Part II

Chemical Constituents from the Stems of *Derris trifoliata*

CHAPTER 2.1

INTRODUCTION

2.1.1 Introduction

Derris trifoliata Lour., a plant belonging to the Leguminoseae family, local name in Thai: “Khwaep Thale” (แควบทะเล), “Thop Thaep Nam” (ถอบแถบน้ำ), “Phak Thaep” (ผักแถบ) in the middle part region;“Thop Thaep Thale” (ถอบแถบทะเล, Phetchaburi); “Tua Nam” (ถั่วน้ำ, Narathiwat); “Thap Thaep” (ทับแถบ, Samut Songkhram).

Derris trifoliata is typically found growing as a scrambling climber in the back mangrove. The plant is also encountered in coastal, non mangrove vegetation. The leaves are ovate in the lower half and lanceolate in upper portion. It is a usually slender, woody climber with pinnate leaves of 3, 5 or 7 leaflets. The inflorescence is slender with few to numerous, 1 cm long, light pink, pea-like flowers. The slightly wrinkled fruits are disc-like, 3-4 cm by 2 cm, with 2-3 seeds. This species is utilized medicinally and as a fish-poison. It is one of the few climbers in the local mangroves.

Figure 3 *Derris trifoliata* (Leguminoseae)

2.1.2 Review of Literatures

Chemical constituents isolated from 24 species of the genus *Derris* were summarized by Suwanna Deachathai in 2001 (Deachathai, 2001). Information from NAPRALERT database developed by University of Illinois at Chicago and Chemical Abstracts of the year 2003 reported additional constituents from three new species of the *Derris* genus and they can be classified into groups, such as benzonoids, flavanone, flavonol, isoflavone.

These compounds are presented in **Table 27** together with previously missing informations.

Table 27 Compounds from plants of *Derris* species

a : Benzonoids **b** : Flavanone **c** : Flavonol **d** : Isoflavone

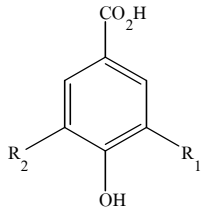
Scientific name	Compound	Bibliography
D. malacensis -stems	12-Deoxo-12α-acetoxyelliptone, **6d** 12a-Hydroxyelliptone, **7d**	Thasana, *et.al.* 2001
D. reticulata -stems	2",3"-Dihydroxylupinifolin, **3b** 4',5-Dihydroxy-8-hydroxymethyl-6",6"-dimethylpyrano(2",3": 7,6)flavanone, **4b**	Mahidol, *et.al.* 2002
D. scanden -stems	3'-Formylalpinumisoflavone, **8d** 4',5,7-Trihydroxy-6,8-diprenylisoflavone, **1d** 2,3-Dihydro-3-hydroxy-2-(1-hydroxy-1-methylethyl)furanoalpinumisflavone, **14d** Lupalbigenin, **10d** Lupinisoflavone G, **13d** Senegalensin, **12d** 4-Hydroxy-3,5-dimethylbenzoic acid, **1a** 4-Hydroxy-3-dimethylbenzoic acid, **2a** Daidzein-7-*O*-rhamnosyl(1$\rightarrow$6)glucoside, **15d** Derriscandenoside A, **18d** Derriscandenoside B, **22d** Derriscandenoside C, **23d**	Chuankamnerdkarn, *et.al.* 2002 Rukachaisirikul, *et.al.* 2002

Table 27 (Continued)

Scientific name	Compound	Bibliography
D. scanden -stems	Derriscandenoside D, **24d** Derriscandenoside E, **25d** 7-Hydroxy-4',8-dimethoxyisoflavone-7-*O*-β-D-glucoside, **20d** 8-Hydroxy-4',7-dimethoxyisoflavone-8-*O*-β-D-glucoside, **21d** Formononetin-7-*O*-α-rhamnopyranosyl-(1$\rightarrow$6)-β-D-glucopyranoside, **16d** Genistein-7-*O*-rhamnosyl(1$\rightarrow$6) glucoside, **17d** Lupinisol A, **9d** Ononin, **19d** Retusin, **5c**	Rukachaisirikul, *et.al.* 2002

Structures

a : **Benzonoids**



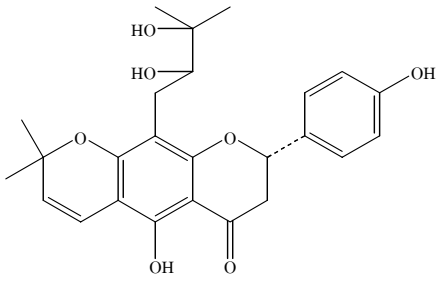
1a: R_1= R_2= OMe;

4-Hydroxy-3,5-dimethoxybenzoic acid

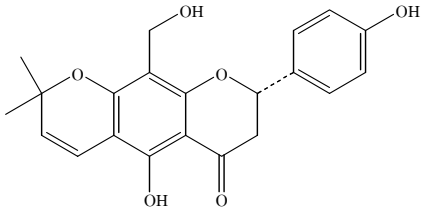
2a: R_1= OMe, R_2= H;

4-Hydroxy-3-dimethoxybenzoic acid

b : **Flavanones**

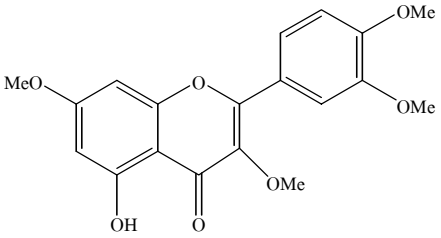


3b: 2",3"-Dihydroxylupinifolin



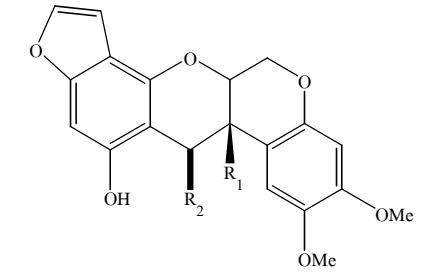
4b: 4',5-Dihydroxy-8-hydroxymethyl-6",6"-dimethylpyrano(2",3":7,6) flavanone

c : Flavonol



5c: Retusin

d : Isoflavones

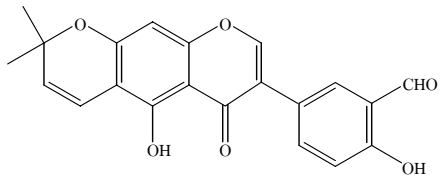


6d: R_1= OH, R_2= =O;

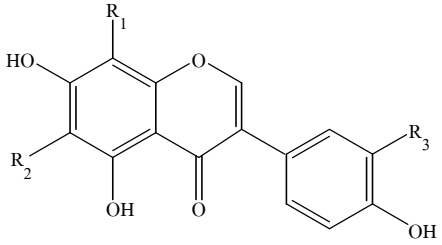
12-Deoxo-12α-acetoxyelliptone

7d: R_1= H, R_2= OAc;

12a-Hydroxyelliptone



8d: 3'-Formylalpinumisoflavone



9d: R_1= H, R_2= -$CH_2CH(OH)CCH_2CH_3$,

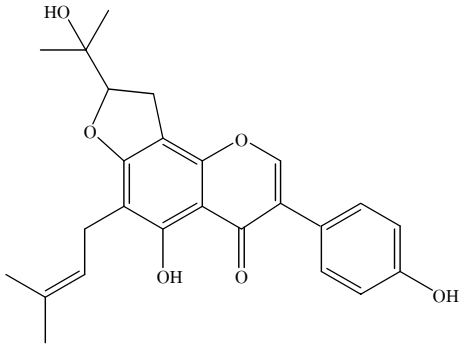
R_3= isoprenyl ; Lupinisol A

10d: R_1= H, R_2= R_3= isoprenyl ;

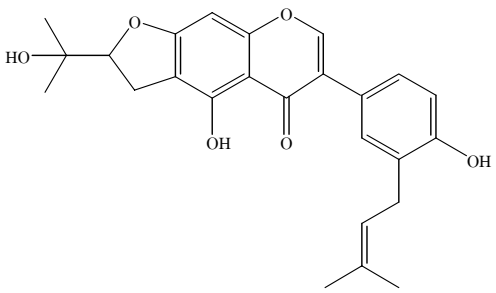
Lupalbigenin

11d:, R_1= R_2= isoprenyl, R_3= H;

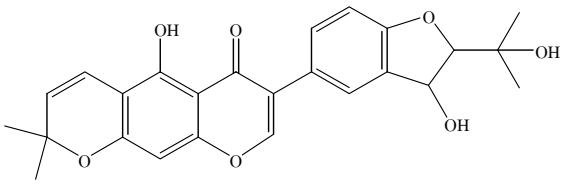
4',5,7-Trihydroxy-6,8-diprenyl-isoflavone



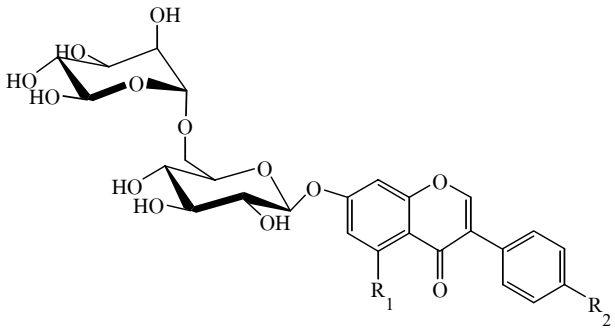
12d: Senegalensin



13d: Lupinisoflavone G



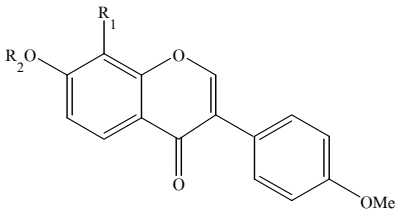
14d: 2,3-Dihydro-3-hydroxy-2-(1-hydroxy-1-methylethyl) furanoalpinumisoflavone



15d: R_1= H, R_2= OH; Daidzein-7-*O*-rhamnosyl(1→6)glucoside

16d: R_1= H, R_2= OMe; Formononetin-7-*O*-α-rhamnopyranosyl(1→6)-β-D-glucopyranoside

17d: R_1= OH, R_2= H; Genistein-7-*O*-rhamnosyl(1→6)glucoside

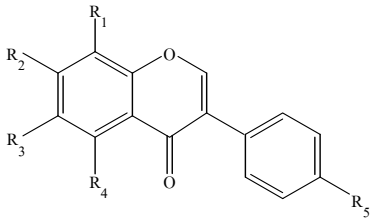


18d: R_1= *O*-β-D-glucosyl, R_2= H; Derriscandenoside A

19d: R_1= H, R_2= β-D-glucosyl; Ononin

20d: R_1= OMe, R_2= β-D-glucosyl; 7-Hydroxy-4',8-dimethoxyisoflavone-7-*O*-β-D-glucoside

21d: R_1= O-β-D-glucosyl, R_2= Me; 8-Hydroxy-4',7-dimethoxyisoflavone-8-*O*-β-D-glucoside



22d: R_1= OH, R_2= OX, R_3= R_4= H, R_5= OMe;

Derriscandenoside B

23d: R_1= OX, R_2= R_5= OMe, R_3= R_4= H;

Derriscandenoside C

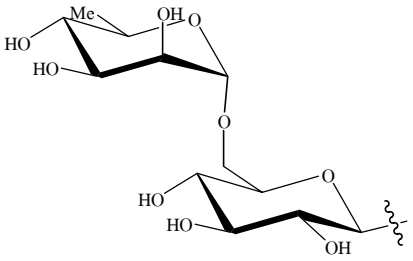
24d: R_1= R_4= H, R_2= OX, R_3= R_5= OMe;

Derriscandenoside D

25d : R_1= H, R_2= OX, R_3= R_5= OMe, R_4= OH;

Derriscandenoside E

X =



2.1.3 Biological activities of Derris species

The importance of *Derris* plants in traditional medicine throughout the tropical world is apparent from NAPRALERT database. The significant biological activities of the extract of *Derris* species are summarized in **Table 28**.

Table 28 Biological activity of *Derri*s species

Scientific name	Biological activity	Bibliography
D. scanden -dried stems	Immunostimulant activity Antioxidant activity Antiinflammatory activity Leukotriene synthesis inhibition Myeloperoxidase inhibition Thromboxane B-2 synthesis inhibition	Sriwanthana and Chavalittumrong, 2001 Laupattarakasem, *et. al*, 2003

This research involved isolation, purification and structure elucidation of chemical constituents isolated from the stems of *D. trifoliata.*

CHAPTER 2.2

EXPERIMENTAL

2.2.1 Instruments and Chemicals

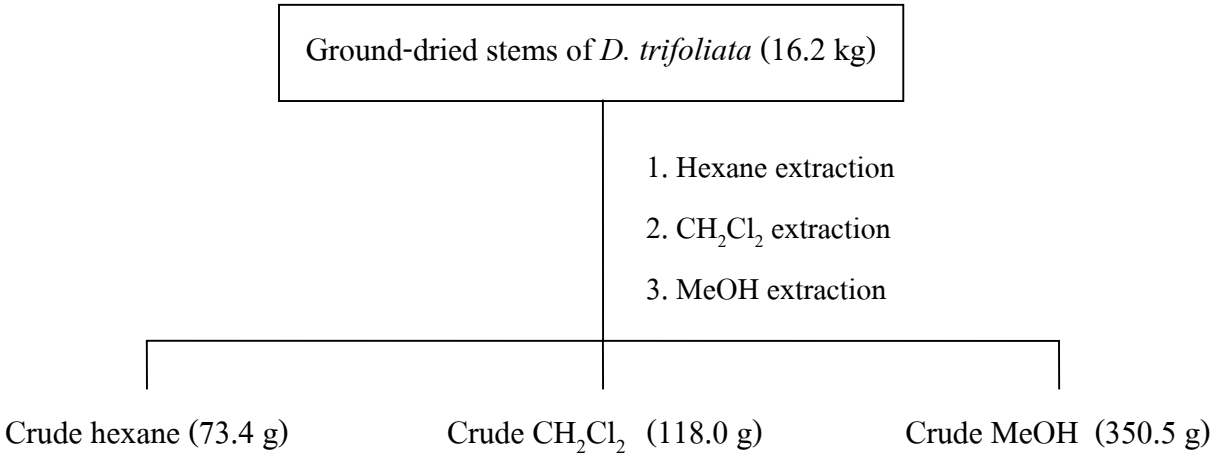
Melting point was recorded in °C and was measured on an Electrothermal Melting Point Apparatus. Infrared spectra were recorded using FTS FT-IR spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using a SPECORD S 100 (Analytikjena) and UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform and methanol solution. Nuclear magnetic resonance spectra were recorded on 500 MHz Varian UNITY INOVA spectrometer and FTNMR Bruker Ultra Shield™ 300 MHz. Spectra were recorded in deuterochloroform, deuteroacetone and deuteromethanol solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Single-crystal X-ray diffraction measurements were collected using SMART 1-K CCD diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 A°) using ω-scan mode and SHELXTL for structure solution and refinement. Optical rotation was measured in chloroform solution with sodium D line (590 nm) on an AUTOPOL® II automatic polarimeter. Solvent for extraction and chromatography were distilled at their boiling point ranges prior to use except diethyl ether was analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063 – 0.200). Precoated plates of silica gel 60 GF_{254} or reversed-phase C_{18} were used for analytical purposes.

2.2.2 Plant material

The stems of *Derris trifoliata* (Leguminoseae) were collected at the Mangrove Research Station in Nakhon Si Thammarat Province, Thailand.

2.2.3 Extraction

Ground-dried stems of *D. trifoliata* (16.2 kg) were immersed at room temperature in hexane (2x10L, 5 days), methylene chloride (3x12L, 5 days) and methanol (2x10L, 5 days). After evaporation, the viscous crude hexane extract (73.4 g), crude methylene chloride extract (118.0 g) and crude methanol extract (350.5 g), were obtained. The process of extraction was shown in **Scheme 3**.



Scheme 3 Extraction of the stems of *D. trifoliata*

2.2.4 Isolation and Chemical Investigation

2.2.4.1 Investigation of the crude hexane extract from the stems of *D. trifoliata*

A portion of crude hexane (21.7 g) was subjected to QCC using silica gel as the stationary phase and eluted with gradient elution of hexane, ethyl acetate and methanol. On the basis of their TLC characteristic, the collected fractions which contained the same major components were combined; fractions A1-A10 were obtained. The selected fractions were further purified to yield fifteen pure compounds as shown in **Scheme 4**.

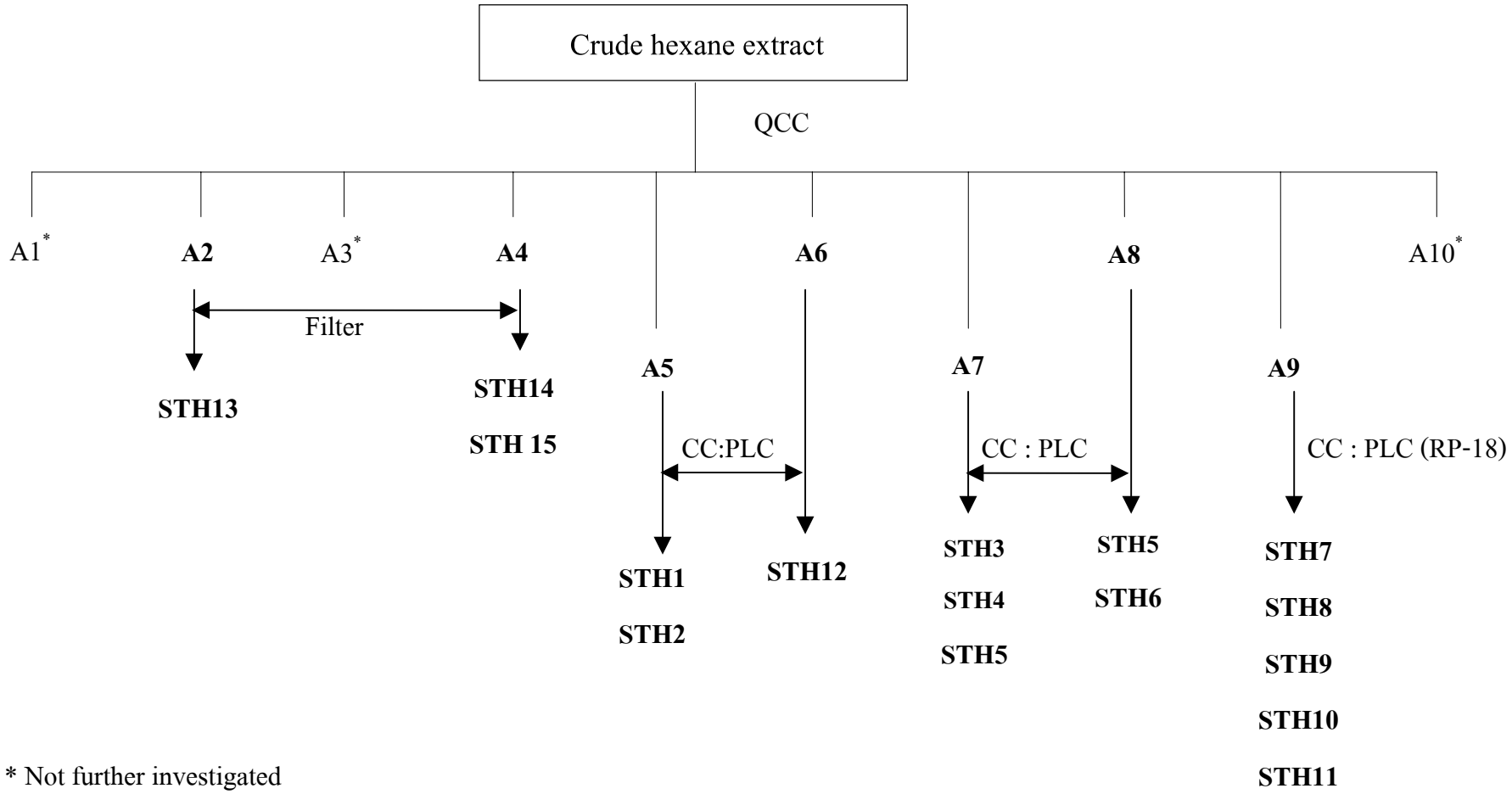
Fraction A2 was filtered and washed with hexane to give a white solid of **STH13** (35.7 mg, R_f= 0.27, 15% ethyl acetate in hexane).

Fraction A4 was filtered and washed with hexane to give a white solid mixture of **STH14** and **STH15** (21.8 mg, R_f= 0.15, 15% ethyl acetate in hexane).

Fraction A5 (0.212 g) was rechromatographed on column chromatography and eluted with 15% acetone in hexane. The major component of this fraction, **STH1** (29.1 mg, R_f = 1.25, 25% ethyl acetate in hexane) as yellow viscous oil was collected and the minor component was purified by preparative thin layer chromatography with 3% ethyl acetate in methylene chloride to yield **STH2** as yellow viscous oil (5.0 mg, R_f = 0.26, 30% acetone in hexane).

Fraction A6 (1.08 g) was rechromatographed on column chromatography and eluted with the mixed solvent of 35% ethyl acetate in hexane to give two subfractions.

Subfraction A6-2 was recrystallized in acetone-chloroform mixture (1:1, v/v). The yellow solid of **STH12** was collected (10.0 mg, R_f = 0.28, 50% diethyl ether in hexane).



