STUDY ON CONSTITUENTS IN MYANMAR TRADITIONAL MEDICINAL PLANT, *Holoptelea integrifolia* (ROXB.) PLANCH

Arnt Win[1], Aye Mon Thida Nyo[2], Yoshiaki Takaya[3], Masatake Niwa[4], Than Than Aye[5]

Abstract

Holoptelea integrifolia (Roxb.) Planch (Ulmaceae) is a tree widely distributed on the Myanmar, India, Indochina and Asia, and it is locally called in Myanmar as "Pyauk-seik". The various extracts of stem bark of *H.integrifolia* were analyzed by Thin Layer and Column Chromatography method. The stem barks of *H. integrifolia* were collected from Chaik Village, Pakokku Township, Magway Region, Myanmar. They were washed, driedin shade, weighed and cut into small pieces and extracted in methanol. The fractionation of crude extract, followed by the addition of distilled water, n-hexane , ethyl acetate and n-butanol to an aqueous portion of each solvent, to obtain the dried masses of each four layers. Three pure compounds such as new 2-(2-hydroxyethylamino)-1,4 -naphthoquinone (**1**),(-)-syringaresinol (**2**), and(+) (4S)-4-hydroxy-α-tetralone (**3**) are isolated from ethyl acetate portion of this plant. The structures of these compounds were elucidated by analysis of NMR spectroscopic and mass spectrometric data. Although compound (**1**) is synthetically known, to the best of our knowledge, there is no previous report of its natural occurrence. The compounds (**2** and **3**) are described for first time from *H. integrifolia* species. Moreover, the antioxidant activity of compound**3**was evaluated by measuring the half inhibition concentration (IC_{50}) using chemiluminescent method. This isolated compound was found to exhibit significant antioxidant property which is comparable to standard ascorbic acid, at a specific concentration.

Keywords: *Holoptelea integrifolia* (Roxb.) Planch, 2-(2-hydroxyethyl amino)-1,4-naphthoquinone, (-)-syringaresinol, (+)(4S)-4-hydroxy- α-tetralone, chemiluminescent method, antioxidant activity

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[1] Associate Professor, Dr, Department of Chemistry, Kyaukse University

[2] Associate Professor, Dr, Department of Chemistry,University of Mandalay

[3] Associate Professor, Dr, Department of Chemistry(Shigen), Meijo University,Japan

[4] Professor, Dr, Department of Chemistry(Shigen), Meijo University,Japan

[5] Professor and Head, Dr, Department of Chemistry, Kyaukse University

Introduction

Holoptelea integrifolia(Roxb.) Planch belongs to the family Ulmaceae, having 15 genera and about 200 species,commonly known as Myanmar name "Pyauk-seik". The native distribution of the plant can be seen in Asia-Tropical region including India, Nepal, Sri Lanka, Indo-China, Cambodia, Laos, Myanmar, Vietnam and China (Bamhole and Jiddewar, 1985).

H. integrifolia is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism. Bark and leaves are used as bitter, astringent, thermogenic, anti-inflammatory digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism. Bark and leaf paste of the plant are applied externally on the white patches or leucoderma (Kumar *et al*., 2012).

The stem bark of *H.integrifolia* consists of the β-sitosterol, lupeol, ellagic acid and β-sitosterol-glucoside. 2-aminonaphthoquinone, Friedlin, β-sitosterol, and β-D-glucose are also isolated from stem bark (Sharma *et al*., 2005). 1,4-Naphthalenedione has been isolated from leaves of *H.integrifolia* and is reported to possess antibacterial activity against *Staphylococcus aureus* (Vinod *et al*., 2010). Hexacosanol, octacosanol, β-sitosterol, β-amyrin are isolated from leaves. β-sitosterol, 2",3"-dihydroxyoelan-12-en-28 oic acid and hederagenin are isolated from heartwood (Rastogi and Mehrotra., 1991).

H.integrifolia has been reported that various parts and different kinds of extracts of this plant showed the several biological activities, such as, anti-inflammatory activity, antioxidant activity, antimicrobial activity, antiviral activity, wound-healing activity, anthelminitic activity, antidiabetic activity, antidiarhoeal activity, adaptogenic activity, antitumor activity, and cross reactivity(Kumar *et al*., 2012).Althoughvarious parts and different kinds of extracts of this plant have been measured on the many biological activities, a very little test has been done on the biological activity and plausible medicinal applications of isolated compounds. A drug development programme should be undertaken to develop modern drugs with the compounds isolated from *H. integrifolia*. Hence, extensive investigation is needed to exploit their therapeutic utilities to combat diseases. And *H. integrifolia*, the versatile

medicinal plant is the unique source of various types of bioactive compounds having diverse chemical structures. Therefore, extensive research is needed to get more bioactive and interesting new compounds from this versatile medicinal plant,*H. integrifolia*.

