acetone-d_6, see Table 38 page 115

^{13}C NMR : δ ppm, 75 MHz, in acetone-d_6, see Table 38 page 115

5.19 Characterization of Compound 180

Compound **180** was obtained as a white solid. It was soluble in acetone.

EIMS : *m/z* (%relative intensity) 154 $C_7H_6O_4$ (M^+, 100), 137 (96), 109 (23), 81 (11), 63 (11)

UV : λ_{max} nm, in methanol 220, 258, 295

^{1}H NMR : δ ppm, 300 MHz, in acetone-d_6, see Table 39 page 116

^{13}C NMR : δ ppm, 75 MHz, in acetone-d_6, see Table 39 page 116

6. The DPPH Free Radical Scavenging Activity

In this study, separation of the extracts from *O. integerrima* gave 19 compounds. Due to the limited amount of the isolates, only 9 compounds were tested. Compounds **171**, **177** and **179** show moderate activities whereas compounds **1**, **21**, **27**, **60**, **173** and **175** exhibited weak activities. The IC_{50} values of compounds **171**, **177** and **179** were calculated (Table 20).

Table 20 The DPPH radical scavenging activity of pure compounds from *O. integerrima*

Compound	Concentration (mg/ml)	Concentration (mM)	% inhibition	IC_{50} (μM)
1	$4X10^{-4}$	0.76	30.7	-[d]
21	$3X10^{-4}$	0.63	7.6	-[d]
27	$4X10^{-4}$	0.78	15.5	-[d]
60	$4X10^{-4}$	1.21	30.7	-[d]
171	$4X10^{-4}$	0.76	77.0	20.0
173	$4X10^{-4}$	0.76	17.1	-[d]
175	$3X10^{-4}$	1.02	15.9	-[d]
177	$3X10^{-4}$	0.95	60.9	78.9
179	$4X10^{-4}$	1.34	85.5	18.0
quercetin (83)	$2x10^{-4}$	0.52	85.9	1.7

-[d] = not determined