Scheme 4 Isolation of **STH1- STH15** from crude hexane extract

Fraction A7 (0.94 g) was purified by column chromatography and eluted with the mixed solvent of 20% ethyl acetate in hexane to give three subfractions.

Subfraction A7-2 (100.0 mg) was further separated by column chromatography using 25% ethyl acetate in hexane and followed by preparative thin layer chromatography with 25% ethyl acetate in hexane to give **STH3** as yellow viscous oil (5.0 mg, R_f = 0.15, 60% methylene chloride in hexane) and **STH4** as yellow viscous oil (15.6 mg, R_f = 0.07, 30% ethyl acetate in hexane).

Subfraction A7-3 (30.6 mg) was further purified by column chromatography using 25% ethyl acetate in hexane to give **STH5** as yellow viscous oil (4.4 mg, R_f = 0.16, 25% ethyl acetate in hexane).

Fraction A8 (0.473 g) was rechromatographed on column chromatography and eluted with the mixed solvent of ethyl acetate-hexane to yield three subfractions.

Only subfraction A8-2 (21.2 mg) was further separated by preparative thin layer chromatography with 20% ethyl acetate in hexane to give **STH5** (12.7 mg) and **STH6** as yellow viscous oil (8.2 mg, R_f = 0.25, 20% ethyl acetate in hexane).

Fraction A9 (1.38 g) was purified by column chromatography and eluted with the mixed solvent of 25% ethyl acetate in hexane to give three subfractions.

Subfraction A9-2 (176.8 mg) was further separated by column chromatography using 10% acetone to give **STH10** as yellow viscous oil (1.7 mg, R_f= 0.22, 10% acetone in hexane) and **STH11** as yellow viscous oil (55.5 mg, R_f = 0.07, 20% ethyl acetate in hexane).

A portion of subfraction A9-3 (40.0 mg) was purified by reversed-phase C_{18} with 60% methanol in H_2O to give **STH7** as yellow viscous oil (9.7 mg, R_f = 0.11, 20% ethyl acetate in hexane), **STH8** as yellow viscous oil (16.7 mg, R_f = 0.12, 20% ethyl acetate in hexane) and **STH9** as yellow viscous oil (2.5 mg, R_f = 0.24, 60% methanol in H_2O).

Compound STH1 : Yellow viscous oil; $[\alpha]_D^{27}$: -17.8 ° (*c* = 0.028, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 203 (4.02), 225 (3.97), 266 (4.08), 273 (4.10), 296 (3.67), 310 (3.59) and 359 (3.07); IR (neat) ν (cm^{-1}): 3437 (O-H stretching), 3014 (C-H aromatic stretching), 2914 (C-H stretching), 1670 (C=O stretching), 1513 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 29**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 29**; DEPT -135°($CDCl_3$): see **Table 29**.

Compound STH2 : Yellow viscous oil; $[\alpha]_D^{27}$: -83.3 ° (*c* = 0.012, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 203 (4.04), 222 (3.93), 266 (4.12), 274 (4.16), 297 (3.59), 311 (3.57) and 364 (2.80); IR (neat) ν (cm^{-1}): 3383 (O-H stretching), 2925 (C-H stretching), 1633 (C=O stretching), 1455 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 31**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 31**; DEPT -135°($CDCl_3$): see **Table 31**.

Compound STH3 : Yellow viscous oil; $[\alpha]_D^{27}$: +29.4° (*c* = 0.034, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 205(4.38), 226(4.18), 273(4.35), 295(4.08), 310(3.97) and 367(3.42); IR (neat) ν (cm^{-1}): 3439 (O-H stretching), 3014 (aromatic stretching), 2934 (C-H stretching), 1641 (C=O stretching), 1514 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 33**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 33**; DEPT -135°($CDCl_3$): see **Table 33**.

Compound STH4 : Yellow viscous oil; $[\alpha]_D^{27}$: +47.60° (*c* = 0.021 , $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 206 (3.91), 231 (3.80), 260 (3.70) and 330 (3.13); IR (neat) ν (cm^{-1}): 2936 (C-H stretching), 1673 (C=O stretching), 1505 (C=C

stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 36**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 36**; DEPT -135°($CDCl_3$): see **Table 36**.

Compound STH5 : Yellow viscous oil; $[\alpha]_D^{27}$: -19.23° (c = 0.026 , $CHCl_3$); UV ($CHCl_3$) λ_{max} (nm) (log ε): 243 (3.94), 274 (4.01), 298 (3.72) and 318 (3.63); IR (neat) ν (cm^{-1}): 3014 (aromatic stretching), 2914 (C-H stretching), 1698 (C=O stretching), 1513 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 37**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 37**; DEPT -135°($CDCl_3$): see **Table 37**.

Compound STH6 : Yellow viscous oil; $[\alpha]_D^{27}$: -27.7° (c = 0.018 , $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 206 (4.34), 235 (4.03) and 292 (3.96); IR (neat) ν (cm^{-1}): 2929 (C-H stretching), 1673 (C=O stretching), 1514 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 41**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 41**; DEPT -135°($CDCl_3$): see **Table 41**.

Compound STH7 : Yellow viscous oil; $[\alpha]_D^{27}$: -66.60° (c = 0.015 , $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 208 (4.30), 234 (3.95) and 294 (4.00); IR (neat) ν (cm^{-1}): 3440 (O-H stretching), 2920 (C-H stretching), 1660 (C=O stretching), 1514(C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 44**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 44**; DEPT -135°($CDCl_3$): see **Table 44**.

Compound STH8 : Yellow viscous oil; $[\alpha]_D^{27}$: +21.7° (c = 0.023 , $CHCl_3$); UV ($CHCl_3$) λ_{max} (nm) (log ε): 243 (3.01), 281 (2.58), 294 (2.48) and 322 (2.35); IR (neat) ν (cm^{-1}): 3445 (O-H stretching), 3014 (aromatic stretching), 2923 (C-H stretching), 1681 (C=O stretching), 1511 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ

ppm) (500 MHz): see **Table 48**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 48**; DEPT -135°($CDCl_3$): see **Table 48**.

Compound STH9 : Yellow viscous oil; $[\alpha]_D^{27}$: -41.60° (*c* = 0.012 , $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 205 (4.22), 225 (4.05), 236 (4.04), 247 (4.02), 270 (4.05), 300 (3.73) and 315 (3.71); IR (neat) ν (cm^{-1}): 3446 (O-H stretching), 2919 (C-H stretching), 1681 (C=O stretching), 1511 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 52**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 52**; DEPT -135°($CDCl_3$): see **Table 52**.

Compound STH10 : Yellow viscous oil; UV (MeOH) λ_{max} (nm) (log ε): 205 (3.41), 235 (3.36), 273 (3.19) and 336 (2.99); IR (neat) ν (cm^{-1}): 2933 (C-H stretching), 1675 (C=O stretching), 1510 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 56**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 56**; DEPT -135°($CDCl_3$): see **Table 56**.

Compound STH11 : Yellow viscous oil; $[\alpha]_D^{27}$: +38.46° (*c* = 0.026 , $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 205 (4.47), 236 (4.50), 257 (3.90) and 293 (3.78); IR (neat) ν (cm^{-1}): 3127 (aromatic stretching), 2956 (C-H stretching), 1673 (C=O stretching), 1515 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 57**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 57**; DEPT -135° ($CDCl_3$): see **Table 57**.

Compound STH12 : Yellow solid; mp: 137-140°C; UV (MeOH) λ_{max} (nm) (log ε): 203 (3.89), 228 (3.83), 264 (4.11), 272 (4.16), 296 (3.59), 309 (3.51) and 363 (2.91); IR (KBr) ν (cm^{-1}): 3333 (C-H stretching), 2971 (C-H stretching), 1633

(C=O stretching), 1520 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 59**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 59**.

Compound STH13 : White solid; mp: 194-195°C; $[\alpha]_D^{27}$: +31.9 (*c* = 0.0094, $CHCl_3$); IR (KBr) ν (cm^{-1}): 3416 (O-H stretching), 2929 (C-H stretching)

Compounds STH14 and STH15 : White solid; mp: 155-156°C; IR (KBr) ν (cm^{-1}): 3321 (O-H stretching), 2929 (C-H stretching)

2.2.4.2 Investigation of the crude methylene chloride extract from the stems of *D. trifoliata*

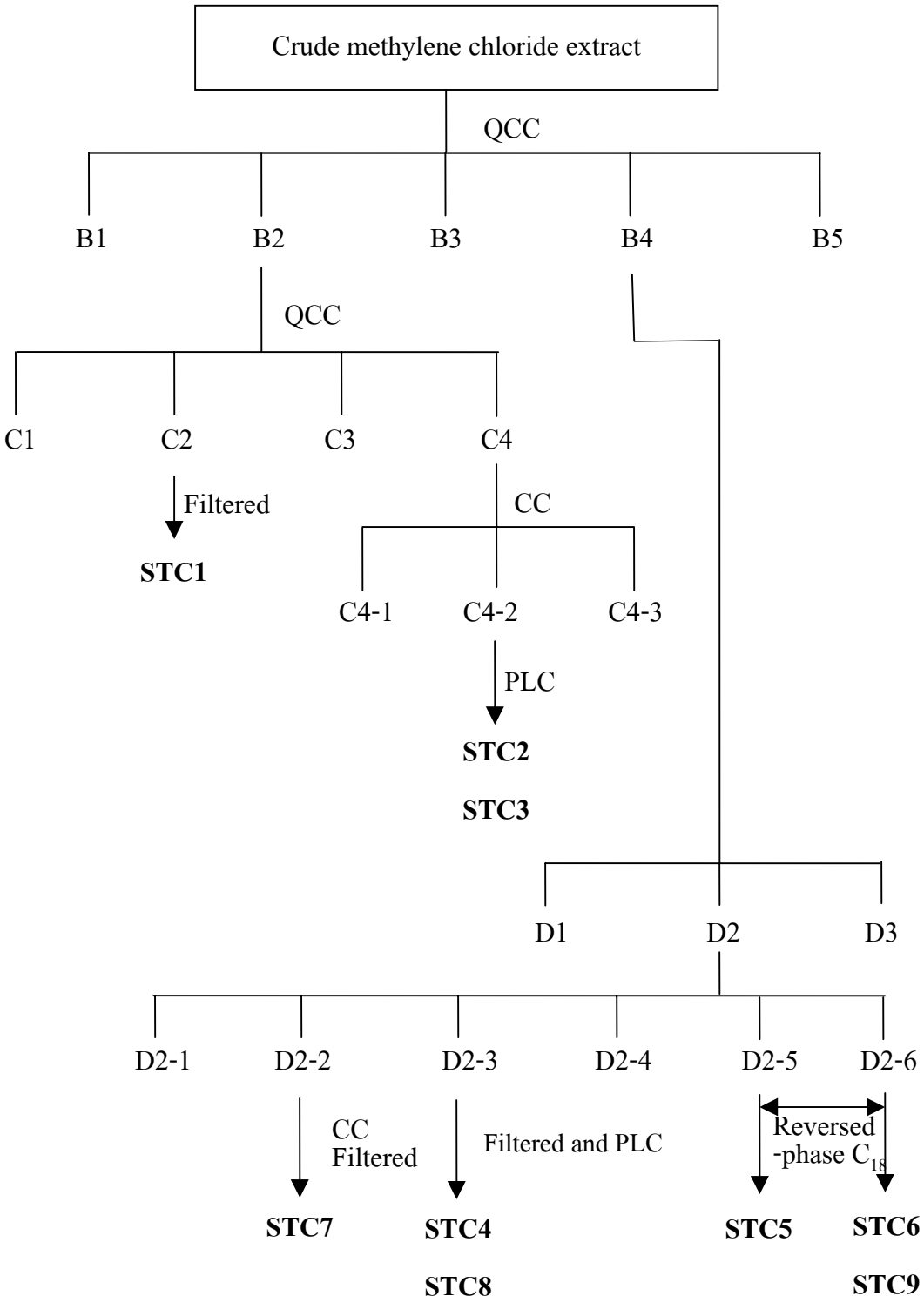
The crude methylene chloride extract (118.0 g) was separated by QCC with hexane and increasing polarity with ethyl acetate and methanol, successively, to give five fractions B1-B5 (**Scheme 5**).

Fraction B2 (6.82 g) was further purified by QCC and eluted with hexane, methylene chloride and methanol by gradually increasing the polarity. On the basis of TLC characteristic, the collected fraction with the same major components were combined, fraction C1-C4 were obtained (**Scheme 5**).

Fraction C2 (1.02 g) was filtered and washed with methanol to give **STC1** as yellow solid (17.2 mg, R_f = 0.25, 25% ethyl acetate in hexane).

Fraction C4 (2.26 g) was separated by CC with 20% ethyl acetate in hexane to give three subfractions.

Subfraction C4-2 (40.0 mg) was further purified by preparative TLC using 20% ethyl acetate in hexane to give **STC2** as yellow viscous oil (10.5 mg, R_f = 0.16, 20% ethyl acetate in hexane) and **STC3** as yellow viscous oil (10.3 mg, R_f = 0.17, 20% ethyl acetate in hexane).



Scheme 5 Isolation of **STC1- STC9** from crude methylene chloride extract

Fraction B4 (31.35 g) was further purified by QCC and eluted with hexane, ethyl acetate and methanol by gradually increasing the polarity. On the basis of TLC characteristic, the collected fraction with the same major components were combined, fraction D1-D3 were obtained (**Scheme 5**).

A portion of fraction D2 (4.96 g) was purified by column chromatography with 100% methylene chloride to give six subfractions.

Subfraction D2-2 (1.40 g) was further purified by column chromatography with 20% ethyl acetate in hexane and followed by filtration and washing with methanol to give **STC7** as yellow viscous oil (2.2 mg, R_f = 0.05, 25% ethyl acetate in hexane).

Subfraction D2-3 (1.04 g) was purified by filtering and washing with methanol to give **STC4** as yellow solid (101.1 mg, R_f = 0.20, 25% ethyl acetate in hexane). A portion of the mother liquor (100 mg) was separated by reversed-phase C_{18} with 60% methanol in H_2O to give **STC8** as yellow solid (6.3 mg, R_f = 0.2, 50% ethyl acetate in hexane).

Subfraction D2-5 (10.0 mg) was separated by reversed phase C_{18} with 70% methanol in H_2O to give **STC5** as yellow viscous oil (3.0 mg, R_f = 0.12, 25% ethyl acetate in hexane).

The last subfraction D2-6 (14.0 mg) was separated by reversed- phase C_{18} with 80% methanol in H_2O to give **STC6** as yellow viscous oil (2.2 mg, R_f = 0.07, 80% methanol in H_2O) and **STC9** as yellow solid (5.2 mg, R_f = 0.30, 90% methanol in H_2O).

Compound STC1 : Yellow solid; mp: 258-259°C; UV ($CHCl_3$) λ_{max} (nm) (log ε): 242 (4.00), 276 (4.18), 311 (3.79) and 331 (3.75); IR (KBr) ν (cm^{-1}): 3470

(O-H stretching), 2912 (C-H stretching), 1646 (C=O stretching), 1511 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 61**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 61**; DEPT -135°($CDCl_3$): see **Table 61**.

Compound STC2 : Yellow viscous oil; $[\alpha]_D^{27}$: -29.41° (*c* = 0.012, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 204 (3.95), 221 (3.93), 295 (3.67) and 344 (3.13); IR (neat) ν (cm^{-1}): 3445 (O-H stretching), 2980 (C-H stretching), 1635 (C=O stretching), 1511 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 64**; ^{13}C NMR ($CDCl_3$) (δppm) (125 MHz): see **Table 64**; DEPT -135°($CDCl_3$): see **Table 64**.

Compound STC3 : Yellow viscous oil; $[\alpha]_D^{27}$: -52.63° (*c* = 0.019, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 211 (4.13), 223 (3.98), 280 (3.69), 286 (3.72) and 319 (3.13); IR (neat) ν (cm^{-1}): 3401 (O-H stretching), 2927 (C-H stretching), 1511 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 66**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 66**; DEPT -135°($CDCl_3$): see **Table 66**.

Compound STC4 : Yellow solid; mp : 209-211°C; $[\alpha]_D^{27}$: +22.72° (*c* = 0.022, $CHCl_3$); UV ($CHCl_3$) λ_{max} (nm) (log ε): 245 (4.02), 280 (3.95) and 310 (3.84); IR (KBr) ν (cm^{-1}): 2915 (C-H stretching) and 1635 (C=O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 69**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 69**; DEPT -135°($CDCl_3$): see **Table 69**.

Compound STC5 : Yellow viscous oil; UV (MeOH) λ_{max} (nm) (log ε): 211 (4.03), 249 (3.58), 260 (3.61), 290 (3.40) and 342(3.08); IR (neat) ν (cm^{-1}): 3421 (O-H stretching), 1732 (C=O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see

Table 73; ^{13}C NMR ($CDCl_3$) (δppm) (125 MHz): see **Table 73**; DEPT -135°($CDCl_3$): see **Table 73**.

Compound STC6 : Yellow viscous oil; $[\alpha]_D^{27}$: +90.91° (*c* = 0.011, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 203 (4.06), 224 (3.96), 267 (4.15), 274 (4.17), 300 (3.68), 314 (3.69) and 369 (3.17); IR (neat) ν (cm^{-1}): 3420 (O-H stretching), 2925 (C-H stretching), 1627 (C=O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 75**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 75**; DEPT -135°($CDCl_3$): see **Table 75**.

Compound STC7 : Yellow viscous oil; ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 79**.

Compound STC8 : Yellow solid; mp : 132-134°C; UV (MeOH) λ_{max} (nm) (log ε): 221 (4.07), 246 (3.65), 254 (3.57) and 293 (2.77); IR (KBr) ν (cm^{-1}): 3447 (O-H stretching), 2919 (C-H stretching) and 1652 (C=O stretching); ^{1}H NMR ($CDCl_3$) (δppm) (500 MHz): see **Table 83**; ^{13}C NMR ($CDCl_3$) (δppm) (125 MHz): see **Table 83**; DEPT -135°($CDCl_3$): see **Table 83**.

Compound STC9 : Yellow solid; mp : 132-133.5°C; UV (MeOH) λ_{max} (nm)(log ε): 221 (4.12), 248 (3.69), 254 (3.64) and 288 (3.04); IR (KBr) ν (cm^{-1}): 3461 (O-H stretching), 2915 (C-H stretching) and 1631 (C=O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 84**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 84**; DEPT -135°($CDCl_3$): see **Table 84**.

CHAPTER 2.3

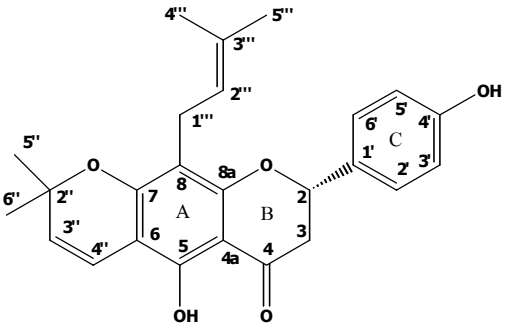
RESULTS AND DISCUSSION

2.3.1 Structural elucidation of compounds from the stems of D. trifoliata

The stems of *D. trifoliata* were dried at room temperature, ground and extracted with hexane, methylene chloride and methanol, successively. The crude hexane extract was subjected to chromatography and/or crystallization and/or PLC to give twelve phenolic compounds, **STH1**-**STH12** and three triterpenoids, **STH13**-**STH15**. Three of them are new compounds (**STH4**, **STH10** and **STH11**). The crude methylene chloride extract was separated by chromatography and/or PLC to give nine phenolic compounds, **STC1**-**STC9**.

Their structures were determined using 1D and 2D NMR spectroscopic data. All carbons were assigned by ^{13}C NMR, HMQC and HMBC data. In addition, the structure of **STH12** was confirmed by X-ray diffraction.

2.3.1.1 Compound STH1

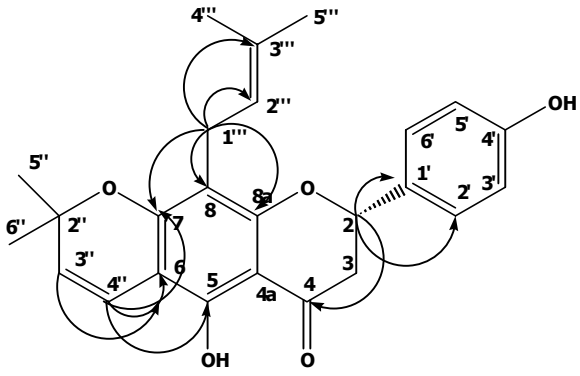


Compound **STH1** was obtained as a yellow viscous oil, $[\alpha]_D^{27}$: -17.8° (*c* = 0.028, $CHCl_3$). The IR spectrum showed absorption bands at 3437 and 1670 cm^{-1} corresponding to a hydroxyl group and conjugated carbonyl group, respectively. The UV spectrum showed maxima at 203, 225, 266, 273, 296, 310 and 359 nm, which is a typical absorption of flavanone.