In this paper, we report a simple and rapid antioxidant assay by chemiluminescence. The method is based on antioxidant-dependent quenching of chemiluminescence generated from lipid hydroperoxide and isoluminol/microperoxidase reagent. This method was used to evaluate the antioxidant ability of various antioxidants by measuring half inhibition concentration (IC_{50}). When an antioxidant is present in the assay mixture, it scavenges the oxyradical and quenches the production of light. Thus, by using a constant amount of lipid hydroperoxide (oxyradical donor) the ability of antioxidants can be estimated as the decrease in chemiluminescence. We employed cumene hydroperoxide as the oxyradical donor and improved the assay conditions to ensure reproducibility. The method was used effectively to evaluate the antioxidative ability of a wide range of natural and synthetic components and the results were compared to the half inhibition concentration (IC_{50}) of standard ascorbic acid (Osamu *et al*., 1997).

The aim of this paper is to study the more affective and new chemical constituents of*H. integrifolia*.In the present investigation, we report the isolation and structural elucidation of one new 2-(2-hydroxyethylamino)-1,4-naphthoquinone (**1**),(-)-syringaresinol (**2**),and(+) (4S)-4-hydroxy-α-tetralone (**3**) from the ethyl acetate extract of bark of *H.integrifolia*. Compounds(**2** and **3)**have not been previously isolated or reported from the bark of this variety.

Materials and Methods

IR spectrum was recorded on FT-IR-410 spectrophotometre.^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL ECA-500 (^{1}H: 500 MHz and ^{13}C: 125 MHz). Chemical shifts for^{1}H- and ^{13}C-NMR are given in parts per million (δ) relative to solvent signal (Chloroform-D: δH 7.24 and δC 76.9) as internal standard. EI-Mass spectrum was obtained with a JEOL JMSMS-700 and HX-110, respectively. Optical rotation was recorded on a JASCO P-1020 polarimeter (cell length 100 mm).Analytical TLC was performed on Silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel BW-

820MH (Fuji Silysia Chemicals, Co. Ltd, Seto, Japan). Isoluminol (6-amino-2,3-dihydro-1,4-phthazinedione), microperoxidase, ascorbic acid and cumene hydroperoxide were purchased from Wako Pure Chemical Industries (Osaka Japan).

Plant Material

The stem bark of *Holoptelea integrifolia* (Roxb.) Planch (Ulmaceae) (Figure 1), Myanmar name Pyauk-seik was collected from Chaik Village, Pakokku Township, Magway Region, Myanmar, in April 2012.

Figure 1 *Holoptelea integrifolia* (Roxb.) Planch (Ulmaceae)

Extraction and Isolation

The air-dried stem bark of *H.integrifolia* was extracted with MeOH at room temperature for one month. The MeOH extract was concentrated and the residue (30.0 g) was suspended in water. This suspension was successively extracted with n-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate soluble extract was concentrated by rotary evaporator to produce a residue (2.85 g). The extract was fractionated on a silica gel column using *n*-hexane and ethyl acetate gradient to afford seven fractions (frs. 2.1 - 2.7). Fraction 2.2was rechromatographed over silica gel eluted with n-hexane and ethyl acetate (4:1) to yield compound **3** (116.1 mg). Fraction 2.5 was subjected to column chromatography over silica gel using chloroform and methanol and the eluate was separated into fractions (2.5.1 – 2.5.3). Fraction 2.5.1 was rechromatographedover silica gel eluted with same solvent mixtures followed by preparative TLC (MeOH−$CHCl_3$, 1:19) to yield compound **1** (1.6 mg). Fraction 2.5.2 was further subjected to column chromatography on silica gel using same eluent mixtures of increasing polarity followed by preparative TLC (MeOH−$CHCl_3$, 1:19) to give compound **2** (3.6 mg).

Determination of Antioxidant Activity

Preparation of chemiluminescent reagent and oxyradical donor

Aqueous sodium borate solution (0.5 M) containing 0.2 mM EDTA was prepared, and its pH was adjusted to 10 with sodium hydroxide. Isoluminol (18 mg) was dissolved in 70 mL of methanol and 30 mL of the above described borate solution, and microperoxidase (10 mg) was dissolved in 10 mL of 70% methanol. Both solutions were stored at -20°C. The isoluminol and microperoxidase solution were mixed in a volume ratio of 100:1 before use, and used as the chemiluminescent reagent.