The ^{1}H NMR spectrum (**Table 29**) showed a sharp *singlet* signal of a chelated hydroxyl group 5-OH at δ 12.24. The resonances for an ABX system at δ 3.04 (1H, *dd, J* = 17.5 and 13 Hz), 2.80 (1H, *dd, J* = 17.5 and 3 Hz) and 5.33 (1H, *dd, J* = 13 and 3 Hz), which is diagnostic for H-3α, H-3β and H-2β of a flavanone nucleus. The *doublet* resonances at δ 5.50 (1H, *J* = 10 Hz) and 6.63 (1H, *J* = 10 Hz), each equivalent to one proton, and the two *singlet* resonances at δ 1.43 (3H) and 1.45 (3H), were characteristics of the *cis* double bond and *gem*-dimethyl group of a 2,2-dimethyl-chromene moiety, respectively (Rao and Srimannarayana, 1983). Two *ortho*-coupled *doublets* centered at δ 7.31 (2H, *J* = 8.5 Hz) and 6.87 (2H, *J* = 8.5 Hz) were assigned to the protons of a *para*-disubstituted benzene ring (C ring). The presence of an isoprenyl group was inferred from the *singlet* at δ 1.65 (each 6H, Me-4′′′/Me-5′′′), the *broad doublet* at δ 3.21 (2H, *J* = 7 Hz, H-1′′′) and the *quintet of triplet* at δ 5.14 (1H, *J* = 9 and 1.5 Hz, H-2′′′). The ^{13}C NMR spectrum and the DEPT spectral data (**Table 29**) indicated the existence of four methyl carbons (δ 28.36, 28.26, 25.78 and 17.79),

two methylene carbons (δ 43.14 and 21.43), six methine carbons (δ 127.67, 125.98, 122.43, 115.59, 115.50 and 78.49), ten quaternary carbons (δ 159.92, 159.36, 156.53, 155.96, 131.09, 130.84, 108.63, 102.78, 102.62 and 78.12) and a carbonyl carbon (δ 196.57).

The location of the isoprenyl unit was deduced to be at C-8 by the result of the 2D HMBC correlations of H-1‴ to C-7 (δ 159.92), C-8 (δ 108.63), C-8a (δ 159.36), C-2‴ (δ 122.43) and C-3‴ (δ 131.09). The carbon signals at C-5 (δ 156.53), C-6 (δ 102.78), C-7 (δ 159.92) and C-2″ (δ 78.12) showed correlation to H-4″ (δ 6.63) and C-6 (δ 102.78), C-2″ (δ 78.12) and C-5″/6″ (δ 28.26/28.36) to H-3″ (δ 5.50), respectively. These correlations confirmed the presence of dimethylchromene ring and suggested that this unit fused to aromatic nuclei at C-6 and C-7.



Selected HMBC correlation of compound **STH1**

Table 29 ^{1}H, ^{13}C and HMBC spectral data of compound **STH1**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
2	78.49	CH	5.33 (1H, *dd*, *J* = 13, 3 Hz)	C-4, C-1′ and C-2′
3α	43.14	CH_2	3.04 (1H, *dd*, *J* = 17.5, 13 Hz)	C-2, C-4 and C-1′
β			2.80 (1H, *dd*, *J* = 17.5, 3 Hz)	C-4 and C-4a
4	196.57	C	-	-
4a	102.62	C	-	-
5	156.53	C	12.24 (OH, *s*)	C-4a, C-5 and C-6
6	102.78	C	-	-
7	159.92[a]	C	-	-
8	108.63	C	-	-
8a	159.36[a]	C	-	-
1′	130.84	C	-	-
2′/ 6′	127.67	CH	7.31 (2H, *d*, *J* = 8.5 Hz)	C-2, C-4′ and C-6′/2′
3′/ 5′	115.50	CH	6.87 (2H, *d*, *J* = 8.5 Hz)	C-1′, C-4′ and C-5′/3′
4′	155.96	C	-	-
2″	78.12	C	-	-
3″	125.98	CH	5.50 (1H, *d*, *J* = 10 Hz)	C-6, C-2″, C-5″ and C-6″
4″	115.59	CH	6.63 (1H, *d*, *J* = 10 Hz)	C-5, C-6, C-7 and C-2″
5″	28.26[b]	CH_3	1.43 [*] (3H, *s*)	C-2″, C-3″and C-6″
6″	28.36[b]	CH_3	1.45 [*] (3H, *s*)	C-2″, C-3″and C-5″
1‴	21.43	CH_2	3.21 (2H, *br d*, *J* = 7 Hz)	C-7, C-8, C-8a, C-2‴ and C-3‴
2‴	122.43	CH	5.14 (1H, *triplet of guintet*, *J* = 9, 1.5 Hz)	} C-8, C-1‴, C-4‴ and C-5‴
3‴	131.09	C	-	-
4‴	25.78[c]	CH_3	1.65 (3H, *s*)	C-8, C-2‴, C-3‴ and C-5‴
5‴	17.79[c]	CH_3	1.65 (3H, *s*)	C-8, C-2‴, C-3‴ and C-4‴

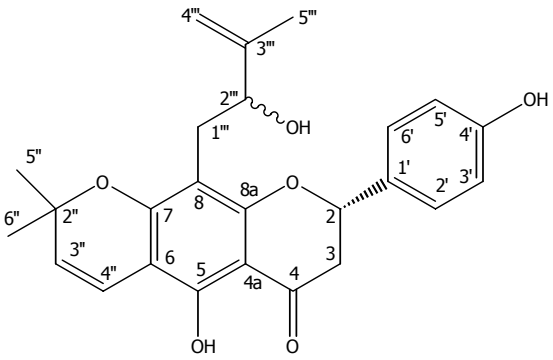
[a, b, c, *] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Comparison of ^{1}H NMR spectrum (**Table 30**) between compound **STH1** and lupinifolin (Mahidol, *et.al*., 1997) showed similarity. Thus compound **STH1** was identified as lupinifolin.

Table 30 Comparison of ^{1}H NMR spectral data between compound **STH1** and lupinifolin

Position	**Compound STH1 δ_H (ppm) (recorded in $CDCl_3$)**	**Lupinifolin δ_H (ppm) (recorded in $CDCl_3$)**
2	5.33 (1H, *dd*, *J* = 13, 3 Hz)	5.30 (1H, *dd*, *J* = 12.7, 3.3 Hz)
3α	3.04 (1H, *dd*, *J* = 17.5, 13 Hz)	3.03 (1H, *dd*, *J* = 17.3, 12.7 Hz)
β	2.80 (1H, *dd*, *J* = 17.5, 3 Hz)	2.78 (1H, *dd*, *J* = 17.3, 3.3 Hz)
5-OH	12.24 (OH, *s*)	12.20 (OH, *s*)
2′, 6′	7.31 (2H, *d*, *J* = 8.5 Hz)	7.28 (2H, *d*, *J* = 8.5 Hz)
3′, 5′	6.87 (2H, *d*, *J* = 8.5 Hz)	6.84 (2H, *d*, *J* = 8.5 Hz)
4′-OH	-	6.30 (1H, *br s*)
3″	5.50 (1H, *d*, *J* = 10 Hz)	5.48 (1H, *d*, *J* = 10 Hz)
4″	6.63 (1H, *d*, *J* = 10 Hz)	6.63 (1H, *d*, *J* = 10 Hz)
5″/ 6″	1.43/1.45 (each 3H, *s*)	1.44 (6H, *s*)
1‴	3.20 (2H, *br d*, *J* = 7 Hz)	3.20 (2H, *br d*, *J* = 7 Hz)
2‴	5.14 (1H, *triplet of guintet*, *J* = 9, 1.5 Hz)	5.14 (1H, *t*, *J* = 7.0 Hz)
4‴/ 5‴	1.65 (6H, *s*)	1.64 (6H, *s*)

2.3.1.2 Compound STH 2



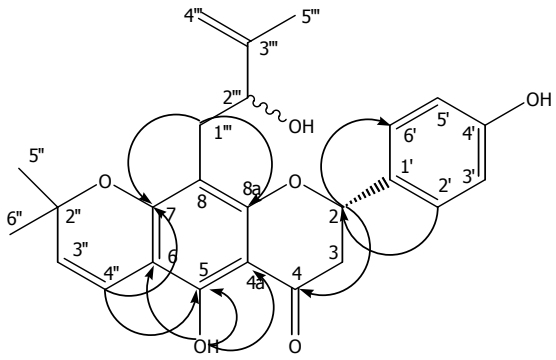
Compound **STH2** was obtained as a yellow viscous oil, $[\alpha]_D^{27}$: -83.3 ° (*c* = 0.012, $CHCl_3$). The IR spectrum showed absorption bands at 3383 and 1633 cm^{-1} corresponding to a hydroxyl group and conjugated carbonyl group, respectively. The UV spectrum showed maxima at 203, 222, 266, 274, 297, 311 and 364 nm, which is a typical absorption of flavanone.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **STH2** (**Table 31**) were assigned with informations provided from HMQC, HMBC (**Table 31**) and COSY spectrum, along with comparison of ^{1}H NMR spectral data to compound **STH1** (**Table 32**). The ^{13}C NMR spectrum (**Table 31**) of compound **STH2** showed 25 signals for 25 carbon atoms. Analysis of DEPT spectra of this compound suggested the presence of three methyl carbons (δ 28.55, 28.35 and 18.27), three methylene carbons (δ 110.10, 43.16 and 29.69), six methine carbons (δ 127.70, 125.84, 115.62, 115.54, 78.84 and 75.73), ten quaternary carbons (δ 159.94, 159.84, 157.08, 156.04, 147.22, 130.60, 105.31, 102.82, 102.70 and 78.78) and a carbonyl carbon (δ 196.34).

Compound **STH2**, a derivative of compound **STH1**, showed similar characteristic IR and UV spectrum with those of **STH1**. Comparison of the ^{1}H NMR (**Table 32**) spectral data of the two compounds revealed close structural similarity.

Differences in the spectrum of compound **STH2** was shown as terminal olefinic protons at δ 4.91 and 4.82 (each 1H, *m*) which were not observed in compound **STH1**. The characteristics of flavanone were shown as resonance signals of ABX system at δ 5.36 (1H, *dd*, *J* = 13.5 and 3 Hz) and a *doublet of doublet* at δ 3.11 (1H, *J* = 17.5 and 13.5 Hz) and 2.84 (1H, *J* = 17.5 and 3 Hz) which were assigned to H-2β, H-3α and H-3β, respectively. A sharp *singlet* signal of a chelated hydroxy group 5-OH appeared at δ 12.35. The ^{1}H NMR spectrum of compound **STH2** also exhibited characteristic signals for H-2′/6′ (δ 7.37, *d*, *J* = 8.5 Hz, 2H) and H-3′/5′ (δ 6.93, *d*, *J* = 8.5 Hz, 2H). Furthermore the signals of H-3″ and H-4″ of 2″,2″-dimethylpyrane ring were shown at δ 5.70 (1H, *d*, *J* = 10 Hz) and 6.70 (1H, *d*, *J* = 10 Hz). In the COSY spectrum, the 2H-1‴ (δ 2.94, *dd*, *J* = 14, 5 Hz and 2.77, *dd*, *J* = 14, 8.5 Hz) methylene protons demonstrated vicinal coupling with the H-2‴ (δ 4.24, *dd*, *J* = 8.5, 5 Hz) methine proton which was connected to the oxygenated carbon at δ 75.73 in the HMQC spectrum; hence, the hydroxyl group was located at C-2‴.

The HMBC correlations of compound **STH2** (**Table 31**) were the same as compound **STH1**. Thus compound **STH2** was identified as dereticulatin (Mahidol, *et.al*., 1997).



Selected HMBC correlation of compound **STH2**

Table 31 ^{1}H, ^{13}C and HMBC spectral data of compound **STH2**

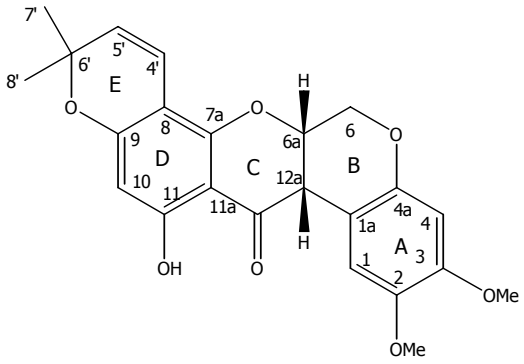
Position	δ_C (ppm)		δ_H (ppm)	HMBC
2	78.84	CH	5.36 (1H, *dd*, *J* = 13.5, 3 Hz)	-
3α	43.16	CH_2	3.11 (1H, *dd*, *J* = 17.5, 13.5 Hz)	C-2, C-4 and C-1′
β			2.84 (1H, *dd*, *J* = 17.5, 3 Hz)	
4	196.34	C	-	-
4a	102.70	C	-	-
5	157.08	C	12.35 (OH, *s*)	C-4a, C-5 and C-6
6	102.82	C	-	-
7	159.94[a]	C	-	-
8	105.31	C	-	-
8a	159.84[a]	C	-	-
1′	130.60	C	-	-
2′ / 6′	127.70	CH	7.37 (2H, *d*, *J* = 8.5 Hz)	C-2, C-1′, C-4′ and C-6′/2′
3′ / 5′	115.62	CH	6.93 (2H, *d*, *J* = 8.5 Hz)	C-1′, C-4′ and C-5′/3′
4′	156.04	C	-	-
2″	78.78	C	-	-
3″	125.84	CH	5.70 (1H, *d*, *J* = 10 Hz)	C-6 and C-2″
4″	115.54	CH	6.70 (1H, *d*, *J* = 10 Hz)	C-5, C-7 and C-2″
5″	28.35[b]	CH_3	1.52 (6H, *s*)	C-2″, C-3″ and C-6″
6″	28.55[b]	CH_3		C-2″, C-3″ and C-5″
1‴α	29.69	CH_2	2.94 (1H, *dd*, *J* = 14, 5 Hz)	C-7 , C-8, C-8a and C-2‴
β			2.77 (1H, *dd*, *J* = 14, 8.5 Hz)	
2‴	75.73	CH	4.24 (1H, *dd*, *J* = 8.5, 5 Hz)	-
3‴	147.22	C	-	-
4‴	110.10	CH_2	4.82 (1H, *m*)	C-2‴and C-5‴
			4.91(1H, *m*)	C-2‴and C-5‴
5‴	18.27	CH_3	1.76 (3H, *s*)	C-2‴, C-3‴ and C-4‴

[a, b]Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 32 Comparison of ^{1}H NMR spectral data between compounds **STH1** and **STH2**

Position	**Compound STH1 δ_H (ppm) (recorded in $CDCl_3$)**	**Compound STH2 δ_H (ppm) (recorded in $CDCl_3$)**
2	5.33 (1H, *dd*, *J* = 13, 3 Hz)	5.36 (1H, *dd*, *J* = 13.5, 3 Hz)
3α	3.04 (1H, *dd*, *J* = 17.5, 13 Hz)	3.11 (1H, *dd, J* = 17.5, 13.5 Hz)
β	2.80 (1H, *dd*, *J* = 17.5, 3 Hz)	2.84 (1H, *dd*, *J* = 17.5, 3 Hz)
5-OH	12.24 (OH, *s*)	12.35 (OH, *s*)
2′, 6′	7.31 (2H, *d*, *J* = 8.5 Hz)	7.37 (2H, *d*, *J* = 8.5 Hz)
3′, 5′	6.87 (2H, *d*, *J* = 8.5 Hz)	6.93 (2H, *d*, *J* = 8.5 Hz)
3″	5.50 (1H, *d*, *J* = 10 Hz)	5.70 (1H, *d*, *J* = 10 Hz)
4″	6.63 (1H, *d*, *J* = 10 Hz)	6.70 (1H, *d*, *J* = 10 Hz)
5″/ 6″	1.43/1.45 (each 3H, *s*)	1.52 (6H, *s*)
1‴	3.21 (2H, *br d*, *J* = 7 Hz)	2.94 (1H, *dd*, *J* = 14, 5 Hz) 2.77 (1H, *dd*, *J* = 14, 8.5 Hz)
2‴	5.14 (1H, *triplet of guintet*, *J* = 9, 1.5 Hz)	4.24 (1H, *dd*, *J* = 8.5, 5 Hz)
4‴	1.65 (3H, *s*)	4.82, 4.91 (each 1H, *m*)
5‴	1.65 (3H, *s*)	1.76 (3H, *s*)

2.3.1.3 Compound STH 3

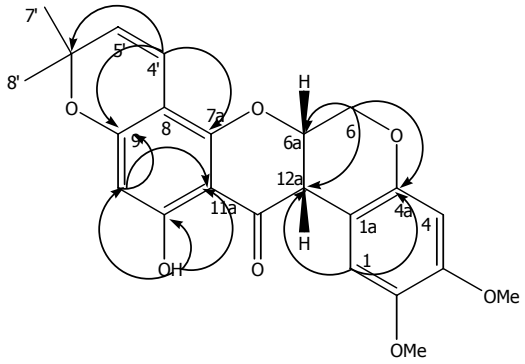


Compound **STH3** was obtained as a yellow viscous oil, $[\alpha]_D^{27}$: +29.4 ° (*c* = 0.034, $CHCl_3$). **STH3** exhibited IR absorption bands at 3439 and 1641 cm^{-1} which indicated the presence of hydroxyl group and conjugated carbonyl group, respectively. The UV spectrum showed maxima at 205, 226, 273, 295, 310 and 367 nm.

The ^{1}H NMR spectrum (**Table 33**) showed a sharp *singlet* of a chelated hydroxy 11-OH at δ 12.21. The dimethylchromene system is well characterized by two *doublets* at δ 5.46 (*J* = 9.9 Hz) and 6.55 (*J* = 9.9 Hz) for the vinyl protons and a *singlet* integrated for 6 protons at δ 1.35 and 1.45. Three *singlet* signals were shown at δ 5.95 (1H), 6.45 (1H) and 6.86 (1H) which were assigned as H-10, H-4 and H-1, respectively. Two methoxy groups resonated as *singlets* at δ 3.78 (2-OMe) and 3.81 (3-OMe). The following signals due to protons on carbon atoms bearing oxygen atoms were exhibited at δ 4.86 (1H, *m*, H-6*a*β), 4.61 (1H, *dd*, *J* = 12 and 3 Hz, H-6α) and 4.17 (1H, *d*, *J* = 12, H-6β). In addition, a *doublet* at δ 3.84 (1H, *J* = 4.2 Hz, H-12aβ) was also observed. The ^{13}C NMR and the DEPT spectral data (**Table 33**) indicated the existence of four methyl carbons (δ 56.30, 55.85, 28.57 and 28.31), a methylene carbon (δ 65.99), seven methine carbons (δ 126.39, 115.41, 110.20, 100.99, 97.76,

71.88 and 43.51), ten quaternary carbons (δ 164.50, 162.82, 155.91, 149.63, 147.25, 143.94, 104.39, 101.75, 101.14 and 78.32) and a carbonyl carbon (δ 194.23).

In the HMBC spectrum (**Table 33**), the correlation of H-4′ (δ 6.55) to C-9 (δ 162.82), C-7a (δ 155.91) and C-6′ (δ 78.32), of H-5′ (δ 5.46) to C-8 (δ 101.75) and C-6′ (δ 78.32), and of H-10 (δ 5.95) to C-9 (δ 162.82) and C-11a (δ 101.14) were observed. These correlations confirmed the presence of dimethylchromene ring and suggested that this unit fused to aromatic nucleus at C-8 and C-9. In NOE experiment, irradiation of methine proton at H-12a (δ 3.84) resulted in the enhancement of the signals at H-6a (δ 4.86) and H-1 (δ 6.86), whereas the small coupling constant of H-12a (4.2 Hz) was observed. These observations indicated that B/C ring fusion*s are cis*. Thus, compound **STH3** was identified to be α-toxicarol (Andrei, *et.al.* 1997).