Cumene hydroperoxide [30 μL, corresponding to 160 μmol, which was determined by idometry] was dissolved in 50 mL of ethanol containing 5 mM EDTA and stored at -20°C. The solution was diluted 200 times with methanol before use, and used as the oxyradical donor (16 μM).

Preparation of sample

The pure compound was prepared inmethanol with various concentrations such as 0.02 $mgmL^{-1}$, 0.04 $mgmL^{-1}$, 0.06 $mgmL^{-1}$, 0.08 $mgmL^{-1}$, and 0.1 $mgmL^{-1}$,respectively.

Standard assay for antioxidant activity

A photo counter (Model AB-2200/-R Luminescence PSN, Japan) was used for the determination of chemiluminescence. 130 μL cumene hydroperoxide solution and 25 μL sample were placed in a 0.8 mL glass tube, and the tube was set in the instrument. The chemiluminescent reagent (200 μL) was put into the vial. The chemiluminescence produced was measured for 3 min. The control was performed in the same manner in the mixture, replacing the sample solution with methanol.

Results and Discussion

The concentrated methanolic extract of the stem bark of *H.integrifolia* was fractionated with distilled water, n-hexane, ethyl acetate and n-butanol. The ethyl acetate crude extract was separated by chromatography on a

silica gel column using hexane-ethyl acetate and chloroform-methanol from non-polar to polar with various ratios to obtain one new compound, 2-(2-hydroxyethylamino)-1,4-naphthoquinone, (-)-syringaresinol,and(+) (4S)-4-hydroxy-α tetralone compounds.

2-(2-hydroxyethylamino)-1,4-naphthoquinone (**1**) was obtained as orangecrystal. The molecular formula of **1** was determined to be $C_{12}H_{11}O_3N$ from the observation of a molecular ion peak at m/z 217 [M^+] on EI mass spectrometry. The FT IR spectrum exhibited absorption bands at 3600-3200, 3066.26, 2932.23, 2863.77, 1682.59, 1600.63, 1561.09, 1518.67, 1367.28, and 1082.83 cm^{-1}, ascribable to hydroxyl, amine, sp^2 H/C, sp^3 H/C, carbonyl, aromatic ring, and C-N amine functional groups, respectively. The ^{1}H- and ^{13}C-NMR(DMSO, Table-1) spectra of **1** showed the presence of four aromatic protons coupled with each other[δH 7.98 (1H, dd, J= 7.6,1.3 Hz; H-8), 7.93 (1H, dd, J= 7.6,1.3 Hz; H-5), 7.83 (1H, td, J= 7.6,1.3 Hz; H-6) and 7.72 (1H, td, J= 7.6,1.3 Hz; H-7],one singlet sp^2 methine proton bearinga carbonyl group[δH5.73 (1H, s; H-3)], oneoxygenated methylene [δH3.59 (2H, t, *J* = 5.7 Hz; H-2′)], another one nitrogen bearing methylene [δH 3.25 (2H, t, *J* = 5.7 Hz; H-1′)] , three quaternary carbons [148.7 (C-2),130.3 (C- 9), 133.2 (C- 10)], and two carbonyl groups [181.5 (C- 1), 181.4 (C- 4)]. All above data indicated that **1** is 1,4-naphthoquinone derivative. Moreover, the ^{1}H–^{1}H COSY experiment on **1**indicated the presence of two partial structures which are ortho-disubstituted benzene ring andethyl alcohol group (-CH_2-CH_2-OH).In the^{1}H and ^{13}C -NMR and ^{1}H–^{1}H COSY spectra, the proton signals due to an ortho-disubstituted aromatic ring, singlet methane proton and two carbonyl carbons suggested the 2-amino-1,4-naphthoquinone moiety in **1**. The two fragments, 1,4-naphthoquinone and ethyl group could be connected by NH group flank between these two groups in which the downfield chemical shift methylene group [δH 3.25 (2H, t, *J* = 5.7 Hz; H-1′)] and downfield chemical shift sp^2 quaternary carbon, [148.7 (C-2)]are observed in ^{1}H NMR and ^{13}C NMR spectra respectively (Table 1). It was confirmed by NOESY experiment. On the basis of the above evidence, the structure of **1** was determined to be 2-(2-hydroxyethylamino)-1,4-naphthoquinone **1**,as shown in

Figure 2. In addition, the spectral data of **1** were identical to the previously reported for synthesized 2-(2-hydroxyethylamino)-1,4-naphthoquinone (Cao *et al.,* 2009).

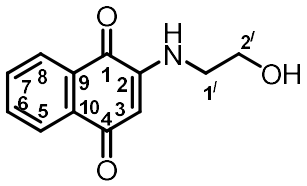

Figure 2 Structure of Compound **1**

(-)-Syringaresinol**2** isolated as colorless crystal, possessed the molecular formula $C_{22}H_{26}O_8$ as assigned by EI-MS ion at m/z 418 $[M]^+$ which is stable base peak, calculating ten degrees of unsaturation. In EIMS spectrum, the observation of molecular ion peak m/z at 418 indicated that this pure compound is dimmer (lignan). The ^{1}H NMR spectral data(Table 2) revealed the four typical singlet with same chemical shift of methoxy groups at δ_H 3.90, two same chemical shift of ether bearing methylene groups at δ_H 4.28 and 3.91, two same chemical shift of methine protons at δ_H4.73 (oxygenated and benzylic), another two same chemical shift methine protons at δ_H 3.07 and two same tetrasubstituted benzene rings which contain two equivalent singlet protons at δ_H 6.58. The ^{13}C NMR, HMQC and DEPT spectral data displayed the presence of eight quaternary carbons (six ether bearing and two phenolic), four sp^2 methine carbons (same chemical shift), four sp^3 methine carbons (two oxyganated and benzylic), two methylene carbons (same chemical shift), and four methoxy carbons (same chemical shift). In DQF-COSY spectrum, the observation of medium graphic area of methine proton (H-1 and H-5) with other ones (H-2 and H-6) and methylene protons (H-4a, H-4b, H-8a and H-8b) indicated the CH-2– CH-1– CH_2-8 and CH-4– CH-5– CH_2-6 fragments. Moreover, the connectivity of these fragments to C-1′, and C-1″ was established by HMBC correlations H-1′/C-2, H-6′/C-2, H-1″/C-6, H-6″/C-6, H-2/C-1′, H-2/C-6′, H-6/C-2″, and H-6/C-6″. Since two aromatic rings of **2** accounted for eight out of ten units of unsaturation, the remaining two units indicated two pentacyclic systems. The above all spectral data strongly

suggested that **2** was a syringaresinol lignan. Moreover, these spectral values were in good agreement with values previously reported for syringaresinol (Abulajiang *et al*., 2012).

The relative configuration (+)-syringaresinol (Reference compound)at H-1 and H-2 could be determined by the3J1,2, H-C-C-H (12.4 Hz) coupling constant which indicated that the two protons were located opposite side with dihedral angle 180° and H-2 and H-6 were both axial (Figure 3).By comparing the ^{1}H-NMR data of (-)-syringaresinol (Reference compound) [3J1,2, H-C-C-H (4.3 Hz)] with those of compound **2**, the relative configuration of compound **2**at H-1 and H-2 could be assigned by the3J1,2, H-C-C-H (4.5 Hz) coupling constant which revealed that the two protons were located axial-equatorial like position. According to the above previously reported data and NMR data of **2** showed that in the isolated compound, H-2 (d, 4.5 Hz) and H-6 (d, 4.5 Hz) were both in equatorial position (Figure 4). Moreover, the relative configurations between H-2 (δ_H4.73) with H-8b (δ_H 3.91) and H-1 (δ_H3.07) with H-2'(δ_H6.58) were also established as cis-configuration by an NOE experiment. Hence, **2** was detected to be (-) syringaresinol (Figure 4).