Selected HMBC correlation of compound **STH3**

Table 33 ^{1}H, ^{13}C and HMBC spectral data of compound **STH3**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1a	104.39	C	-	-
1	110.20	CH	6.86 (1H, *s*)	C-1a , C-2, C-3, C-4a and C-12a
2	143.94	C	-	-
3	149.63	C	-	-
4	100.99	CH	6.45 (1H, *s*)	C-1a, C-2, C-3 and C-4a
4a	147.25	C	-	-
6α	65.99	CH_2	4.61 (1H, *dd*, *J* = 12, 3 Hz)	C-4a and C-12a
β			4.17 (1H, *d*, *J* = 12 Hz)	C-6a
6a	71.88	CH	4.86 (1H, *m*)	C-6
7a	155.91	C	-	-
8	101.75[a]	C	-	-
9	162.82	C	-	-
10	97.76	CH	5.95 (1H, *s*)	C-9 and C-11a
11-OH	164.50	C-OH	12.21 (1H, *s*)	C-11, C-11a and C-10
11a	101.14[a]	C	-	-
12	194.23	C	-	-
12a	43.51	CH	3.84 (1H, *d*, *J* = 4.2 Hz)	C-1a, C-4a, C-11a and C-12
4′	115.41	CH	6.55 (1H, *d*, *J* = 9.9 Hz)	C-7a, C-9 and C-6′
5′	126.39	CH	5.46 (1H, *d*, *J* = 9.9 Hz)	C-8 and C-6′
6′	78.32	C	-	-
7′	28.31[b]	CH_3	1.35 [*] (3H, *s*)	C-5′, C-6′ and C-8′
8′	28.57[b]	CH_3	1.45[*] (3H, *s*)	C-5′, C-6′ and C-7′
2-OMe	55.85	CH_3	3.78 (3H, *s*)	C-2
3-OMe	56.30	CH_3	3.81 (3H, *s*)	C-3

[a, b, *] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 34 Comparison of ^{1}H NMR spectral data between compound **STH3** and α-toxicarol

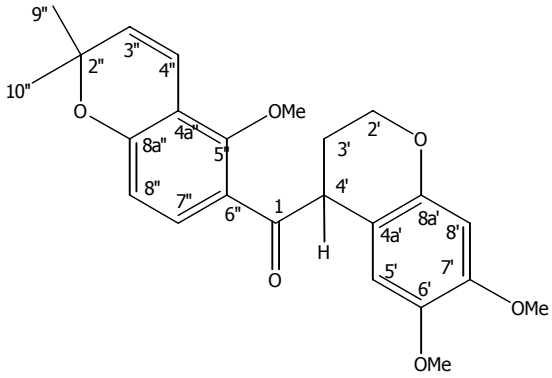
Position	**Compound STH3 δ_H (ppm) (recorded in $CDCl_3$)**	**α-Toxicarol δ_H (ppm) (recorded in $CDCl_3$)**
1	6.86 (1H, *s*)	6.86 (1H, *s*)
4	6.45 (1H, *s*)	6.46 (1H, *s*)
6α	4.61 (1H, *dd*, *J* = 12, 3 Hz)	4.60 (1H, *dd*, *J* = 12, 3.2 Hz)
β	4.17 (1H, *d*, *J* = 12 Hz)	4.16 (1H, *d*, *J* = 12 Hz)
6a	4.86 (1H, *m*)	4.87 (1H, *t*, *J* = 3.2 Hz)
10	5.95 (1H, *s*)	5.95 (1H, *s*)
11-OH	12.21 (1H, *s*)	12.19 (1H, *s*)
12a	3.84 (1H, *d, J* = 4.2 Hz)	3.84 (1H, *d*, *J* = 4 Hz)
4′	6.55 (1H, *d*, *J* = 9.9 Hz)	6.55 (1H, *d*, *J* = 10 Hz)
5′	5.46 (1H, *d*, *J* = 9.9 Hz)	5.47 (1H, *d*, *J* = 10 Hz)
7′/8′	1.35 /1.45 (each 3H, *s*)	1.37 /1.43 (each 3H, *s*)
2-OMe	3.78 (3H, *s*)	3.79 (3H, *s*)
3- OMe	3.81 (3H, *s*)	3.81 (3H, *s*)

Table 35 Comparison of ^{13}C NMR spectral data between compound **STH3** and α-toxicarol

Position	**Compound STH3 δ_C (ppm) (recorded in $CDCl_3$)**	**α-Toxicarol δ_C (ppm) (recorded in $CDCl_3$)**
1a	104.4	104.4
1	110.2	110.3
2	143.9	143.9
3	149.6	149.6
4	101.0	101.0
4a	147.3	147.3
6	66.0	66.0
6a	71.9	71.9
7a	155.9	155.9
8	101.8 [a]	101.8 [a]
9	162.8	162.8
10	97.8	97.8
11	164.5	164.5
11a	101.1 [a]	101.2 [a]
12	194.2	194.3
12a	43.5	43.5
4′	115.4	115.4
5′	126.4	126.4
6′	78.3	78.3

[a, b] Interchangeable values in each column.

2.3.1.4 Compound STH 4

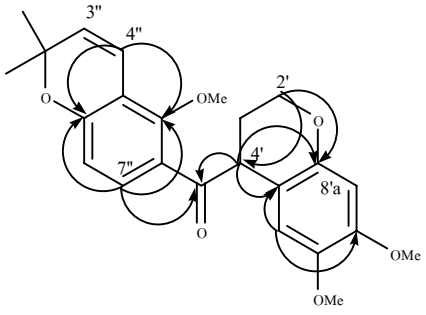


Compound **STH4** was obtained as a pale yellow viscous oil and gave a molecular ion at m/z 411.1808 $[M+H]^+$ by HR-ESITOF MS, corresponding to $C_{24}H_{26}O_6$. The IR spectrum showed absorption band at 1673 cm^{-1} corresponding to a conjugated carbonyl group. The UV spectrum showed maxima at 206, 231, 260 and 330 nm.

The ^{1}H NMR spectrum of **STH4** (**Table 36**) showed a *doublet* resonance at δ 5.71 (*J* = 9.9 Hz) and δ 6.62 (*d*, *J* = 9.9 Hz) each equivalent to one proton and two *singlet* resonances at δ 1.45 (3H) and δ 1.46 (3H) which were characteristic of the *cis* double bond and *gem*-dimethyl group of a 2,2-dimethylchromene moiety, respectively. An *ortho*-coupled *doublet* centered at δ 6.62 (1H, *d*, *J* = 8.4 Hz) and δ 7.35 (1H, *d*, *J* = 8.4 Hz) were assigned to the protons of a tetra-substituted benzene ring. Other aromatic protons at δ 6.40 (2H, *s*) were assigned as H-5′ and H-8′, respectively. Three *singlet* signals at δ 3.64 (3H, *s*), 3.80 (3H, *s*) and 3.81 (3H, *s*) were assigned to the methoxy group of benzene ring at C-6′, C-5′′ and C-7′, respectively. The protons at δ 4.61 (1H, *dd*, *J* = 6.0, 4.2 Hz) and two *multiplets* at δ 2.22 (2H) and 4.19 (2H) were assigned as H-4′, H-3′ and H-2′, respectively. The assignments were confirmed by COSY spectrum in which cross-peaks were observed between H-4′ and H-3′, H-3′ and H-2′, H-7′′ and H-8′′ and H-3′′and H-4′′.

The ^{13}C NMR spectrum (**Table 36**) showed a total of 24 carbons with one carbonyl group at δ 202.67 and DEPT experiment showed five methyl carbons (δ 63.47, 56.17, 55.76, 28.00 and 27.96), two methylene carbons (δ 63.52 and 25.27), seven methine carbons (δ 130.89, 130.63, 116.38, 112.75, 112.67, 100.89 and 44.62) and nine quaternary carbons (δ 157.42, 155.53, 149.10, 149.02, 142.91, 125.24, 114.88, 109.96 and 76.89). The assignments of all carbons were achieved by ^{13}C, HMQC and HMBC experiments. In the HMBC spectrum (**Table 36**), a correlation of both H-8″ and H-3″ with the carbon at δ 114.88 confirmed its assignment as C-4″a. Furthermore, C-8″a was assigned at δ 157.42 as it showed correlations with δ 7.35 (H-7″) and 6.62 (H-4″). The features of the HMBC experiment confirmed the attachment of the pyrano ring through C-8″a (δ 157.42) and C-4″a (δ 114.88). The assignments were additional confirmed by correlation of H-4′ (δ 4.61) to δ 202.67 (C-1), 109.96 (C-4′a) and 149.10 (C-8′a), of H-2′ (δ 4.19) to δ 149.10 (C-8′a), 25.27 (C-3′) and 44.62 (C-4′).

The relative stereochemistry at C-4′ of compound **STH4** could not be assigned based on the observation of NOE experiment and coupling constant. Thus, the structure of the new compound **STH4** was elucidated as [6′,7′-dimethoxy-3′,4′-dihydro-2*H*-chromen-4′-yl](5″-methoxy-2″,2″-dimethyl-2*H*-chromen-6″-yl) methanone, (trifolinone A).



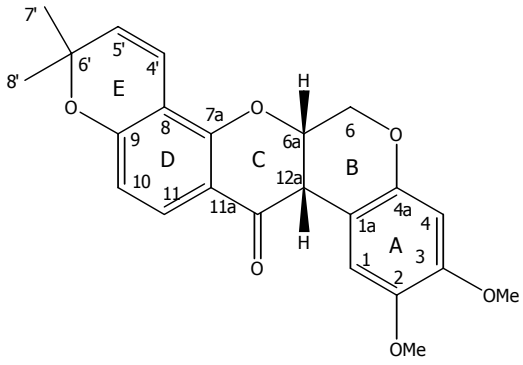
Selected HMBC correlation of compound **STH4**

Table 36 ^{1}H, ^{13}C and HMBC spectral data of compound **STH4**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1	202.67	C	-	-
2′	63.52	CH_2	4.19 (2H, *m*)	C-3′, C-4′ and C-8′a
3′	25.27	CH_2	2.22 (2H, *m*)	C-1, C-2′, C-4′ and C-4′a
4′	44.62	CH	4.61 (1H, *dd*, *J* = 6.0, 4.2 Hz)	C-1, C-2′, C-4′a, C-5′ and C-8′a
4′a	109.96	C	-	-
5′	100.89	CH	6.40 (1H, *s*)	C-4′, C-4′a, C-7′ and C-8′a
6′	149.02	C	-	-
7′	142.91	C	-	-
8′	112.67	CH	6.40 (1H, *s*)	C-4′a and C-7′
8′a	149.10	C	-	-
2″	76.89	C	-	-
3″	130.63	CH	5.71 (1H, *d*, *J* = 9.9 Hz)	C-2″, C-4″a and C-9″/ 10″
4″	116.38	CH	6.62 (1H, *d*, *J* = 9.9 Hz)	C-2″, C-5″ and C-8″a
4″a	114.88	C	-	-
5″	155.53	C	-	-
6″	125.24	C	-	-
7″	130.89	CH	7.35 (1H, *d*, *J* = 8.4 Hz)	C-1, C-5″ and C-8″a
8″	112.75	CH	6.62 (1H, *d*, *J* = 8.4 Hz)	C-4″a and C-6″
8″a	157.42	C	-	-
9″	28.00[a]	CH_3	1.45[†] (3H, *s*)	} C-2 ″, C-3″ and C-4″
10″	27.96[a]	CH_3	1.46[†] (3H, *s*)	
6′-OMe	56.17[b]	CH_3	3.64[*] (3H, *s*)	C-6′
7′-OMe	55.76[b]	CH_3	3.81[*] (3H, *s*)	C-7′
5″-OMe	63.47	CH_3	3.80 (3H, *s*)	C-5″

[a, †, *] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

2.3.1.5 Compound STH 5

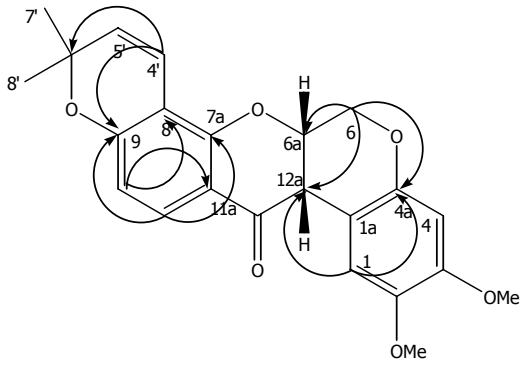


Compound **STH5** was obtained as a yellow viscous oil, $[\alpha]_D^{27}$: -19.2° (*c* = 0.026, $CHCl_3$). In the UV spectrum, strong absorptions at 243, 274, 298 and 318 nm were detected. The IR spectrum exhibited absorption bands of conjugated carbonyl group at 1698 cm^{-1}.

Comparison of the ^{1}H NMR spectral data (**Table 38**) of compounds **STH5** and **STH3** revealed close structural similarity. Difference in the spectrum of compound **STH5** was shown as a signal of *ortho*-coupling protons at δ 7.74 (1H, *d*, *J* = 8.7 Hz) and 6.46 (1H, *dd*, *J* = 8.7 and 0.6 Hz), which was not observed in compound **STH3**. The signals of two olefinic protons at δ 6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz) and 5.57 (1H, *d*, *J* = 9.9 Hz) and two methyl groups at δ 1.45 and 1.38 which corresponded to a part of dimethylchromene ring were detected (Rao and Srimannarayana, 1983). Two methoxy groups resonated as two *singlets* at δ 3.80 (3-OMe) and 3.77 (2-OMe). A *singlet* signal at δ 6.46 (1H) was assigned as H-4, while the H-1 appeared at δ 6.78 (1H, *d*, *J* = 0.6 Hz). It also exhibited the signals due to protons on carbon atoms bearing oxygen atoms at δ 4.64 (1H, *dd*, *J* = 12 and 3.3 Hz, H-6α), 4.19 (1H, *d*, *J* = 12 Hz, H-6β) and 4.91 (1H, *m*, H-6aβ). In addition, a *doublet* at δ 3.83 (1H, *J* = 3.9 Hz, H-12aβ) was apparent. The ^{13}C NMR spectrum and the DEPT spectral data (**Table 37**)

indicated the existence of four methyl carbons (δ 56.27, 55.82, 28.46 and 28.12), a methylene carbon (δ 66.27), eight methine carbons (δ 128.64, 128.53, 115.72, 110.34, 111.45, 100.88, 72.39 and 44.35), nine quaternary carbons (δ 160.07, 156.92, 149.42, 147.37, 143.82, 112.72, 109.10, 104.71 and 77.67) and a carbonyl carbon (δ 189.23).

In the HMBC spectrum (**Table 37**), the carbon signals at C-6 (δ 66.27) and C-9 (δ 160.07) showed correlation to H-4′ (δ 6.65) and C-9 (δ 160.07) and C-7a (δ 156.92) to H-11 (δ 7.74). These correlations confirmed the presence of dimethyl-chromene ring and suggested that this unit fused to aromatic nuclei at C-8 and C-9. In NOE experiment, irradiation of methine proton at H-12a (δ 3.83) resulted in the enhancement of the signals at H-6a (δ 4.91) and H-1 (δ 6.78). These observations suggested that the B/C ring fusions are *cis*. Thus, compound **STH5** was identified to be deguelin (Andrei, *et.al.* 1997).



Selected HMBC correlation of compound **STH5**

Table 37 ^{1}H, ^{13}C and HMBC spectral data of compound **STH5**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1a	104.71	C	-	-
1	110.34	CH	6.78 (1H, *d*, *J* = 0.6 Hz)	C-2, C-3, C-4a and C-12a
2	143.82	C	-	-
3	149.42	C	-	-
4	100.88	CH	6.46 (1H, *s*)	C-1a, C-2, C-3 and C-4a
4a	147.37	C	-	-
6α	66.27	CH_2	4.64 (1H, *dd*, *J* = 12, 3.3 Hz)	C-4a, C-6a and C-12a
β			4.19 (1H, *d*, *J* = 12 Hz)	C-6a and C-12a
6a	72.39	CH	4.91 (1H, *m*)	C-6 and C-1a
7a	156.92	C	-	-
8	109.10	C	-	-
9	160.07	C	-	-
10	111.45	CH	6.46 (1H, *dd*, *J* = 8.7, 0.6 Hz)	C-8 and C-11a
11	128.53	CH	7.74 (1H, *d*, *J* = 8.7 Hz)	C-7a and C-9
11a	112.72	C	-	-
12	189.23	C	-	-
12a	44.35	CH	3.83 (1H, *d*, *J* = 3.9 Hz)	C-1a
4′	115.72	CH	6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz)	C-9 and C-6′
5′	128.64	CH	5.57 (1H, *d*, *J* = 9.9 Hz)	C-8 and C-6′
6′	77.67	C	-	-
7′	28.12[a]	CH_3	1.38[*] (3H, *s*)	C-5′, C-6′ and C-8′
8′	28.46[a]	CH_3	1.45[*] (3H, *s*)	C-5′, C-6′ and C-7′
2-OMe	55.82	CH_3	3.77 (3H, *s*)	C-2
3-OMe	56.27	CH_3	3.80 (3H, *s*)	C-3

[a, *] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 38 Comparison of ^{1}H NMR spectral data between compounds **STH5** and **STH3**

Position	**Compound STH5 δ_H (ppm) (recorded in $CDCl_3$)**	**Compound STH3 δ_H (ppm) (recorded in $CDCl_3$)**
1	6.78 (1H, *d*, *J* = 0.6 Hz)	6.86 (1H, *s*)
4	6.46 (1H, *s*)	6.45 (1H, *s*)
6α	4.64 (1H, *dd*, *J* = 12, 3.3 Hz)	4.61 (1H, *dd*, *J* = 12, 3 Hz)
β	4.19 (1H, *d*, *J* = 12 Hz)	4.17 (1H, *d*, *J* = 12 Hz)
6a	4.91 (1H, *m*)	4.86 (1H, *m*)
10	6.46 (1H, *dd*, *J* = 8.7, 0.6 Hz)	5.95 (1H, *s*)
11	7.74 (1H, *d*, *J* = 8.7 Hz)	12.21 (1H, *s*)
12a	3.83 (1H, *d*, *J* = 3.9 Hz)	3.84 (1H, *d, J* = 4.2 Hz)
4′	6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz)	6.55 (1H, *d*, *J* = 9.9 Hz)
5′	5.57 (1H, *d*, *J* = 9.9 Hz)	5.46 (1H, *d*, *J* = 9.9 Hz)
7′/8′	1.45/1.38 (each 3H, *s*)	1.45 /1.35 (each 3H, *s*)
2-OMe	3.77 (3H, *s*)	3.78 (3H, *s*)
3-OMe	3.80 (3H, *s*)	3.81 (3H, *s*)

Table 39 Comparison of ^{1}H NMR spectral data between compound **STH5** and deguelin

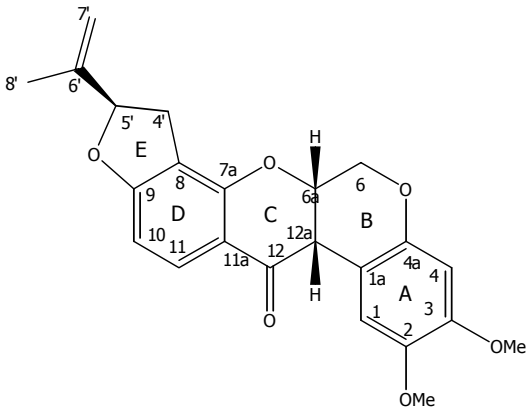
Position	**Compound STH5 δ_H (ppm) (recorded in $CDCl_3$)**	**Deguelin δ_H (ppm) (recorded in $CDCl_3$)**
1	6.78 (1H, *d*, *J* = 0.6 Hz)	6.73 (1H, *s*)
4	6.46 (1H, *s*)	6.38 (1H, *s*)
6α	4.64 (1H, *dd*, *J* = 12, 3.3 Hz)	4.56 (1H, *dd*, *J* = 12.4, 3.2 Hz)
β	4.19 (1H, *d*, *J* = 12 Hz)	4.11 (1H, *d*, *J* = 12.4 Hz)
6a	4.91 (1H, *m*)	4.84 (1H, *m*)
10	6.46 (1H, *dd*, *J* = 8.7, 0.6 Hz)	6.38 (1H, *d*, *J* = 8.8 Hz)
11	7.74 (1H, *d*, *J* = 8.7 Hz)	7.67 (1H, *d*, *J* = 8.8 Hz)
12a	3.83 (1H, *d*, *J* = 3.9 Hz)	3.77 (1H, *d*, *J* = 4 Hz)
4′	6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz)	6.57 (1H, *d*, *J* = 10 Hz)
5′	5.57 (1H, *d*, *J* = 9.9 Hz)	5.48 (1H, *d*, *J* = 10 Hz)
7′/8′	1.38/1.45 (each 3H, *s*)	1.32 /1.38 (each 3H, *s*)
2-OMe	3.77 (3H, *s*)	3.70 (3H, *s*)
3- OMe	3.80 (3H, *s*)	3.73 (3H, *s*)

Table 40 Comparison of ^{13}C NMR spectral data between compound **STH5** and deguelin

Position	**Compound STH5 δ_C (ppm) (recorded in $CDCl_3$)**	**Deguelin δ_C (ppm) (recorded in $CDCl_3$)**
1a	104.7	105.0
1	110.3	110.7 [a]
2	143.8 [a]	144.1
3	149.4 [a]	149.8
4	100.9	101.2
4a	147.4	147.7
6	66.3	66.5
6a	72.4	72.7
7a	156.9	158.0
8	109.1	109.4
9	160.1	160.3
10	111.5	117.7 [a]
11	128.5 [b]	128.8 [b]
11a	112.7	113.0
12	189.2	189.4
12a	44.4	44.6
4′	115.7	116.0
5′	128.6 [b]	128.9 [b]
6′	77.7	77.9

[a, b] Interchangeable values in each column.

2.3.1.6 Compound STH 6



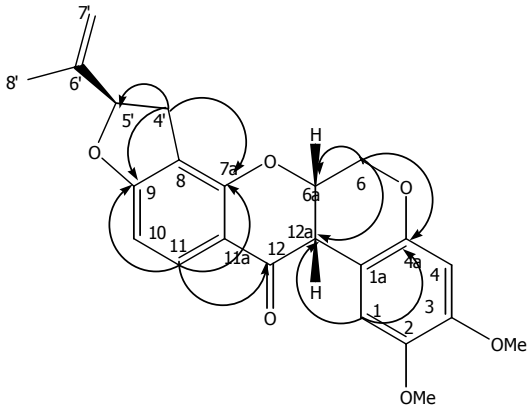
Compound **STH6** was isolated as a yellow viscous oil, $[\alpha]_D^{27}$: -27.7° (*c* = 0.018, $CHCl_3$). In the UV spectrum, strong absorptions at 206, 235 and 292 nm were detected. The presence of a conjugated carbonyl group at 1673 cm^{-1} was suggested from the IR spectrum.