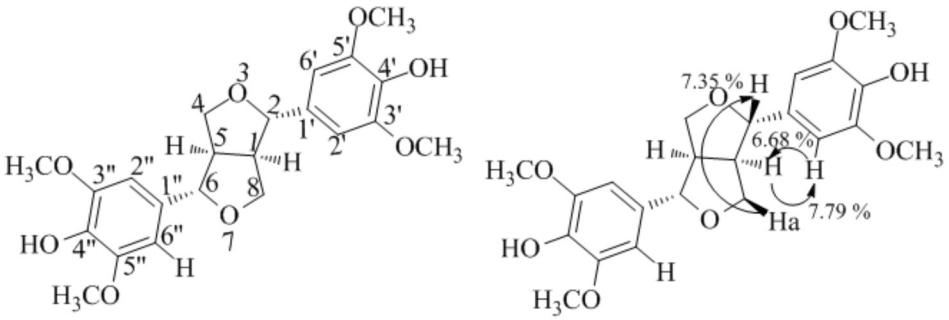

Figure 3 Structure of (+)-syringaresinol (reference compound) and NOE experiment

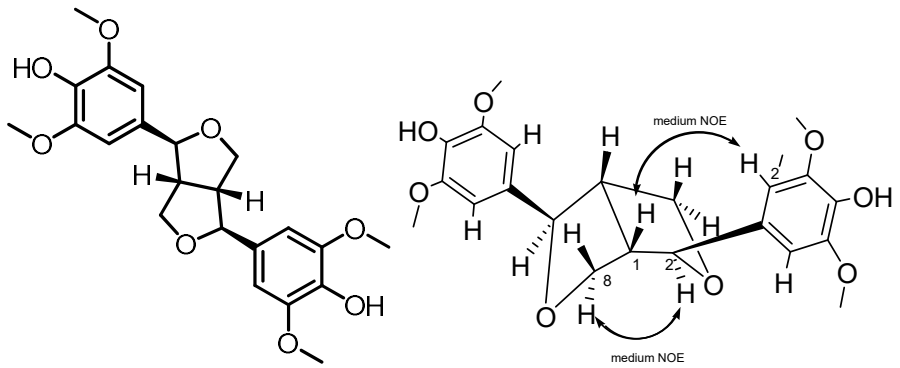

Figure 4 Structure of Compound **2** and NOE experiment

The molecular formula of compound **3** was assigned as $C_{10}H_{10}O_2$based on the FT IR,^{1}H and ^{13}C NMR, HMQC, DEPT and EIMS [M^+] (*m*/*z* 162). Its mass spectrum exhibited a molecular ion at *m*/*z* 162 which is its molecular mass. The stable ion of *m*/*z* 105 which is base peak was formed by the liberation of CH_2=CH-CHO molecule and hydrogen radical ($H^{\bullet}$) from the molecular ion (m/z 162). In the ^{1}H NMR(500 MHz) (Table 3),the observation of proton signals at δ 8.03 (1H, d, J= 7.7 Hz), 7.60 (2H, m), and 7.43 (1H, m) and proton coupling with each other in DQF-COSY experiment, led to the ortho-disubstituted benzene moiety. The^{1}H NMR(500 MHz)also represented a set of proton signals due to two methylenes at *δ*2.95 (1H, ddd, *J* = 17.3, 7.4, 4.5 Hz) and 2.62 (1H, ddd, *J* = 17.3, 9.7, 4.9 Hz), and 2.42 (1H, m), 2.19 (1H, m), a carbinol methine at *δ* 4.99 (1H, dd, J= 8.0, 3.9 Hz). In addition,^{13}C NMR(125 MHz), and HMQC spectra displayed signals for two methylene carbons at *δ* 35.1 and 32.1, a carbinylic carbon at *δ*67.9, and a carbonyl carbon at *δ*197.3. These above data indicated that **3** is4-hydroxy-α-tetralone.More detailed analysis of DQF-COSY, HMQC and HMBC supported a 4-hydroxy-α-tetralone structure. The carbinol methine proton (*δ* 4.99) at C-4 could be deduced to be equatorial from its coupling constant value of 3.9 Hz with H_{ax}-3 (*δ* 2.42).By comparing the spectroscopic data, such as MS, NMR and $[\alpha]_D^{25}$ of **3**with those of reported reference compound, compound **3**was identified to be (4S)-4-hydroxy-α-tetralone (**3)** (Joyl and Nair, 2001).Based on the above

evidence, the structure of compound **3** was assigned as(+) (4S)-4-hydroxy-α-tetralone (Figure 5).

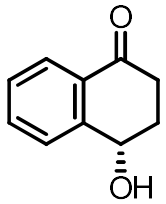

Figure 5 Structure of Compound **3**

Table 1 ^{13}C, ^{1}H NMR Data of 2-(2-hydroxyethylamino) -1,4-naphthoquinone and ^{1}H-^{13}C, ^{1}H-^{1}H Correlations Exhibited in the 2D NMR Spectra in $CDCl_3$

Carbon	$\delta^{13}C$ (DEPT)	Proton	Proton $\delta^{1}H$ (J Hz)	HMBC correlation	COSY correlation
1	181.5(C=O)	-	-	-	-
2	148.7	-	-	-	-
3	99.6 (CH)	3	5.73 (s)	C-1, C-4, C-10	-
4	181.4(C=O)	-	-	-	-
5	125.3 (CH)	5	7.93(dd, 7.6 and 1.3)	C-7, C-9	H-6, H-7
6	134.9 (CH)	6	7.83(td, 7.6 and 1.3)	C-8	H-5, H-7,H-8
7	132.2 (CH)	7	7.72(td, 7.6 and 1.3)	-	H-5, H-6, H-8
8	125.9 (CH)	8	7.98(dd, 7.6 and 1.3)	-	H-6,H-7
9	130.3	-	-	-	-
10	133.2		-	-	-
1′	44.6 (CH_2)	1′	3.25(t, 5.7)	-	H-2′
2′	58.4 (CH_2)	2′	3.59(t, 5.7)	-	H-1′

Table 2 ^{13}C, ^{1}H NMR Data of (-)-syringaresinol and ^{1}H-^{13}C, ^{1}H-^{1}H Correlations Exhibited in the 2D NMR Spectra in $CDCl_3$

Carbon	$\delta^{13}C$ (DEPT)	Proton	Proton $\delta^{1}H$ (*J* Hz)	HMBC correlation	COSY correlation
1	54.4(CH)	1	3.07 (m)	-	H-2, H-8a, H-8b
2	86.1(CH)	2	4.73(d, 4.5)	C-4,C-8,C-2′,C-6′	H-1
3	-	-	-	-	-
4	71.8(CH_2)	4a 4b	4.28(dd, *J* = 9.1, 6.8) 3.91(m)	C-2, C-6 C-2, C-6	H-4b, H-5 H-4a, H-5
5	54.4(CH)	5	3.07(m)	-	H-4a, H-4b, H-6
6	86.1(CH)	6	4.73(d, 4.5)	C-4,C-8,C-2′,C-6′	H-5
7	-	-	-	-	-
8	71.8(CH_2)	8a 8b	4.28(dd, *J* = 9.1, 6.8) 3.91(m)	C-2, C-6 C-2, C-6	H-8b, H-1 H-8a, H-1
1′	132.1	-	-	-	-
2′	102.8(CH)	2′	6.58(s)	C-2,C-1′,C-3′,C-4′,C-6′	-
3′	147.2	-	-	-	-
4′	134.4	-	-	-	-
5′	147.2	-	-	-	-
6′	102.8(CH)	6′	6.58(s)	C-2,C-1′,C-2′,C-4′,C-5′	-
1″	132.1	-	-		
2″	102.8(CH)	2″	6.58(s)	C-6,C-1′,C-3′,C-4′,C-6′	-
3″	147.2	-	-	-	-
4″	134.4	-	-	-	-
5″	147.2	-	-	-	-
6″	102.8(CH)	6″	6.58(s)	C-6,C-1″,C-2″,C-4″,C-5″	-
3′-O$\underline{C}H_3$	56.4	3′-O$\underline{C}H_3$	3.90(s)	C-3′	-
5′-O$\underline{C}H_3$	56.4	5′-O$\underline{C}H_3$	3.90(s)	C-5′	-
3″-O$\underline{C}H_3$	56.4	3″-O$\underline{C}H_3$	3.90(s)	C-3″	-
5″-O$\underline{C}H_3$	56.4	5″-O$\underline{C}H_3$	3.90(s)	C-5″	-

Table 3 ^{13}C, ^{1}H NMR Data of (4S)-4-hydroxy-1-tetralone and ^{1}H-^{13}C, ^{1}H-^{1}H Correlations Exhibited in the 2D NMR Spectra in $CDCl_3$

Carbon	$\delta^{13}C$ (DEPT)	Proton	Proton $\delta^{1}H$ (*J* Hz)	HMBC correlation	COSY correlation
1	197.3(C=O)	-	-	-	-
2	35.1(CH_2)	2a	2.95(ddd, 17.3,7.4,4.5)	C-1, C-3, C-4	H-2b, H-3a, H-3b
		2b	2.62(ddd, 17.3,9.7,4.9)	C-1, C-3, C-4	H-2a, H-3a, H-3b
3	32.1(CH_2)	3a	2.42(m)	C-1, C-2, C-4	H-2a, H-2b, H-3b
		3b	2.19(m)	C-1, C-2, C-4	H-2a, H-2b, H-3a
4	67.9(CH)	4	4.99(dd, *J* = 8.0, 3.9)	-	H-3a, H-3b
5	126.9(CH)	5	7.60(m)	C-7, C-8, C-9,C-10	H-7
6	134.1(CH)	6	7.60(m)	C-7, C-8, C-9,C-10	H-7
7	128.4(CH)	7	7.43(m)	C-5, C-6, C-8,C-9	H-5, H-6, H-8
8	127.2(CH)	8	8.03(d, *J* = 7.7)	C-1, C-6, C-10	H-7
9	131.2	-	-	-	-
10	145.3	-	-	-	-