The ^{1}H NMR spectral data (**Table 41**), revealed characteristic signals of rotenoid at δ 4.92 (1H, *m*, H-6aβ), 4.61 (1H, *dd*, *J* = 12 and 3.3 Hz, H-6α), 4.18 (1H, *d*, *J* = 12 Hz, H-6β) and 3.84 (1H, *d*, *J* = 3 Hz, H-12aβ). Signals of four aromatic protons were discernible at δ 7.84 and 6.51 (each 1H, *d*, *J* = 8.4 Hz, H-11, H-10) and δ 6.77 (1H, *d*, *J* = 0.6 Hz, H-1) and 6.46 (1H, *s*, H-4). Compound **STH6** also contained two phenolic methyl ethers appearing at δ 3.81 and 3.76 (each 3H, *s*), and a terminal olefinic protons at δ 5.07 (1H, *s*) and 4.93 (1H, *m*) which were assigned to be signals of H-7′. Two *doublets of doublet* signals at δ 3.32 (1H, *J* = 15.9, 9 Hz), 2.96 (1H, *J* = 15.9, 9 Hz) and a *triplet* signal at δ 5.24 (1H, *J* = 9 Hz) were suggested to be signals of H-4′α, H-4′β and H-5′α, respectively. A *singlet* signal of a methyl group at δ 1.77 (3H, *s*, H-8′) was shown. The ^{13}C NMR and the DEPT spectral data (**Table 41**) indicated the existence of three methyl carbons (δ 56.28, 55.83 and 17.11), three methylene carbons (δ 112.57, 66.25 and 31.24), seven methine carbons (δ 129.97, 110.27, 104.88, 100.86,

87.83, 72.18 and 44.56), nine quaternary carbons (δ 167.36, 157.93, 149.44, 147.33, 143.84, 143.00, 113.30, 112.95 and 104.76) and a carbonyl carbon (δ 188.97).

In the HMBC experiments, correlation of H-11 (δ 7.84) to C-12 (δ 188.97), C-9 (δ 167.36) and C-7a (δ 157.93) and of H-4′ (δ 3.32) to C-9 (δ 167.36), C-7a (δ 157.93), C-5′ (δ 87.83) and C-6′ (δ 143.00), suggested that the furan ring was connected to the 8, 9-position. The H-6 (δ 4.61 and 4.18) showed correlation to C-4a (δ 147.33), C-6a (δ 72.18) and C-12a (δ 44.56) and of H-12a (δ 3.84) showed correlation to C-1a (δ 104.76), C-12 (δ 188.97) and C-1 (δ 110.27). This information enabled us to deduce the location of the proton to be at the C-6 position. The NOE studies displayed enhancement of the H-6a (δ 4.92) and H-1 (δ 6.77) signals upon irradiation of H-12a (δ 3.84). These observations suggested the B/C to be *cis* ring fusion.

Comparison of ^{1}H NMR spectral data (**Table 42**) of compounds **STH6** and **STH5** revealed close structural similarity. Difference in the spectrum of compounds **STH6** and **STH5** was shown as a furan ring in **STH6** but a pyran ring in **STH5**. Thus, compound **STH6** was identified as rotenone (Carlson, *et.al.*, 1973).



Selected HMBC correlation of compound **STH6**

Table 41 ^{1}H, ^{13}C and HMBC spectral data of compound **STH6**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1a	104.76	C	-	-
1	110.27	CH	6.77 (1H, *d*, *J* = 0.6 Hz)	C-2, C-3, C-4a and C-12a
2	143.84	C	-	-
3	149.44	C	-	-
4	100.86	CH	6.46 (1H, *s*)	C-2, C-3, C-1a and C-4a
4a	147.33	C	-	-
6α	66.25	CH_2	4.61 (1H, *dd*, *J* = 12, 3.3 Hz)	C-4a, C-6a and C-12a
β			4.18 (1H, *d*, *J* = 12 Hz)	C-1a, C-6a and C-12a
6a	72.18	CH	4.92 (1H, *m*)	C-1a
7a	157.93	C	-	-
8	112.95	C	-	-
9	167.36	C	-	-
10	104.88	CH	6.51 (1H, *d*, *J* = 8.4 Hz)	C-8 and C-9
11	129.97	CH	7.84 (1H, *d*, *J* = 8.4 Hz)	C-7a, C-9 and C-12
11a	113.30	C	-	-
12	188.97	C	-	-
12a	44.56	CH	3.84 (1H, *d*, *J* = 3 Hz)	C-1, C-1a and C-12
4′α	31.24	CH_2	3.32 (1H, *dd*, *J* = 15.9, 9 Hz)	C-7a, C-8, C-9, C-5′ and C-6′
β			2.96 (1H, *dd*, *J* = 15.9, 9 Hz)	C-7a, C-8, C-9, C-5′ and C-6′
5′	87.83	CH	5.24 (1H, *t*, *J* = 9 Hz)	C-8, C-6′ and C-8′
6′	143.00	C	-	-
7′	112.57	CH_2	5.07 (1H, *s*)	C-5′, C-6′ and C-8′
			4.93 (1H, *m*)	C-5′ and C-8′
8′	17.11	CH_3	1.77 (3H, *s*)	C-5′, C-6′ and C-7′
2-OMe	55.83	CH_3	3.76 (3H, *s*)	C-2
3-OMe	56.28	CH_3	3.81 (3H, *s*)	C-3

[#] Carbon type deduced from DEPT experiment.

Table 42 Comparison of ^{1}H NMR spectral data between compounds **STH6** and **STH5**

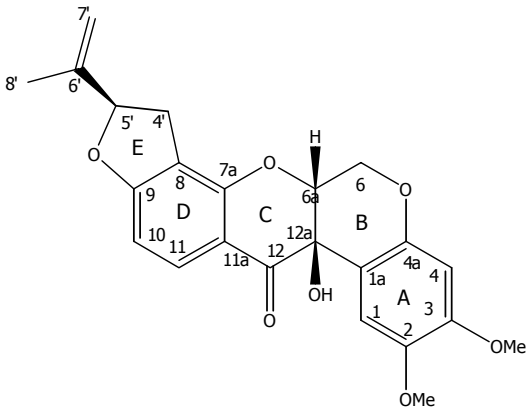
Position	Compound STH6 δ_H (ppm) (recorded in $CDCl_3$)	Compound STH5 δ_H (ppm) (recorded in $CDCl_3$)
1	6.77 (1H, *d*, *J* = 0.6 Hz)	6.78 (1H, *d*, *J* = 0.6 Hz)
4	6.46 (1H, *s*)	6.46 (1H, *s*)
6α	4.61 (1H, *dd*, *J* = 12, 3.3 Hz)	4.64 (1H, *dd*, *J* = 12, 3.3 Hz)
β	4.18 (1H, *d*, *J* = 12 Hz)	4.19 (1H, *d*, *J* = 12 Hz)
6a	4.92 (1H, *m*)	4.91 (1H, *m*)
10	6.51 (1H, *d*, *J* = 8.4 Hz)	6.46 (1H, *dd*, *J* = 8.7, 0.6 Hz)
11	7.84 (1H, *d*, *J* = 8.4 Hz)	7.74 (1H, *d*, *J* = 8.7 Hz)
12a	3.84 (1H, *d*, *J* = 3 Hz)	3.83 (1H, *d*, *J* = 3.9 Hz)
4′α	3.32 (1H, *dd*, *J* = 15.9, 9 Hz)	6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz)
β	2.96 (1H, *dd*, *J* = 15.9, 9 Hz)	-
5′	5.24 (1H, *t*, *J* = 9 Hz)	5.57 (1H, *d*, *J* = 9.9 Hz)
7′	5.07 (1H, *s*)	1.38* (3H, *s*)
	4.93 (1H, *m*)	-
8′	1.77 (3H, *s*)	1.45* (3H, *s*)
2-OMe	3.76 (3H, *s*)	3.77 (3H, *s*)
3-OMe	3.81 (3H, *s*)	3.80 (3H, *s*)

*Assignment with the same superscripts may be interchanged.

Table 43 Comparison of ^{1}H NMR spectral data between compound **STH6** and rotenone

Position	**Compound STH6 δ_H (ppm) (recorded in $CDCl_3$)**	**Rotenone δ_H (ppm) (recorded in $CDCl_3$)**
1	6.77 (1H, *d*, *J* = 0.6 Hz)	6.77 (1H, *d*, *J* = 0.8 Hz)
4	6.46 (1H, *s*)	6.46 (1H, *s*)
6α	4.61 (1H, *dd*, *J* = 12, 3.3 Hz)	4.62 (1H, *dd*, *J* = 12, 3 Hz)
β	4.18 (1H, *d*, *J* = 12 Hz)	4.19(1H, *d*, *J* = 12 Hz)
6a	4.92 (1H, *m*)	4.94 (1H, *m*)
10	6.51 (1H, *d*, *J* = 8.4 Hz)	6.52 (1H, *d*, *J* = 8.6 Hz)
11	7.84 (1H, *d*, *J* = 8.4 Hz)	7.84 (1H, *d*, *J* = 8.6 Hz)
12a	3.84 (1H, *d*, *J* = 3 Hz)	3.8 (1H, partly absoured)
4′α	3.32 (1H, *dd*, *J* = 15.9, 9 Hz)	3.33 (1H, *dd*, *J* = 15.8, 9.8 Hz)
β	2.96 (1H, *dd*, *J* = 15.9, 9 Hz)	2.95 (1H, *dd*, *J* = 15.8, 8.8 Hz)
5′	5.24 (1H, *t*, *J* = 9 Hz)	5.25 (1H, *t*, *J* = 8.8 Hz)
7′	5.07 (1H, *s*)	5.08 (1H, *s*)
	4.93 (1H, *m*)	4.94 (1H, *s*)
8′	1.77 (3H, *s*)	1.77 (3H, *s*)
2-OMe	3.76 (3H, *s*)	3.77 (3H, *s*)
3-OMe	3.81 (3H, *s*)	3.81 (3H, *s*)

2.3.1.7 Compound STH 7



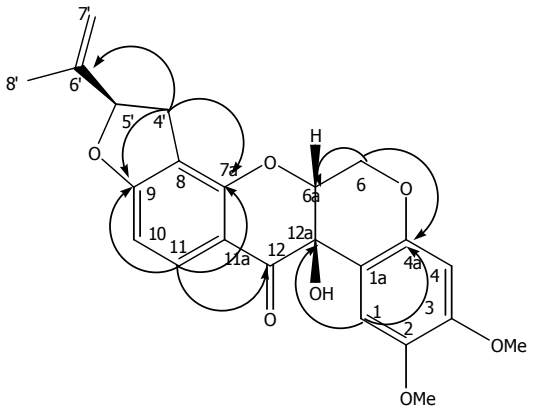
Compound **STH7** was isolated as a yellow viscous oil, $[\alpha]_D^{27}$: -66.6° (*c* = 0.015, $CHCl_3$). The UV spectrum showed maxima at 208, 234 and 294 nm. The presence of a conjugated carbonyl group (1660 cm^{-1}) and the hydroxyl group (3440 cm^{-1}) were suggested from the IR spectrum.

The complete analysis of ^{13}C and ^{1}H NMR spectrum of compound **STH7** (**Table 44**) were assigned with the informations provided from COSY, HMQC and HMBC (**Table 44**). The ^{13}C NMR and the DEPT spectra suggested that **STH7** contained three methyl carbons (δ 56.32, 55.83 and 17.05), three methylene carbons (δ 112.70, 63.80 and 31.08), six methine carbons (δ 130.06, 109.24, 105.29, 101.00, 87.94 and 75.98), ten quaternary carbons (δ 168.00, 157.64, 151.06, 148.31, 143.94, 142.81, 113.17, 111.68, 108.68 and 67.51) and a carbonyl carbon (δ 191.05).

Compound **STH7** showed the same characteristic peaks in the IR and UV spectrum as compound **STH6**. Comparison of the ^{1}H NMR spectra data (**Table 45**) of the two compounds revealed their close structural similarity. Difference in the spectrum of compound **STH7** was shown as a hydroxyl proton at δ 4.50 (1H, *br s*) which was not observed in compound **STH6**. The characteristic signals of isopropenylfuran resonated at δ 5.23 (1H, *t*, *J* = 9 Hz), 5.06 (1H, *br s*), 4.93 (1H, *m*) and *doublet of doublet*

AB system at δ 3.29 (1H, *J* = 15.9, 9.9 Hz) and 2.93 (1H, *J* = 15.9, 8.1 Hz), which were assigned to H-5′, H-7′a, H-7′b, H-4′α and H-4′β respectively. Signals of four aromatic protons appeared at δ 7.82, 6.53 (each 1H, *d*, *J* = 8.7 Hz, H-11, H-10) and δ 6.55, 6.48 (each 1H, *s*, H-1, H-4). Two methoxyl groups resonated at δ 3.81 and 3.72 (each 3H, *s*). Two *doublets of doublet* signals at δ 4.60 (1H, *J* = 13.2, 2.4 Hz) and 4.49 (1H, *J* = 13.2, 2.4 Hz) and a *singlet* signal at δ 4.58 were suggested to be signals of H-6α, H-6β and H-6aβ, respectively.

The HMBC correlations of compound **STH7** (**Table 44**) were similar to compound **STH6** except H-1 (δ 6.55) of compound **STH7** showed correlation peaks with C-4a (δ 148.31) and C-12a (δ 67.51), thus confirmed the position of the hydroxyl group at C-12a. Compound **STH7** was identified as 12a-hydroxyrotenone (Magalhaes, *et.al*., 1996).



Selected HMBC correlation of compound **STH7**

Table 44 ^{1}H, ^{13}C and HMBC spectral data of compound **STH7**

Position	δ_C [#](ppm)		δ_H (ppm)	HMBC
1a	108.68	C	-	-
1	109.24	CH	6.55 (1H, *s*)	C-2, C-3, C-4a and C-12a
2	143.94	C	-	
3	151.06	C	-	-
4	101.00	CH	6.48 (1H, *s*)	C-1a, C-2, C-3 and C-4a
4a	148.31	C	-	-
6α	63.80	CH_2	4.60 (1H, *dd*, *J* = 13.2, 2.4 Hz)	C-1a, C-4a, C-6a, C-12 and
β			4.49 (1H, *dd*, *J* = 13.2, 2.4 Hz)	C12a
6a	75.98	CH	4.58 (1H, *s*)	C-1a and C-12
7a	157.64	C	-	-
8	111.68	C	-	-
9	168.00	C	-	-
10	105.29	CH	6.53 (1H, *d*, *J* = 8.7 Hz)	C-8, C-9 and C-11a
11	130.06	CH	7.82 (1H, *d*, *J* = 8.7 Hz)	C-7a, C-9 and C-12
11a	113.17	C	-	-
12	191.05	C	-	-
12a-OH	67.51	C	4.50 (1H, *br s*)	-
4′α	31.08	CH_2	3.29 (1H, *dd*, *J* = 15.9, 9.9 Hz)	C-7a, C-9, C-5′, C-6′and C-7′
β			2.93 (1H, *dd*, *J* = 15.9, 8.1 Hz)	C-7a, C-9, C-5′, C-6′and C-7′
5′	87.94	CH	5.23 (1H, *t*, *J* = 9 Hz)	C-8, C-4′, C-6′ and C-8′
6′	142.81	C	-	-
7′	112.70	CH_2	5.06 (1H, *br s*)	C-5′, C-6′ and C-8′
			4.93 (1H, *m*)	C-5′, C-6′ and C-8′
8′	17.05	CH_3	1.75 (3H, *s*)	C-5′, C-6′ and C-7′
2-OMe	55.83	CH_3	3.72 (3H, *s*)	C-2
3-OMe	56.32	CH_3	3.81 (3H, *s*)	C-3

[#] Carbon type deduced from DEPT experiment.

Table 45 Comparison of ^{1}H NMR spectral data between compound **STH7** and **STH6**

Position	**Compound STH7 δ_H (ppm) (recorded in $CDCl_3$)**	**Compound STH6 δ_H (ppm) (recorded in $CDCl_3$)**
1	6.55 (1H, *s*)	6.77 (1H, *d*, *J* = 0.6 Hz)
4	6.48 (1H, *s*)	6.46 (1H, *s*)
6α	4.60 (1H, *dd*, *J* = 13.2, 2.4 Hz)	4.61 (1H, *dd*, *J* = 12, 3.3 Hz)
β	4.49 (1H, *dd*, *J* = 13.2, 2.4 Hz)	4.18 (1H, *d*, *J* = 12 Hz)
6a	4.58 (1H, *s*)	4.92 (1H, *m*)
10	6.53 (1H, *d*, *J* = 8.7 Hz)	6.51 (1H, *d*, *J* = 8.4 Hz)
11	7.82 (1H, *d*, *J* = 8.7 Hz)	7.84 (1H, *d*, *J* = 8.4 Hz)
12a	4.50 (1H, *br s*, OH)	3.84 (1H, *d*, *J* = 3 Hz)
4′α	3.29 (1H, *dd*, *J* = 15.9, 9.9 Hz)	3.32 (1H, *dd*, *J* = 15.9, 9 Hz)
β	2.93 (1H, *dd*, *J* = 15.9, 8.1 Hz)	2.96 (1H, *dd*, *J* = 15.9, 9 Hz)
5′	5.23 (1H, *t*, *J* = 9 Hz)	5.24 (1H, *t*, *J* = 9 Hz)
7′	5.06 (1H, *br s*)	5.07 (1H, *s*)
	4.93 (1H, *m*)	4.93 (1H, *m*)
8′	1.75 (3H, *s*)	1.77 (3H, *s*)
2-OMe	3.72 (3H, *s*)	3.76 (3H, *s*)
3-OMe	3.81 (3H, *s*)	3.81 (3H, *s*)

Table 46 Comparison of ^{1}H NMR spectral data between compound **STH7** and 12a-hydroxyrotenone

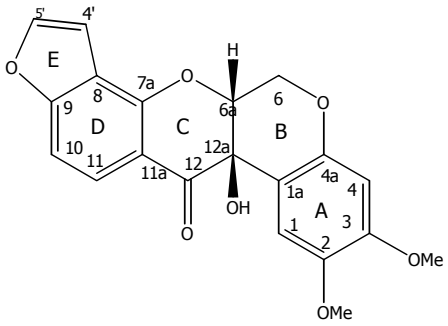
Position	**Compound STH7 δ_H (ppm) (recorded in $CDCl_3$)**	**12a-Hydroxyrotenone δ_H ppm) (recorded in $CDCl_3$)**
1	6.55 (1H, *s*)	6.55 (1H, *s*)
4	6.48 (1H, *s*)	6.48 (1H, *s*)
6α	4.60 (1H, *dd*, *J* = 13.2, 2.4 Hz)	4.59 (1H, *m*)
β	4.49 (1H, *dd*, *J* = 13.2, 2.4 Hz)	4.49 (1H, *dd*, *J* = 13.1, 2.3 Hz)
6a	4.58 (1H, *s*)	4.59 (1H, *m*)
10	6.53 (1H, *d*, *J* = 8.7 Hz)	6.53 (1H, *d*, *J* = 8.6 Hz)
11	7.82 (1H, *d*, *J* = 8.7 Hz)	7.83 (1H, *d*, *J* = 8.6 Hz)
4′α	3.29 (1H, *dd*, *J* = 15.9, 9.9 Hz)	3.29 (1H, *dd*, *J* = 16, 9 Hz)
β	2.93 (1H, *dd*, *J* = 15.9, 8.1 Hz)	2.93 (1H, *dd*, *J* = 16, 9 Hz)
5′	5.23 (1H, *t*, *J* = 9 Hz)	5.24 (1H, *t*, *J* = 9 Hz)
7′	5.06 (1H, *br s*)	5.07 (1H, *br s*)
	4.93 (1H, *m*)	4.94 (1H, *br s*)
8′	1.75 (3H, *s*)	1.76 (3H, *s*)
2-OMe	3.72 (3H, *s*)	3.72 (3H, *s*)
3-OMe	3.81 (3H, *s*)	3.82 (3H, *s*)

Table 47 Comparison of ^{13}C NMR spectral data between compound **STH7** and 12a-hydroxyrotenone

Position	**Compound STH7 δ_C (ppm) (recorded in $CDCl_3$)**	**12a-Hydroxyrotenone δ_C (ppm) (recorded in $CDCl_3$)**
1a	108.7	108.9
1	109.2	109.5
2	143.9	143.1 [a]
3	151.1	151.4 [a]
4	101.0	101.2
4a	148.3	148.6
6	63.8	63.9
6a	76.0	76.2
7a	157.6	157.9
8	111.7	113.4
9	168.0	168.3
10	105.3	105.5
11	130.1	130.3
11a	113.2	111.9
12	191.1	191.4
12a	67.5	67.7
4′	31.1	31.2
5′	87.9	88.1
6′	142.8	143.1
7′	112.7	112.9
8′	17.1	17.1
2-OMe	55.8	56.0
3- OMe	56.3	56.3

[a] Interchangeable values in each column.

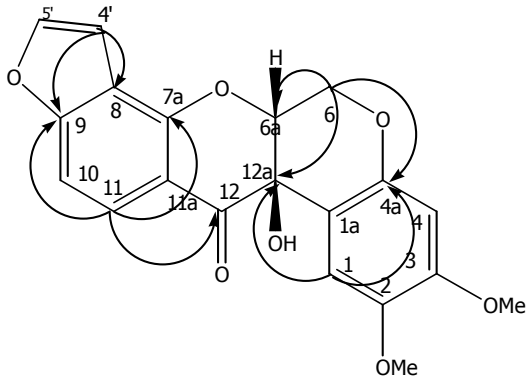
2.3.1.8 Compound STH 8



Compound **STH8** was isolated as a yellow viscous oil, $[\alpha]_D^{27}$: +21.7° (*c* = 0.023, $CHCl_3$). The UV spectrum showed maxima at 243, 281, 294 and 322 nm. In IR spectrum, the absorption bands of OH stretching (3445 cm^{-1}) and C=O stretching (1681 cm^{-1}) were shown.

Comparison of the ^{1}H NMR spectral data (**Table 49**) of **STH8** and **STH7** revealed their close structural similarity. Difference in the spectrum of compound **STH8** was shown as signals of a furan ring at δ 6.91 (1H, *dd*, *J* = 2, 1 Hz, H-4′) and 7.57 (1H, *d*, *J* = 2 Hz, H-5′), which were not observed in compound **STH7**. The ^{1}H NMR spectral data (**Table 48**) showed the existence of two *singlet* signals of the aromatic protons H-1 and H-4 at δ 6.54 and 6.49, respectively, and an *ortho* coupling type of aromatic protons which were assigned to H-10 and H-11 at δ 7.18 (1H, *dd*, *J* = 8.5, 0.5 Hz) and 7.87 (1H, *d*, *J* = 8.5 Hz). The ABX pattern of oxymethylene protons were existed at δ 4.73 (1H, *dd*, *J* = 11.5, 2.5 Hz), 4.56 (1H, *d*, *J* = 11.5 Hz) and 4.74 (1H, *s*) and were assigned to be the resonances of H-6α, H-6β and H-6a, respectively. Two *singlet* signals at δ 3.80 (3H) and 3.71 (3H) were suggested to be signals of 3-OMe and 2-OMe, respectively. The ^{13}C NMR and DEPT spectral data (**Table 48**) indicated the existence of two methyl carbons (δ 56.33 and 55.84), a methylene carbon (δ 63.78), seven methine carbons (δ 145.14, 123.85, 109.12, 107.11, 104.80, 101.09 and 76.68), nine quaternary carbons (δ 160.62, 155.78, 151.17, 148.40, 144.00, 117.25, 112.01, 108.33 and 67.70) and a carbonyl carbon (δ 198.17).

In the HMBC experiments; correlations of H-11 (δ 7.87) to C-7a (δ 155.78), C-9 (δ 160.62) and C-12 (δ 198.17) and of H-4′ (δ 6.91) to C-8 (δ 112.01), C-9 (δ 160.62) and C-5′ (δ 145.14), confirmed the position of a furan ring. The remaining positions of oxymethylene proton 2H-6 (δ 4.73 and 4.56) and hydroxyl group were confirmed by the correlations of 2H-6 to C-4a (δ 148.40), C-6a (δ 76.68) and C-12a (δ 67.70) whereas H-1 (δ 6.54) showed correlations to C-2 (δ 144.00), C-3 (δ 151.17), C-4a (δ 148.40) and C-12a (δ 67.70). The latter result suggested that the hydroxyl group was connected to C-12a. Thus, compound **STH8** was considered to be 12a-hydroxyelliptone (Thasana, *et.al.*, 2001).



Selected HMBC correlation of compound **STH8**

Table 48 ^{1}H, ^{13}C and HMBC spectral data of compound **STH8**

Position	δ_C [#] **(ppm)**		δ_H **(ppm)**	**HMBC**
1a	108.33	C	-	-
1	109.12	CH	6.54 (1H, *s*)	C-2, C-3, C-4a and C-12a
2	144.00	C	-	-
3	151.17	C	-	-
4	101.09	CH	6.49 (1H, *s*)	C-1a, C-2, C-3 and C-4a
4a	148.40	C	-	
6α	63.78	CH_2	4.73 (1H, *dd*, *J* = 11.5, 2.5 Hz)	C-4a, C-6a and C-12a
β			4.56 (1H, *d*, *J* = 11.5 Hz)	C-6a
6a	76.68	CH	4.74 (1H, *s*)	C-1a and C-12
7a	155.78	C	-	-
8	112.01	C	-	-
9	160.62	C	-	-
10	107.11	CH	7.18 (1H, *dd*, *J* = 8.5, 0.5 Hz)	C-8 and C-11a
11	123.85	CH	7.87 (1H, *d*, *J* = 8.5 Hz)	C-7a, C-9 and C-12
11a	117.25	C	-	-
12	198.17	C	-	-
12a-OH	67.70	C	-	-
4′	104.80	CH	6.91 (1H, *dd*, *J* = 2, 1 Hz)	C-8, C-9 and C-5′
5′	145.14	CH	7.57 (1H, *d*, *J* = 2 Hz)	C-8 and C-9
2-OMe	55.84	CH_3	3.71 (3H, *s*)	C-2
3-OMe	56.33	CH_3	3.80 (3H, *s*)	C-3

[#] Carbon type deduced from DEPT experiment.

Table 49 Comparison of ^{1}H NMR spectral data between compounds **STH8** and **STH7**

Position	**Compound STH8 δ_H (ppm) (recorded in $CDCl_3$)**	**Compound STH7 δ_H (ppm) (recorded in $CDCl_3$)**
1	6.54 (1H, *s*)	6.55 (1H, *s*)
4	6.49 (1H, *s*)	6.48 (1H, *s*)
6α	4.73 (1H, *dd*, *J* = 11.5, 2.5 Hz)	4.60 (1H, *dd*, *J* = 13.2, 2.4 Hz)
β	4.56 (1H, *d*, *J* = 11.5 Hz)	4.49 (1H, *dd*, *J* = 13.2, 2.4 Hz)
6a	4.74 (1H, *s*)	4.58 (1H, *s*)
10	7.18 (1H, *dd*, *J* = 8.5, 0.5 Hz)	6.53 (1H, *d*, *J* = 8.7 Hz)
11	7.87 (1H, *d*, *J* = 8.5 Hz)	7.82 (1H, *d*, *J* = 8.7 Hz)
12a	-	4.50 (1H, *br s*, OH)
4′α	6.91 (1H, *dd*, *J* = 2, 1 Hz)	3.29 (1H, *dd*, *J* = 15.9, 9.9 Hz)
β		2.93 (1H, *dd*, *J* = 15.9, 8.1 Hz)
5′	7.57 (1H, *d*, *J* = 2 Hz)	5.23 (1H, *t*, *J* = 9 Hz)
7′	-	5.06 (1H, *br s*)
	-	4.93 (1H, *m*)
8′	-	1.75 (3H, *s*)
2-OMe	3.71 (3H, *s*)	3.72 (3H, *s*)
3-OMe	3.80 (3H, *s*)	3.81 (3H, *s*)

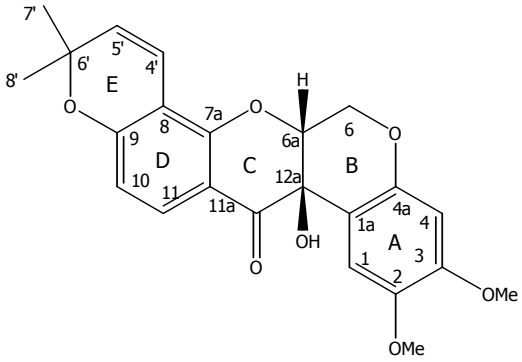
Table 50 Comparison of ^{1}H NMR spectral data between compound **STH8** and 12a-hydroxyellipton

Position	**Compound STH8 δ_H (ppm) (recorded in $CDCl_3$)**	**12a-Hydroxyelliptone δ_H ppm) (recorded in $CDCl_3$)**
1	6.54 (1H, *s*)	6.56 (1H, *s*)
4	6.49 (1H, *s*)	6.45 (1H, *s*)
6 α	4.73 (1H, *dd*, *J* = 11.5, 2.5 Hz)	4.70 (1H, *dd*, *J* = 12.0, 2.3 Hz)
β	4.56 (1H, *d*, *J* = 11.5 Hz)	4.56 (1H, *d*, *J* = 12.0 Hz)
6a	4.74 (1H, *s*)	4.74 (1H, *d*, *J* = 2.3 Hz)
10	7.18 (1H, *dd*, *J* = 8.5, 0.5 Hz)	7.17 (1H, *dd*, *J* = 8.6, 1.1 Hz)
11	7.87 (1H, *d*, *J* = 8.5 Hz)	7.87 (1H, *d*, *J* = 8.6 Hz)
4′	6.91 (1H, *dd*, *J* = 2, 1 Hz)	6.95 (1H, *dd*, *J* = 2.3, 1.1 Hz)
5′	7.57 (1H, *d*, *J* = 2 Hz)	7.56 (1H, *d*, *J* = 2.3 Hz)
2-OMe	3.71 (3H, *s*)	3.70 (3H, *s*)
3-OMe	3.80 (3H, *s*)	3.78 (3H, *s*)

Table 51 Comparison of ^{13}C NMR spectral data between compound **STH8** and 12a-hydroxyelliptone

Position	**Compound STH8 δ_C (ppm) (recorded in $CDCl_3$)**	**12a-Hydroxyelliptone δ_C (ppm) (recorded in $CDCl_3$)**
1a	108.3	108.7
1	109.1	109.4
2	144.0	144.0
3	151.2	151.2
4	101.1	101.2
4a	148.4	148.4
6	63.8	64.0
6a	76.7	76.8
7a	155.8	156.0
8	112.0	112.0
9	160.6	160.7
10	107.1	107.1
11	123.9	124.0
11a	117.3	117.5
12	198.2	192.1
12a	67.7	67.9
4′	104.8	104.8
5′	145.1	145.0
2-OMe	55.8	56.4
3- OMe	56.3	56.0

2.3.1.9 Compound STH 9



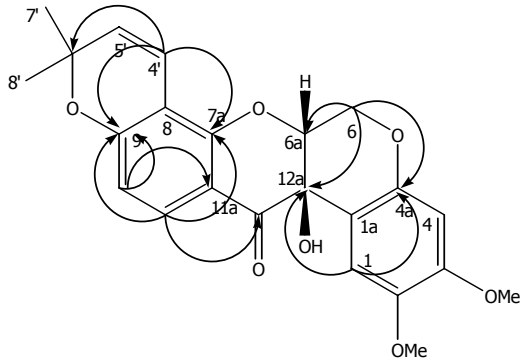
Compound **STH9** was obtained as a yellow viscous oil, $[\alpha]_D^{27}$: -41.6° (*c* = 0.012, $CHCl_3$). The UV spectrum exhibited maxima at 205, 225, 236, 247, 270, 300 and 315 nm. The IR spectrum exhibited absorption bands of conjugated carbonyl group at 1681 cm^{-1} and hydroxyl group at 3446 cm^{-1}.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **STH9** (**Table 52**) were assigned with the information provided from COSY, HMQC and HMBC correlation (**Table 52**). The ^{13}C NMR and the DEPT spectral data (**Table 52**) indicated the existence of four methyl carbons (δ 56.32, 55.85, 28.51 and 28.27), a methylene carbon (δ 63.83), seven methine carbons (δ 128.81, 128.53, 115.38, 111.87, 109.24, 101.03 and 76.21), ten quaternary carbons (δ 160.75, 156.63, 151.05, 148.34, 143.92, 111.05, 109.12, 108.57, 77.98 and 67.40) and a carbonyl carbon (δ 191.35).

Comparison of the ^{1}H NMR spectral data (**Table 53**) of **STH9** and **STH5** revealed close structure similarity. Difference in the spectrum of compound **STH5** was shown as a *doublet* signal at δ 3.83 (1H, *d*, *J* = 3.9 Hz) which was not observed in compound **STH9**. The signals of two olefinic protons at δ 6.60 (1H, *d*, *J* = 9.9 Hz) and 5.56 (1H, *d*, *J* = 9.9 Hz) and a *singlet* signal of two methyl groups at δ 1.45 and 1.39 which corresponded to a part of dimethylchromene ring were detected. Two methoxy

groups resonated as two *singlet*s at δ 3.73 (2-OMe) and 3.82 (3-OMe). Signals of four aromatic protons were discernible at δ 7.73 (1H, *d*, *J* = 8.7 Hz, H-11), 6.56 (1H, *s*, H-1), 6.48 (1H, *s*, H-4) and 6.47 (1H, *d*, *J* = 8.7 Hz, H-10). It also exhibited the following signals due to protons on carbon atoms bearing oxygen atoms at δ 4.63 (1H, *dd*, *J* = 12, 2.4 Hz, H-6α), 4.57 (1H, *d*, *J* = 2.4 Hz, H-6a) and 4.49 (1H, *d*, *J* = 12 Hz, H-6β).

In the HMBC spectrum (**Table 52**), the carbon signals at C-7a (δ 156.63), C-9 (δ 160.75) and C-6′ (δ 77.99) showed correlation to H-4′ (δ 6.60) and C-7a (δ 156.63), C-9 (δ 160.75) and C-12 (δ 191.35) to H-11 (δ 7.73). These correlations confirmed the presence of a dimethylchromene ring and suggested that this unit fused to aromatic nucleus at C-8 and C-9. The remaining positions of oxymethylene proton 2H-6 (δ 4.63 and 4.49) and hydroxyl group were confirmed by the correlations of 2H-6 to C-4a (δ 148.34), C-6a (δ 76.21) and C-12a (δ 67.40), and of H-1 (δ 6.56) to C-4a (δ 148.34) and C-12a (δ 67.40) The latter result suggested that the hydroxyl group was connected to the C-12a position. Thus, this compound was identified to be tephrosin (Andrei, *et.al.* 1997).



Selected HMBC correlation of **STH9**

Table 52 ^{1}H, ^{13}C and HMBC spectral data of compound **STH9**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1a	108.57	C	-	-
1	109.24	CH	6.56 (1H, *s*)	C-2, C-3, C-4a and C-12a
2	143.92	C	-	-
3	151.05	C	-	-
4	101.03	CH	6.48 (1H, *s*)	C-2, C-3 and C-4a
4a	148.34	C	-	-
6α	63.83	CH_2	4.63 (1H, *dd*, *J* = 12, 2.4 Hz)	C-4a, C-6a and C-12a
β			4.49 (1H, *d*, *J* = 12 Hz)	C-6a and C-7a
6a	76.21	CH	4.57 (1H, *d*, *J* = 2.4 Hz)	C-1a, C-6 and C-12a
7a	156.63	C	-	-
8	109.12	C	-	-
9	160.75	C	-	-
10	111.87	CH	6.47 (1H, *d*, *J* = 8.7 Hz)	C-9 and C-11a
11	128.81	CH	7.73 (1H, *d*, *J* = 8.7 Hz)	C-7a, C-9 and C-12
11a	111.05	C	-	-
12	191.35	C	-	-
12a	67.40	C	-	-
4′	115.38	CH	6.60 (1H, *d*, *J* = 9.9 Hz)	C-7a, C-9 and C-6′
5′	128.53	CH	5.56 (1H, *d*, *J* = 9.9 Hz)	C-8, C-6′, C-7′ and C-8′
6′	77.99	C	-	-
7′	28.51[a]	CH_3	1.39[*] (3H, *s*)	C-5′, C-6′ and C-8′
8′	28.27[a]	CH_3	1.45[*] (3H, *s*)	C-5′, C-6′ and C-7′
2-OMe	55.85	CH_3	3.73 (3H, *s*)	C-2
3-OMe	56.32	CH_3	3.82 (3H, *s*)	C-3

[a, *] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 53 Comparison of ^{1}H NMR spectral data between compound **STH9** and **STH5**

Position	Compound STH9 δ_H (ppm) (recorded in $CDCl_3$)	Compound STH5 δ_H (ppm) (recorded in $CDCl_3$)
1	6.56 (1H, *s*)	6.78 (1H, *d*, *J* = 0.6 Hz)
4	6.48 (1H, *s*)	6.46 (1H, *s*)
6α	4.63 (1H, *dd*, *J* = 12, 2.4 Hz)	4.64 (1H, *dd*, *J* = 12, 3.3 Hz)
β	4.49 (1H, *d*, *J* = 12 Hz)	4.19 (1H, *d*, *J* = 12 Hz)
6a	4.57 (1H, *d*, *J* = 2.4 Hz)	4.91 (1H, *m*)
10	6.47 (1H, *d*, *J* = 8.7 Hz)	6.46 (1H, *dd*, *J* = 8.7, 0.6 Hz)
11	7.73 (1H, *d*, *J* = 8.7 Hz)	7.74 (1H, *d*, *J* = 8.7 Hz)
12a	-	3.83 (1H, *d*, *J* = 3.9 Hz)
4′	6.60 (1H, *d*, *J* = 9.9 Hz)	6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz)
5′	5.56 (1H, *d*, *J* = 9.9 Hz)	5.57 (1H, *d*, *J* = 9.9 Hz)
7′/8′	1.39†/1.45† (3H, *s*)	1.38*/1.45* (3H, *s*)
2-OMe	3.73 (3H, *s*)	3.77 (3H, *s*)
3-OMe	3.82 (3H, *s*)	3.80 (3H, *s*)

$^{\dagger, *}$ Interchangeable values in each column.

Table 54 Comparison of ^{1}H NMR spectral data between compound **STH9** and tephrosin

Position	**Compound STH9 δ_H (ppm) (recorded in $CDCl_3$)**	**Tephrosin δ_H ppm) (recorded in $CDCl_3$)**
1	6.56 (1H, *s*)	6.49 (1H, *s*)
4	6.48 (1H, *s*)	6.41 (1H, *s*)
6α	4.63 (1H, *dd*, *J* = 12, 2.4 Hz)	4.55 (1H, *dd*, *J* = 12, 2.4 Hz)
β	4.49 (1H, *d*, *J* = 12 Hz)	4.41 (1H, *dd*, *J* = 12, 1.2 Hz)
6a	4.57 (1H, *d*, *J* = 2.4 Hz)	4.49 (1H, *dd*, *J* = 2.4, 1.2 Hz)
10	6.47 (1H, *d*, *J* = 8.7 Hz)	6.39 (1H, *d*, *J* = 8.8 Hz)
11	7.73 (1H, *d*, *J* = 8.7 Hz)	7.65(1H, *d*, *J* = 8.8 Hz)
12a	-	4.32 (1H, *br s*)
4′	6.60 (1H, *d*, *J* = 9.9 Hz)	6.52 (1H, *d*, *J* = 10 Hz)
5′	5.56 (1H, *d*, *J* = 9.9 Hz)	5.48 (1H, *d*, *J* = 10 Hz)
7′/8′	1.39†/1.45† (3H, *s*)	1.31*/1.37* (3H, *s*)
2-OMe	3.73 (3H, *s*)	3.65 (3H, *s*)
3- OMe	3.82 (3H, *s*)	3.74 (3H, *s*)

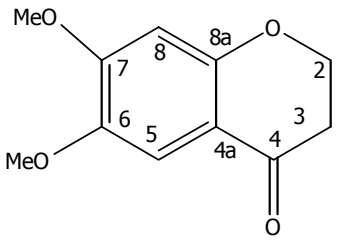
$^{\dagger, *}$ Interchangeable values in each column.

Table 55 Comparison of ^{13}C NMR spectral data between compound **STH9** and tephrosin

Position	**Compound STH9 δ_C (ppm) (recorded in $CDCl_3$)**	**Tephrosin δ_C (ppm) (recorded in $CDCl_3$)**
1a	108.6	108.5 [a]
1	109.2	109.3 [b]
2	143.9	143.8
3	151.1	148.3 [c]
4	101.0	100.9
4a	148.3	150.9 [c]
6	63.8	66.7
6a	76.2	75.9
7a	156.6	156.5
8	109.1	109.0 [a]
9	160.8	160.6
10	111.9	111.7 [b]
11	128.8	128.4 [d]
11a	111.1	111.0
12	191.4	191.3
12a	67.4	67.3
4′	115.4	115.3
5′	128.5	128.7 [d]
6′	78.0	77.9

[a, b, c, d] Interchangeable values in each column.

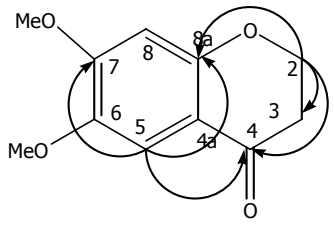
2.3.1.10 Compound STH 10



Compound **STH10** was isolated as yellow viscous oil. In the UV spectrum, strong absorptions at 205, 235, 273 and 336 nm were detected. The presence of a conjugated carbonyl group at 1675 cm^{-1} was suggested from the IR spectrum.

The ^{1}H NMR spectra (**Table 66**) exhibited two singlet signals of two isolated aromatic protons at δ 7.29 and 6.43 which were assigned as H-5 and H-8, respectively. The two *singlet* signals of methoxy groups were shown at δ 3.91 (3H) and 3.87 (3H). In addition, two *triplet* signals at δ 4.50 (2H, *J* = 6.6 Hz, H-2) and 2.75 (2H, *J* = 6.6 Hz, H-3) were observed. The ^{13}C NMR and the DEPT spectral data (**Table 56**) indicated the existence of two methyl carbons (δ 56.26 and 56.18), two methylene carbons (δ 67.55, and 37.27), two methine carbons (δ 106.72 and 100.00), four quaternary carbons (δ 158.35, 156.09, 144.49 and 113.56) and a carbonyl carbon (δ 190.55).

The assignments of methylene protons were confirmed by HMBC experiments, correlation of H-2 (δ 4.50) to C-3 (δ 37.27), C-4 (δ 190.55) and C-8a (δ 158.35), and of H-3 (δ 2.75) to C-2 (δ 67.55) and C-4 (δ 190.55). The remaining position of methine proton H-5 (δ 7.29) was confirmed by correlation of H-5 to C-4 (δ 190.55), C-6 (δ 144.49), C-7 (δ 156.09) and C-8a (δ 158.35), and of H-8 to C-4a (δ 113.56), C-6 (δ 144.49), C-7 (δ 156.09) and C-8a (δ 158.35). Thus, the structure of the new compound **STH10** was elucidated as 6,7-dimethoxy-2,3-dihydro-4*H*-chromen-4-one.