Compound 1: Orange crystal.FT IR (KBr): (cm^{-1}) 3600-3200 (-OH and -NH), 3066.26 (=C-H), 2932.23, 2863.77 (Sat-H/C), 1682.59 (C=O), 1600.63, 1561.09, 1518.67, (ArH and NH), 1367.28 (C-OH): ^{1}H-NMR (500 MHz, DMSO) δ: 7.98 (1H, dd, J= 7.6,1.3 Hz; H-8), 7.93 (1H, dd,J= 7.6,1.3 Hz; H-5), 7.83 (1H, td,J=7.6,1.3 Hz; H-6), 7.72 (1H, td,J= 7.6,1.3 Hz; H-7), 5.73 (1H, s; H-3), 3.59 (2H, t, *J* = 5.7 Hz; H-2′), 3.25 (2H, t, *J* = 5.7 Hz; H-1′), ^{13}C-NMR (125 MHz, DMSO) δ: 181.5 (C- 1), 181.4 (C- 4), 148.7 (C-2), 134.9 (C-6), 133.2 (C- 10), 132.2 (C- 7), 130.3 (C- 9), 125.9 (C- 8), 125.3 (C - 5), 99.6 (C-3), 58.4 (C-2′), 44.6 (C-1′), . EIMS *m*/*z* (rel. int.): 231 [M^+] ($C_{12}H_{11}O_3N$).

Compound 2: colourless crystal. $[\alpha]_D^{25}$= - 8.4° (*c* 0.058, $CHCl_3$).FT IR (KBr): (cm^{-1})3421.1(-OH), 3084.58 (=C-H), 2935.13, 2862.81 (Sat-H/C), 1614.13, 1518.67, 1459.85 (ArH), 1215.9, 1113.69 (C-O-C): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.58 (1H, s ; H-2′), 6.58 (1H,s ; H-6′), 6.58 (1H, s ; H-2″), 6.58 (1H, s ; H-6″), 4.73 (1H, d, *J* = 4.5 Hz; H-2), 4.73 (1H, d, *J* = 4.5 Hz; H-6), 4.28 (1H, dd, *J* = 9.1, 6.8 Hz; H-4a), 4.28 (1H, dd, *J*= 9.1, 6.8 Hz; H-8a), 3.91 (1H, m ; H-4b), 3.91 (1H, m ; H-8b), 3.90 (3H, s ; C-3′-OC<u>H</u>$_3$), 3.90 (3H, s ; C-5″-OC<u>H</u>$_3$), 3.90 (3H, s ; C-3′-OC<u>H</u>$_3$), 3.90 (3H, s ; C-5′-OC<u>H</u>$_3$), 3.09 (1H, m ; H-1), 3.09 (1H, m ; H-5), ^{13}C-NMR (125 MHz,$CDCl_3$) δ: 147.2 (C- 3′), 147.2 (C- 5′), 147.2 (C- 3″), 147.2 (C- 5″), 134.4 (C- 4′), 134.4 (C- 4″), 132.1 (C- 1′), 132.1 (C - 1″), 102.8 (C- 2′), 102.8 (C- 6′), 102.8 (C- 2″), 102.8 (C- 6″), 86.1 (C- 2), 86.1 (C- 6), 71.8 (C- 4), 71.8 (C- 5), 56.4 (C-3′-O<u>C</u>H$_3$), 56.4 (C-5′-O<u>C</u>H$_3$), 56.4 (C-3″-O<u>C</u>H$_3$), 56.4 (C-5″-O<u>C</u>H$_3$), 54.4 (C-1), 54.4 (C-5). EIMS *m*/*z* (rel. int.): 418 [M^+] ($C_{22}H_{26}O_8$).