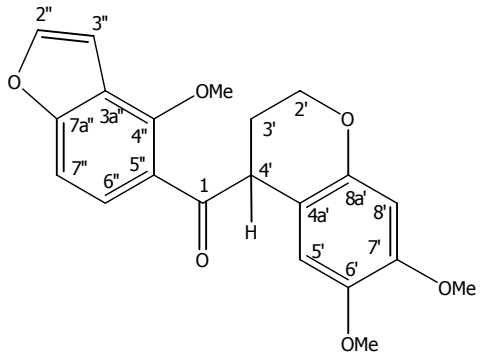
Selected HMBC correlation of **STH10**

Table 56 ^{1}H, ^{13}C and major HMBC spectral data of compound **STH10**

Position	δ_C **(ppm)**		δ_H **(ppm)**	**HMBC**
2	67.55	CH_2	4.50 (*t*, *J* = 6.6 Hz)	C-3, C-4 and C-8a
3	37.27	CH_2	2.75 (*t*, *J* = 6.6 Hz)	C-2 and C-4
4	190.55	C	-	-
4a	113.56	C	-	-
5	106.72	CH	7.29 (*s*)	C-4, C-6, C-7 and C-8a
6	144.49	C	-	-
7	156.09	C	-	-
8	100.00	CH	6.43 (*s*)	C-4a, C-6, C-7 and C-8a
8a	158.35	C	-	-
6-OMe	56.26	CH_3	3.87 (*s*)	C-6
7-OMe	56.18	CH_3	3.91 (*s*)	C-7

[#] Carbon type deduced from DEPT experiment.

2.3.1.11 Compound STH 11

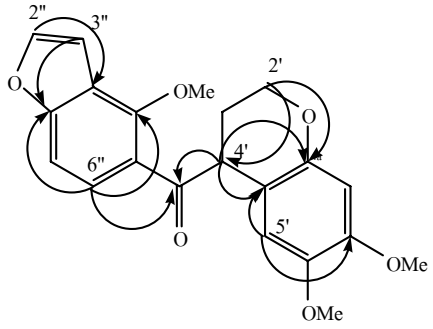


Compound **STH11** was obtained as a pale yellow viscous oil and gave a molecular ion at m/z 411.1808 $[M+H]^+$ by HR-ESITOF MS, corresponding to $C_{24}H_{26}O_6$. The IR spectrum showed absorption band at 1673 cm^{-1} corresponding to a conjugated carbonyl group. The UV spectrum showed maxima at 205, 236, 257 and 293 nm.

The ^{1}H NMR spectrum of **STH11** (**Table 57**) is very similar to that of compound **STH4** (**Table 36**) except for the characteristic signals of two benzofuranic protons at δ 7.63 (1H, *d*, *J* = 2.4 Hz) and δ 7.01 (1H, *dd*, *J* = 2.4, 0.6 Hz) in place of the chromene group of **STH4**. The location of a furan ring was assigned to be fused on to a neighboring ring at the C-7″a (δ 158.83) and C-3″a (δ 117.94) position based on the HMBC correlation. An *ortho*-coupled protons at δ 7.46 (1H, *d*, *J* = 8.7 Hz) and δ 7.23 (1H, *dd*, *J* = 8.7, 0.6 Hz) was assigned to the protons of a 1,2,3,4-tetra-substituted benzene ring. Other aromatic protons appeared as two *singlets* at δ 6.42 and 6.39 (each 1H) were assigned as H-8′ and H-5′, respectively. Three *singlet* signals at δ 3.60 (3H, *s*), 3.81 (3H, *s*) and 4.18 (3H, *s*) were assigned to the methoxyl group of benzene ring at C-7′, C-6′ and C-4″, respectively. The protons at δ 4.69 (1H, *dd*, *J* = 5.7, 4.2 Hz) and two *multiplets* at δ 2.23 (2H) and 4.21 (2H) were assigned as H-4′, H-3′ and H-2′, respectively. These assignments were confirmed by COSY cross-peak between H-4′

and H-3′, H-3′ and H-2′, H-6′′ and H-7′′, and H-2′′ and H-3′′. The ^{13}C NMR spectral data (**Table 57**) showed a total of 21 carbons including one carbonyl carbon at δ 204.40. DEPT experiment showed three methyl carbons (δ 60.53, 56.78 and 56.24), two methylene carbons (δ 63.49 and 25.27), seven methine carbons (δ 144.91, 126.51, 112.83, 106.65, 105.48, 100.91 and 45.79) and eight quaternary carbons (δ 158.83, 152.70, 149.18, 149.08, 142.96, 125.55, 117.94 and 110.54). The assignments of all carbons were achieved by ^{13}C, HMQC and HMBC experiments.

The HMBC correlations of compound **STH11** (**Table 57**) were the same as compound **STH4**. The absolute stereochemistry at C-4′ of compound **STH11** could not be assigned based on the observation of NOE experiment and coupling constant. Thus, the structure of the new compound **STH11** was elucidated as [6′,7′-dimethoxy-3′,4′-dihydro-2*H*-chromen-4′-yl](4′′-methoxy-1′′-benzofuran-5′′-yl)methanone, (trifolinone B).



Selected HMBC correlation of **STH11**

Table 57 ^{1}H, ^{13}C and HMBC spectral data of compound **STH11**

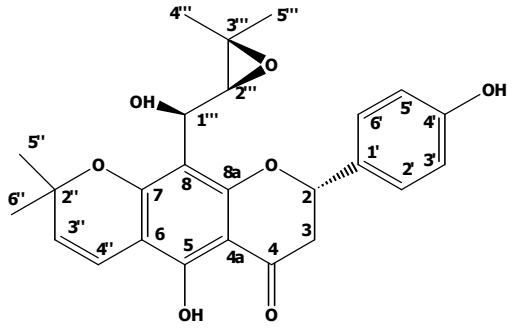
Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1	204.40	C	-	-
2′	63.49	CH_2	4.21 (2H, *m*)	C-8′a, C-3′ and C-4′
3′	25.27	CH_2	2.23 (2H, *m*)	C-4′a, C-2′, C-4′ and C-1
4′	45.79	CH	4.69 (1H, *dd*, *J* = 5.7, 4.2 Hz)	C-5′, C-4′a, C-8′a, C-6′, C-3′ and C-1
4′a	110.54	C	-	-
5′	112.83	CH	6.39 (1H, *s*)	C-4′a, C-7′, C-8′, C-8′a and C-4′
6′	149.08	C	-	-
7′	142.96	C	-	-
8′	100.91	CH	6.42 (1H, *s*)	C-4′a, C-6′ and C-7′
8′a	149.18	C	-	-
2′′	144.91	CH	7.63 (1H, *d*, *J* = 2.4 Hz)	C-7′′a and C-3′′a
3′′	105.48	CH	7.01 (1H, *dd*, *J* = 2.4, 0.6 Hz)	C-2′′, C-3′′a and C-7′′a
3′′a	117.94	C	-	-
4′′	152.70	C	-	-
5′′	125.55	C	-	-
6′′	126.51	CH	7.46 (1H, *d*, *J* = 8.7 Hz)	C-1, C-7′′a and C-4′′
7′′	106.65	CH	7.23 (1H, *dd*, *J* = 8.7, 0.6 Hz)	C-5′′, C-7′′a, C-3′′a and C-4′′
7′′a	158.83	C	-	-
6′-OMe	56.24	CH_3	3.81 (3H, *s*)	C-6′
7′-OMe	56.78	CH_3	3.60 (3H, *s*)	C-7′
4′′-OMe	60.53	CH_3	4.18 (3H, *s*)	C-4′′

[#] Carbon type deduced from DEPT experiment.

Table 58 Comparison of ^{1}H NMR spectral data between compound **STH11** and **STH4**

Position	Compound STH11 δ_H (ppm) (recorded in $CDCl_3$)	Compound STH4 δ_H ppm) (recorded in $CDCl_3$)
2′	4.21 (2H, *m*)	4.19 (2H, *m*)
3′	2.23 (2H, *m*)	2.22 (2H, *m*)
4′	4.69 (1H, *dd*, *J* = 5.7, 4.2 Hz)	4.61 (1H, *dd*, *J* = 6.0, 4.2 Hz)
5′	6.39 (1H, *s*)	6.40 (1H, *s*)
8′	6.42 (1H, *s*)	6.40 (1H, *s*)
2′′	7.63 (1H, *d*, *J* = 2.4 Hz)	5.71 (1H, *d*, *J* = 9.9 Hz)
3′′	7.01 (1H, *dd*, *J* = 2.4, 0.6 Hz)	6.62 (1H, *d*, *J* = 9.9 Hz)
6′′	7.46 (1H, *d*, *J* = 8.7 Hz)	7.35 (1H, *d*, *J* = 8.4 Hz)
7′′	7.23 (1H, *dd*, *J* = 8.7, 0.6 Hz)	6.62 (1H, *d*, *J* = 8.4 Hz)
6′-OMe	3.81 (3H, *s*)	3.64 (3H, *s*)
7′-OMe	3.60 (3H, *s*)	3.81 (3H, *s*)
4′′-OMe (5′′-OMe)	4.18 (3H, *s*)	3.80 (3H, *s*)

2.3.1.12 Compound STH12



Compound **STH12** was obtained as a yellow crystalline solid, mp: 137-140° C. The IR spectrum showed absorption bands at 3333 and 1633 cm^{-1} corresponding to a hydroxyl group and a conjugated carbonyl group, respectively. The UV spectrum

showed maxima at 203, 228, 264, 272, 296, 309 and 363 nm, which is a typical absorption of a pyranoflavanone.

Compound **STH12** showed one spot on both normal and reversed-phase TLC with various solvent systems. However, ^{1}H and ^{13}C NMR spectral data (**Table 59**) clearly exhibited two sets of signals with partial overlapping. The ^{1}H NMR spectrum of flavanone **STH12** was nearly identifiable with those of lupinifolin **STH1**. The only difference appeared in an isoprenyl group. Compound **STH12** showed the presence of an extra hydroxyl group at the benzylic position at δ 4.82, 4.80 (1H, *d, J* = 6.0 Hz) and an epoxide group at δ 3.39, 3.37 (1H, *d, J* = 6.0 Hz) in the ^{1}H NMR and at δ 66.71, 66.70 and 59.44 in the ^{13}C NMR, respectively. The structure of compound **STH12** was further confirmed by X-ray diffraction (**Figure 9**). Thus, compound **STH12** was considered to be 1‴-hydroxy-2‴,3‴-epoxylupinifolin (Prawat, *et.al.*, 2000).

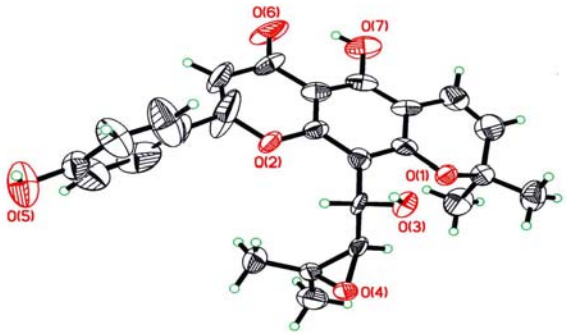


Figure 4 X-ray ORTEP diagram of compound **STH12**

Table 59 ^{1}H and ^{13}C spectral data of compound **STH12**

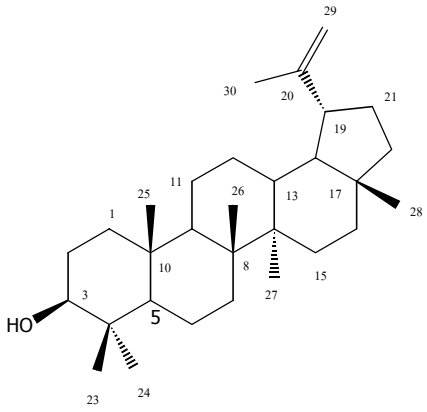
Position	δ_C [#] (ppm)	Compound STH12 δ_H (ppm)
2	79.73, 79.61	5.34, 5.32 (1H, *br d*, *J* = 3 Hz)
3α	43.04, 42.91	3.11 (1H, *dd*, *J* = 17, 13.5 Hz)
β		2.82, 2.78 (1H, *dd*, *J* = 17, 3 Hz)
4	196.73, 196.58	-
4a	103.14	-
5	157.95	12.42, 12.10 (OH, *s*)
6	102.65	-
7	159.50 [a]	-
8	107.42	-
8a	159.43 [a]	-
1′	128.75	-
2′, 6′	127.85, 127.71	7.24 (2H, *d*, *J* = 8.5 Hz)
3′, 5′	115.65	6.86 (2H, *d*, *J* = 8.5 Hz)
4′	159.25 [a]	-
2″	79.34	-
3″	126.08	5.55 (1H, *d*, *J* = 10 Hz)
4″	115.22	6.64 (1H, *d*, *J* = 10 Hz)
5″/6″	28.50 [b], 28.40 [b]	1.48, 1.46, 1.23, 1.21 (3H, *s*)
	28.13 [b], 28.03 [b]	
1‴	66.71	4.82, 4.80 (1H, *d*, *J* = 6 Hz)
2‴	66.70	3.39, 3.37 (1H, *d*, *J* = 6 Hz)
3‴	59.44, 59.37	-
4‴/5‴	19.20 [b], 19.36 [b], 24.83 [b]	1.50 /1.24 (each 3H, *s*)

[a,b] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 60 Comparison of ^{1}H NMR spectral data between compound **STH12** and 1‴ -hydroxy-2‴,3‴-epoxylupinifolin

Position	**Compound STH12 δ_H (ppm) (recorded in $CDCl_3$+CD_3OD)**	**1‴ -Hydroxy-2‴,3‴-epoxylupinifolin δ_H (ppm) (recorded in pyridine-d_5)**
2	5.34, 5.32 (*br d*, *J* = 3 Hz)	5.48, 5.41 (*dd*, *J* = 13.0, 2.9 Hz)
3α	3.11 (*dd*, *J* = 17, 13.5 Hz)	3.24, 3.23 (*dd*, *J* = 17.1, 13.0 Hz)
β	2.82, 2.78 (*dd*, *J* = 17, 3 Hz)	2.91, 2.89 (*dd*, *J* = 17.1, 2.9 Hz)
5-OH	12.42, 12.10 (OH, *s*)	13.06 (OH, *s*)
2′, 6′	7.24 (*d*, *J* = 8.5 Hz)	7.51, 7.48 (*d*, *J* = 8.6 Hz)
3′, 5′	6.86 (*d*, *J* = 8.5 Hz)	7.18 (*d*, *J* = 8.6 Hz)
4′-OH	-	11.81, 11.80 (*br s*)
3″	5.55 (*d*, *J* = 10 Hz)	5.58 (*d*, *J* = 10 Hz)
4″	6.64 (*d*, *J* = 10 Hz)	6.85 (*d*, *J* = 10 Hz)
5″/ 6″	1.48, 1.46, 1.23, 1.21 (*s*)	1.46, 1.43, 1.41, 1.40 (*s*)
1‴	4.81, 4.80 (*d*, *J* = 6 Hz)	5.47, 5.46 (*d*, *J* = 7.2 Hz)
2‴	3.38, 3.37 (*d*, *J* = 6 Hz)	4.16, 4.15 (*d*, *J* = 7.2 Hz)
4‴/ 5‴	1.50, 1.24 (*s*)	1.36, 1.34, 1.32 (*s*)

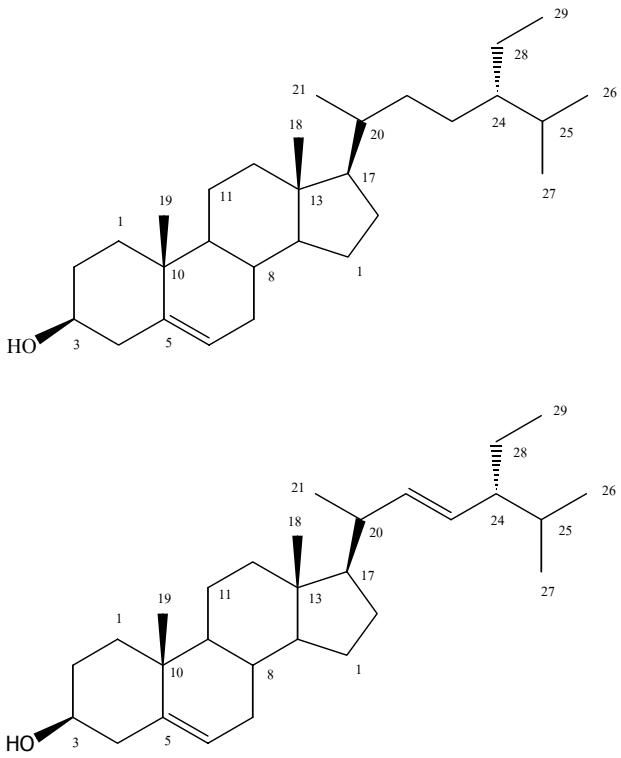
2.3.1.13 Compound STH13



Compound **STH13** was obtained as white needles, mp = 194-195 °C, $[\alpha]_D^{27}$ = +31.9 (*c* = 0.0094 , $CHCl_3$). This compound showed the characteristic of triterpene by visualizing as a purple spot with vanillin sulfuric acid reagent. Its IR spectrum exhibited hydroxyl group (3416 cm^{-1}) absorption.

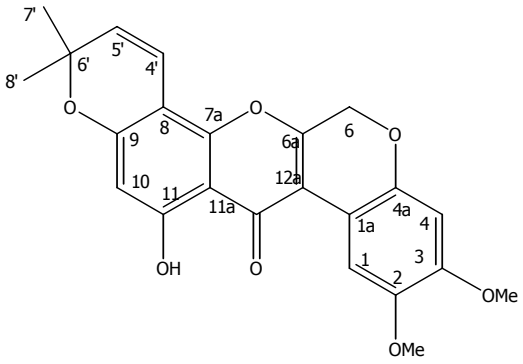
The ^1H NMR spectrum showed six *singlet* methyls at δ 0.78, 0.83, 0.84, 0.85, 0.93 and 1.03, one oxymethine proton at δ 4.47 (*dd, J* = 6, 10 Hz) and one methine proton at δ 2.37 (*dt, J* = 6, 11.5 Hz). The above informations suggested a pentacyclic triterpene skeleton. The signals of two olefinic and one vinylic methyl protons at δ 4.68 (1H, *d, J* = 2.5 Hz), 4.57 (1H, *m*) and 1.68 (3H, *s*), respectively, suggested the structure of isopropenyl unit [$-C(CH_3)=CH_2$]. The ^1H NMR data, optical rotation value and melting point were corresponded to the previous reported data of lupeol (Reynolds, *et.al*., 1986). Therefore, compound **STH13** was assigned to be lupeol.

2.3.1.14 Compound STH14 and STH15



The mixture of compound **STH14** and **STH15** was isolated as a white solid, mp = 155-156 °C. The IR spectrum showed absorption band at 3321 cm^{-1} (O-H stretching). The ^{1}H NMR spectrum contained an oxymethine proton signal at δ 3.56-3.48 (*m*), three olefinic protons at δ 5.36-5.33 (*m*), 5.16 (*dd*) and 5.02 (*dd*). The ^{1}H NMR data and melting point were corresponded to previous reported data of β-sitosterol and stigmasterol. Thus, this mixtures were identified as β-sitosterol and stigmasterol (Sukpondma, 2001).

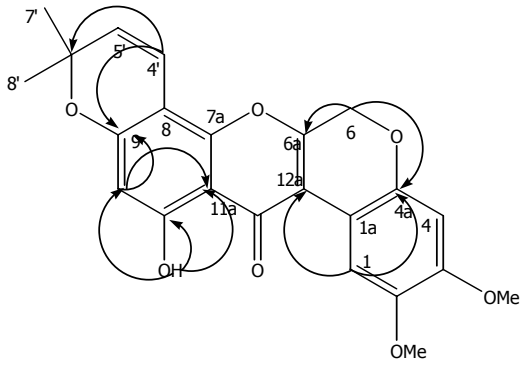
2.3.1.15 Compound STC1



Compound **STC1** was obtained as yellow solid, mp: 258-259°C. The UV spectrum showed maxima at 242, 276, 311 and 331 nm. **STC1** exhibited IR absorption bands at 3470 and 1646 cm^{-1} which indicated the presence of hydroxyl group and conjugated carbonyl group, respectively.

The ^{1}H NMR spectrum (**Table 61**) showed a sharp *singlet* of a chelated hydroxy 11-OH at δ 12.95. The dimethylchromene system is well characterized by two *doublets* at δ 6.62 (J = 10.5 Hz) and 5.59 (J = 10.5 Hz) for the vinyl protons and a *singlet* integrated for six protons at δ 1.46. Three *singlet* signals corresponding to an aromatic proton H-1, H-4 and H-10 were present at δ 8.25, 6.53 and 6.27, respectively. A *singlet* signal of oxymethylene proton at δ 4.97 was assigned to H-6. In addition, NMR spectral data showed that this compound had two methoxy groups resonating as two *singlets* at δ 3.86 (3-OMe) and 3.93 (2-OMe). The ^{13}C NMR and the DEPT spectral data (**Table 61**) indicated the existence of four methyl carbons (δ 56.34, 55.89 and 28.16 (2)), a methylene carbon (δ 64.75), five methine carbons (δ 127.71, 114.34, 109.81, 100.62 and 100.49), twelve quaternary carbons (δ 162.29(2), 159.29, 156.82, 150.80, 149.16, 146.24, 110.78, 109.64, 105.94, 101.05 and 78.09) and a carbonyl carbon (δ 179.26).