Compound 3:yellowish amorphous solid. $[\alpha]_D^{25}$= + 13.5° (*c* 0.066, $CHCl_3$). FT-IR(KBr): (cm^{-1}) 3408.57(-OH), 3062.41 (=C-H), 2926.45, 2869.56 (Sat-H/C), 1684.52 (C=O), 1600.63, 1455.99, 1415.49 (ArH), 1287.25 (C- C -O): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.03 (1H, d, J= 7.7 Hz; H-8), 7.60 (2H, m ; H-5 and H-6), 7.43 (1H, m ; H-7), 4.99 (1H, dd, J= 8.0, 3.9 Hz ; H-4), 2.95 (1H, ddd, *J* = 17.3, 7.4, 4.5 Hz; H-2a), 2.62 (1H, ddd, *J* = 17.3, 9.7, 4.9 Hz; H-2b), 2.42 (1H, m ; H-3a), 2.19 (1H, m ; H-3b), ^{13}C-NMR (125 MHz,$CDCl_3$) δ: 197.3 (C- 1), 145.3 (C-10), 134.1 (C-6), 131.2 (C- 9), 128.4 (C- 7), 127.2 (C- 8), 126.9 (C- 5), 67.9 (C - 4), 35.1 (C-2), 32.1 (C-3). EIMS *m*/*z* (rel. int.): 162 [M^+] ($C_{10}H_{10}O_2$).

Evaluation of Antioxidant Activity

This pure yellowish amorphous solid compound, (+)(4S)-4-hydroxy-α-tetralone isolated from the stem bark of*H. integrifolia*. traditionally used in Myanmar system of medicine was screened for its antioxidant activity using ascorbic acid as standard antioxidant. The free radical scavenging potential of this compound was evaluated by a sensitive and simple chemiluminescent method. By using the resulted number of photon data from the printed graph, the % inhibition of isolated compound was calculated with the following equation.

$$\% \text{ inhibition of sample} = 1 - \frac{number\ of\ photo\ \ of\ the\ sample}{number\ of\ phot\ \ \ of\ blank} \text{x } 100$$

The calculated data of pure compound are described in the following Table 4.

Table 4 % Inhibition of (+)(4S)-4-hydroxy-1-tetralone

Cocentratiom (mg/mL)	0.02	0.04	0.06	0.08	0.1
% Inhibition (%)	17.34	38.9	48.54	61.85	70.85

In accordance with graph (Figure 6), the half inhibition concentration (IC_{50}) of this compound was determined as 0.065 mg mL^{-1}. The pure compound was found to show significant antioxidant property which is comparable to IC_{50}value of standard ascorbic acid, at a specific concentration.

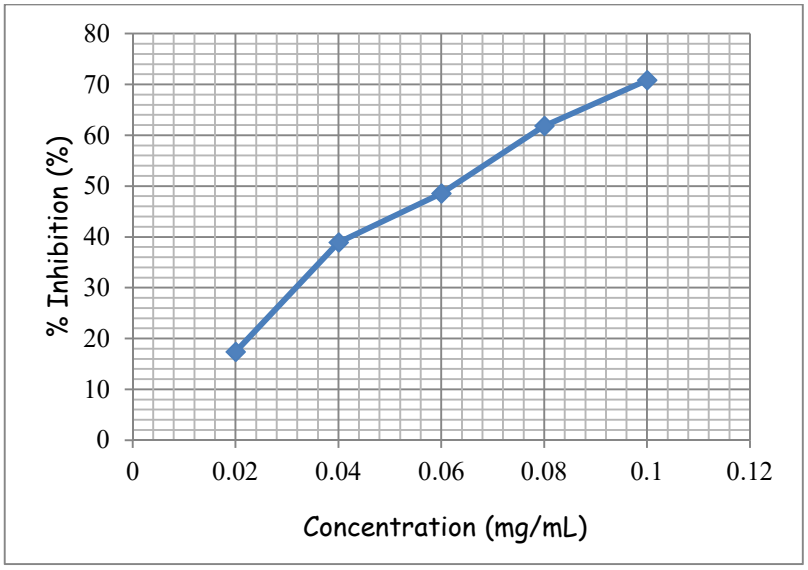

Figure 6 The graph of % inhibition (%) Vs concentration (mg/mL) of (+)(4S)-4-hydroxy-1-tetralone

Conclusion

In the present investigation, we describe the isolation of one new compound (**1**) and other two interesting compounds (**2** and **3**)from the stem bark of *H. integrifolia* (Pyauk-seik). Moreover,the elucidated pure yellowish amorphous solid compound which is described for first time, (+)(4S)-4-hydroxy-α-tetralone isolated from the EtOAc extract of stem bark of *H. integrifolia* (Pyauk-seik) traditionally used in Myanmar system of medicine, responds significant antioxidant activity by comparing the half inhibition concentration (IC_{50}) of ascorbic acid as standard antioxidant using a sensitive and simple chemiluminescent method. Further studies are required and are in progress here.

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