The structure and arrangement of the substituents were deduced by the HMBC experiments. Correlations of H-4′ (δ 6.62) to C-9 (δ 159.29) and C-6′ (δ 78.09) and of H-10 (δ 6.27) to C-9 (δ 159.29), C-11 (δ 162.29) and C-11a (δ 105.94), confirmed the presence of a dimethylchromene ring and suggested that this unit fused to aromatic nucleus at C-8 and C-9. The remaining methylene protons showed correlations with C-1a (δ 110.78), C-4a (δ 149.16) and C-6a (δ 156.82). This result confirmed the position of a double bond at C-6a and C-12a. Thus, compound **STC1** was identified to be 6a,12a-dehydro-α-toxicarol (Andrei, *et.al.* 1997).



Selected HMBC correlation of **STC1**

Table 61 ^{1}H, ^{13}C and HMBC spectral data of compound **STC1**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1a	110.78	C	-	-
1	109.81	CH	8.25 (1H, *s*)	C-1a, C-2, C-3, C-4a and C-12a
2	146.24	C	-	-
3	150.80	C	-	-
4	100.62	CH	6.53 (1H, *s*)	C-1a, C-2, C-3 and C-4a
4a	149.16	C	-	-
6	64.75	CH_2	4.97 (2H, *s*)	C-1a, C-4a and C-6a
6a	156.82	C	-	-
7a	162.29	C	-	-
8	101.05	C	-	-
9	159.29	C	-	-
10	100.49	CH	6.27 (1H, *s*)	C-9, C-11 and C-11a
11-OH	162.29	C-OH	12.95 (1H, *s*)	C-10, C-11 and C-11a
11a	105.94	C	-	-
12	179.26	C	-	-
12a	109.64	C	-	-
4′	114.34	CH	6.62 (1H, *d*, *J* = 10.5 Hz)	C-9 and C-6′
5′	127.71	CH	5.59 (1H, *d*, *J* = 10.5 Hz)	C-8 and C-6′
6′	78.09	C	-	-
7′	28.16[a]	CH_3	1.46 (3H, *s*)	C-5′, C-6′ and C-8′
8′	28.16[a]	CH_3	1.46 (3H, *s*)	C-5′, C-6′ and C-7′
2-OMe	56.34	CH_3	3.93 (3H, *s*)	C-2
3-OMe	55.89	CH_3	3.86 (3H, *s*)	C-3

[a]Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 62 Comparison of ^{1}H NMR spectral data between compound **STC1** and 6a,12a-dehydro-α-toxicarol

Position	**Compound STC1 δ_H (ppm) (recorded in $CDCl_3$)**	**6a,12a-Dehydro-α-toxicarol δ_H (ppm) (recorded in $CDCl_3$)**
1	8.25 (1H, *s*)	8.26 (1H, *s*)
4	6.53 (1H, *s*)	6.54 (1H, *s*)
6	4.97 (2H, *s*)	4.98 (2H, *s*)
10	6.27 (1H, *s*)	6.28 (1H, *s*)
11-OH	12.95 (1H, *s*)	12.99 (1H, *s*)
4′	6.62 (1H, *d*, *J* = 10.5 Hz)	6.62 (1H, *d*, *J* = 10 Hz)
5′	5.59 (1H, *d*, *J* = 10.5 Hz)	5.59 (1H, *d*, *J* = 10 Hz)
7′/8′	1.46(6H, *s*)	1.47(6H, *s*)
OMe(2/3)	3.93/3.86 (each 3H, *s*)	3.94/3.87 (each 3H, *s*)

Table 63 Comparison of ^{13}C NMR spectral data between compound **STC1** and 6a,12a-dehydro-α-toxicarol

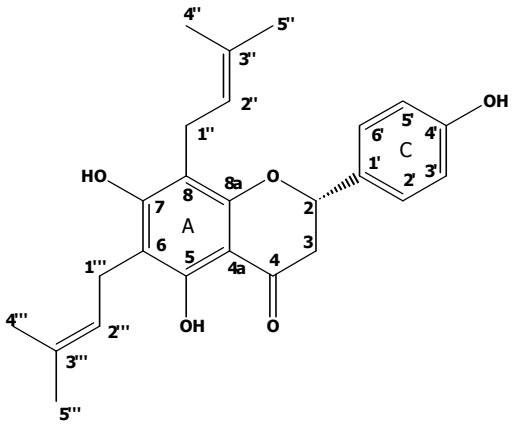
Position	**Compound STC1 δ_C (ppm) (recorded in $CDCl_3$)**	**6a,12a-Dehydro-α-toxicarol δ_C (ppm) (recorded in $CDCl_3$)**
1a	110.8	109.9
1	109.8	109.7
2	146.2	144.2
3	150.8	149.2
4	100.6	100.5[a]
4a	149.2	146.3
6	64.8	64.7
6a	156.8	156.8
7a	162.3	150.9

Table 63 (Continue)

Position	**Compound STC1 δ_C (ppm) (recorded in $CDCl_3$)**	**6a,12a-Dehydro-α-toxicarol δ_C (ppm) (recorded in $CDCl_3$)**
8	101.1	101.1
9	159.3	159.3
10	100.5	100.6 [a]
11	162.3	162.3
11a	105.9	106.0
12	179.3	179.3
12a	109.6	110.8
4′	114.3	114.4
5′	127.7	127.7
6′	78.1	78.1

[a]δ interchangeable values in each column.

2.3.1.16 Compound STC2

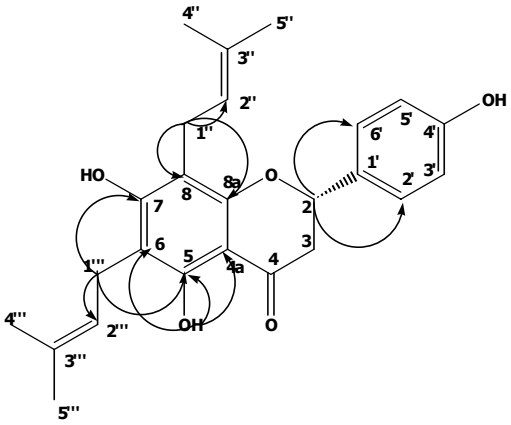


Compound **STC2** was obtained as yellow viscous oil, $[\alpha]_D^{27}$: -29.41° (*c* = 0.012, $CHCl_3$). The IR spectrum showed absorption bands at 3445 and 1635 cm^{-1} corresponding to a hydroxyl group and conjugated carbonyl group, respectively. The UV spectrum showed maximum absorptions at 204, 221, 295 and 344 nm, which are typical absorptions of flavanone.

The ^{1}H NMR spectrum (**Table 64**) showed a sharp *singlet* signal of a chelated hydroxyl group 5-OH at δ 12.30. The resonances for an ABX system at δ 3.03 (1H, *dd, J* = 17.1 and 12.6 Hz), 2.79 (1H, *dd, J* = 17.1 and 3 Hz) and 5.31 (1H, *dd, J* = 12.6 and 3 Hz) is diagnostic for H-3α, H-3β and H-2β of a flavanone nucleus. Four aromatic protons which coupled to each other as AA′BB′ type at δ 7.31 (2H, *d, J* = 8.7 Hz) and 6.87 (2H, *d, J* = 8.7 Hz) were observed. The proton signals of an isoprenyl group appeared as follow: the *gem*-dimethyl protons (CH_3-4′′, CH_3-5′′) at δ 1.81 (*s*) and 1.74 (*s*), benzylic methylene protons (CH_2-1′′) at δ 3.29 (*d, J* = 7.2 Hz), an olefinic methine proton (CH -2′′) at δ 5.23 (*m*). The signals of the second isoprenyl group appeared as follow: the *gem*-dimethyl protons (CH_3-4′′′, CH_3-5′′′) at δ 1.70 (*s*) and 1.69 (*s*), benzylic methylene protons (CH_2-1′′′) at δ 3.34 (*d, J* = 6.9 Hz), an olefinic methine proton (CH -2′′′) at δ 5.18 (*m*). The ^{13}C NMR showed the signals of 25 carbon atoms (**Table 64**). Analysis of the DEPT spectra indicated the presence of a carbonyl

carbon (δ 196.57), four methyl carbons (δ 25.83, 25.82, 17.87 and 17.81), three methylene carbons (δ 43.24, 21.87 and 21.23), five methine carbons (δ 127.68, 121.94, 121.73, 115.49 and 78.49) and ten quaternary carbons (δ 162.33, 159.28, 157.73, 155.89, 134.73, 133.97, 131.02, 107.23, 106.41 and 102.77).

In the HMBC spectrum, correlation of H-1″ (δ 3.29) with C-8 (δ 107.23), C-8a (δ 157.73), C-2″ (δ 121.94) and C-3″ (δ 134.73) were present, it suggested the position of an isoprenyl unit to be at C-8 in A ring. Correlation of H-1‴ (δ 3.34) to C-5 (δ 159.28), C-6 (δ 106.41), C-7 (δ 162.33), C-2‴ (δ 121.73) and C-3‴ (δ 133.97), indicated that the second isoprenyl unit was at C-6. Moreover, the oxymethine proton at δ 5.31 was found to show correlation with the carbon signal at C-2′/6′ (δ 127.68), which confirmed the flavanone skeleton. Thus, this compound **STC2** was identified to be senegalensein (Fomum, *et.al*., 1987).



Selected HMBC correlation of **STC2**

Table 64 ^{1}H, ^{13}C and HMBC spectral data of compound **STC2**

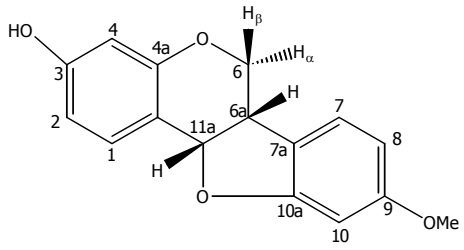
Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
2	78.49	CH	5.31 (1H, *dd*, *J* = 12.6, 3 Hz)	C-2′ and C-6′
3α	43.24	CH_2	3.03 (1H, *dd*, *J* = 17.1, 12.6 Hz)	C-2, C-4 and C-1′
β			2.79 (1H, *dd*, *J* = 17.1, 3 Hz)	C-4
4	196.57	C	-	-
4a	102.77	C	-	-
5-OH	159.28	C	12.30 (1H, *s*)	C-4a, C-5 and C-6
6	106.41	C	-	-
7-OH	162.33	C	6.38 (1H, *s*)	C-6 and C-7
8	107.23	C	-	-
8a	157.73	C	-	-
1′	131.02	C	-	-
2′, 6′	127.68	CH	7.31 (2H, *d*, *J* = 8.7 Hz)	C-2, C-3′/5′, C-4′ and C-6′/2′
3′, 5′	115.49	CH	6.87 (2H, *d*, *J* = 8.7 Hz)	C-1′, C-4′ and C-5′/3′
4′	155.89	C	-	-
1″	21.87[a]	CH_2	3.29 (2H, *d*, *J* = 7.2 Hz)	C-8, C-8a, C-2″ and C-3″
2″	121.94[b]	CH	5.23 (1H, *m*)	C-8, C-1″, C-4″and C-5″
3″	134.73[c]	C	-	-
4″	17.87[d]	CH_3	1.81[*] (3H, *s*)	} C-1″, C-2″ and C-3″
5″	25.82[e]	CH_3	1.74[*] (3H, *s*)	
1‴	21.23[a]	CH_2	3.34 (2H, *d*, *J* = 6.9 Hz)	C-5, C-6, C-7 , C-2‴ and C-3‴
2‴	121.73[b]	CH	5.18 (1H, *m*)	C-6, C-1‴, C-4‴ and C-5‴
3‴	133.97[c]	C	-	-
4‴	17.81[d]	CH_3	1.70[*] (3H, *s*)	} C-2‴ and C-3‴
5‴	25.83[e]	CH_3	1.69[*] (3H, *s*)	

[a, b, c, d, e, *] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 65 Comparison of ^{1}H NMR spectral data between compound **STC2** and senegalensein

Position	**Compound STC2 δ_H (ppm) (recorded in $CDCl_3$)**	**Senegalensein δ_H (ppm) (recorded in $CDCl_3$)**
2	5.31 (1H, *dd*, *J* = 12.6, 3 Hz)	5.20-5.30 (1H, *m*)
3α	3.03 (1H, *dd*, *J* = 17.1, 12.6 Hz)	3.02 (1H, *dd*, *J* = 17, 12 Hz)
β	2.79 (1H, *dd*, *J* = 17.1, 3 Hz)	2.78 (1H, *dd*, *J* = 17, 3 Hz)
5-OH	12.30 (1H, *s*)	12.29 (1H, *s*)
7-OH	6.38 (1H, *s*)	6.41 (1H, *s*)
2′, 6′	7.31 (2H, *d*, *J* = 8.7 Hz)	7.27 (2H, *d*, *J* = 9 Hz)
3′, 5′	6.87 (2H, *d*, *J* = 8.7 Hz)	6.84 (2H, *d*, *J* = 9 Hz)
1″	3.29 (2H, *d*, *J* = 7.2 Hz)	3.33 (2H, *d*, *J* = 6.2 Hz)
2″	5.23 (1H, *m*)	5.20-5.30 (1H, *m*)
4″, 5″	1.81, 1.74 (each 3H, *s*)	1.80, 1.69 (each 3H, *s*)
1‴	3.34 (2H, *d*, *J* = 6.9 Hz)	3.28 (2H, *d*, *J* = 8.7 Hz)
2‴	5.18 (1H, *m*)	5.20-5.30 (1H, *m*)
4‴, 5‴	1.70, 1.69 (each 3H, *s*)	1.78, 1.68 (each 3H, *s*)

2.3.1.17 Compound STC3



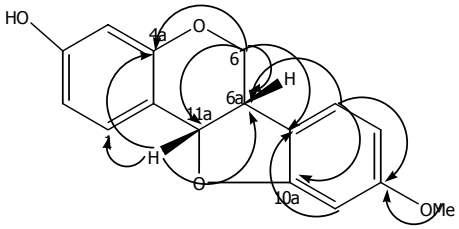
Compound **STC3** was isolated as yellow viscous oil, $[\alpha]_D^{27}$: -52.63° (*c* = 0.019, $CHCl_3$). The UV spectrum showed the maxima at 211, 223, 280, 286 and 319 nm. In IR spectrum, the absorption band of OH stretching (3401 cm^{-1}) was shown.

The ^1H NMR (**Table 66**) showed the existence of two ABM types of aromatic protons which were assigned to H-1, H-2 and H-4 for the first group at δ 7.38 (1H, *d*, *J* = 8.6 Hz), 6.54 (1H, *dd*, *J* = 8.6, 2.1 Hz) and 6.41 (1H, *d*, *J* = 2.1 Hz). The second group of signals was assigned to H-7, H-8 and H-10 at δ 7.12 (1H, *d*, *J* = 8.9 Hz), 6.45 (1H, *dd*, *J* = 8.9, 2.4 Hz) and 6.45 (1H, *d*, *J* = 2.4 Hz), respectively. A *doublet of doublet* signal at δ 4.23 (*J* = 10.5 and 4.5 Hz), two *multiplet* signal at δ 3.60 and 3.52 were found to couple to each other. Moreover a *multiplet* signal at δ 3.52 was further coupled to a *doublet* signal at δ 5.49 (*J* = 6.6 Hz). Thus, these signals were suggested to be signals of H-6β, H-6α, H-6a and H-11a, respectively. A *singlet* signal of methoxy group was shown at δ 3.76 and was found to correlate to C-9 in HMBC, thus it was located at C-9. Accordingly, **STC3** was deduced to be dihydropterocarpan derivative. The ^{13}C NMR and the DEPT spectral data (**Table 66**) indicated the existence of a methyl carbon (δ 55.47), a methylene carbon (δ 66.55), eight methine carbons (δ 132.10, 124.92, 110.10, 106.25, 103.60, 96.89, 78.86 and 39.63) and six quaternary carbons (δ 161.20, 160.88, 156.75 (2), 119.46 and 111.69).

In the HMBC experiments, the methoxy group at δ 3.76 (9-OMe) was found to show the correlation with the carbon signal at C-9 (δ 55.47), this showed that the position of methoxy group at C-9. By NOE experiments, irradiation at 9-OMe (δ

3.76) gave enhancement of the signals of H-8/H-10 (δ 6.45), and irradiation of H-10 (δ 6.45) resulted in the enhancement of the signal at 9-OMe (δ 3.76) thus the position of methoxy group at C-9 was confirmed.

The stereochemistry of H-6a and H-11a were assigned by NOE experiments. H^1-6 (δ 4.23) and H-11a (δ 5.49) signals were enhanced upon irradiation at H-6a (δ 3.52). It was therefore proposed that H^1-6, H-6a and H-11a were *cis*. **STC3** was considered to be 3-hydroxy-9-methoxypterocarpan, (medicarpin) (Herath, *et.al*., 1998).



Selected HMBC correlation of **STC3**

Table 66 ^{1}H, ^{13}C and HMBC spectral data of compound **STC3**

Position	**δ_C (ppm)**		**δ_H (ppm)**	**HMBC**
1	132.10	CH	7.38 (1H, *d*, *J* = 8.6 Hz)	C-3, C-4, C-4a and C-11a
1a	111.69	C	-	-
2	110.10	CH	6.54 (1H, *dd*, *J* = 8.6, 2.1 Hz)	C-4
3	156.75	C	-	-
4	103.60	CH	6.41 (1H, *d*, *J* = 2.1 Hz)	C-1a and C-2
4a	156.75	C	-	-
6α	66.55	CH_2	3.60 (1H, *m*)	C-4a , C-6a, C-7a and C-11a
β			4.23 (1H, *dd*, *J* = 10.5, 4.5 Hz)	
6a	39.63	CH	3.52 (1H, *m*)	C-6, C-7a, C-7 and C-10a
7	124.92	CH	7.12 (1H, *d*, *J* = 8.9 Hz)	C-6a, C-9, C-10 and C-10a
7a	119.46	C	-	-

Table 66 (Continue)

Position	δ_C[#](ppm)		δ_H (ppm)	HMBC
8	106.25	CH	6.45 (1H, *dd*, *J* = 8.9, 2.4 Hz)	C-7a and C-9
9	160.88[a]	C	-	-
10	96.89	CH	6.45 (1H, *d*, *J* = 2.4 Hz)	C-7a , C-8 and C-10a
10a	161.20[a]	C	-	-
11a	78.86	CH	5.49 (1H, *d*, *J* = 6.6 Hz)	C-1, C-1a, C-4a, C-6 and C-6a
9-OMe	55.47	CH_3	3.76 (3H, *s*)	C-9

[a]Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Table 67 Comparison of ^{1}H NMR spectral data between compound **STC3** and medicarpin

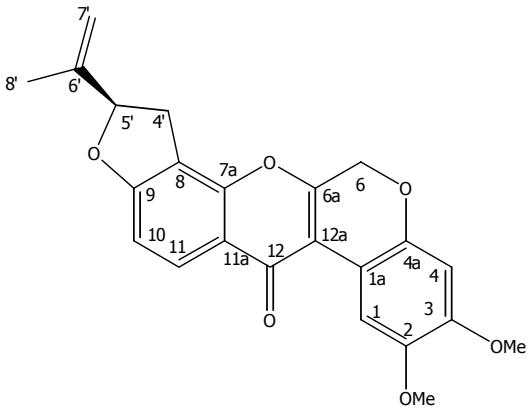
Position	Compound STC3 δ_H (ppm) (recorded in $CDCl_3$)	Medicarpin δ_H (ppm) (recorded in $CDCl_3$)
1	7.38 (1H, *d*, *J* = 8.6 Hz)	7.41 (1H, *d*, *J* = 8.4 Hz)
2	6.54 (1H, *dd*, *J* = 8.6, 2.1 Hz)	6.58 (1H, *dd*, *J* = 8.4, 2.4 Hz)
4	6.41 (1H, *d*, *J* = 2.1 Hz)	6.44 (1H, *d*, *J* = 2.4 Hz)
6α	3.60 (1H, *m*)	3.64 (1H, *dd*, *J* = 11.0, 10.9 Hz)
β	4.23 (1H, *dd*, *J* = 10.5, 4.5 Hz)	4.26 (1H, *dd*, *J* = 11.0, 5.0 Hz)
6a	3.52 (1H, *m*)	3.55 (1H, *ddd*, *J* = 11, 6.7, 5.1 Hz)
7	7.12 (1H, *d*, *J* = 8.9 Hz)	7.15 (1H, *d*, *J* = 8.9 Hz)
8	6.45 (1H, *dd*, *J* = 8.9, 2.4 Hz)	6.48 (1H, *m*)
10	6.45 (1H, *d*, *J* = 2.4 Hz)	6.48 (1H, *m*)
11a	5.49 (1H, *d*, *J* = 6.6 Hz)	5.52 (1H, *d*, *J* = 6.8 Hz)
9-OMe	3.76 (3H, *s*)	3.79 (3H, *s*)

Table 68 Comparison of ^{13}C NMR spectral data between compound **STC3** and medicarpin

Position	**Compound STC3 δ_C (ppm) (recorded in $CDCl_3$)**	**Medicarpin δ_C (ppm) (recorded in $CDCl_3$)**
1	132.1	132.6
1a	111.7	113.0
2	110.1	102.2
3	156.8	157.5
4	103.6	104.1
4a	156.8	157.1
6	66.6	67.0
6a	39.6	39.9
7	124.9	125.2
7a	119.5	119.5
8	106.3	106.8
9	160.9[a]	161.1
10	96.9	97.3
10a	161.2[a]	161.5
11a	78.9	79.0
9-OMe	55.5	55.9

[a]δ interchangeable values in each column.

2.3.1.18 Compound STC4



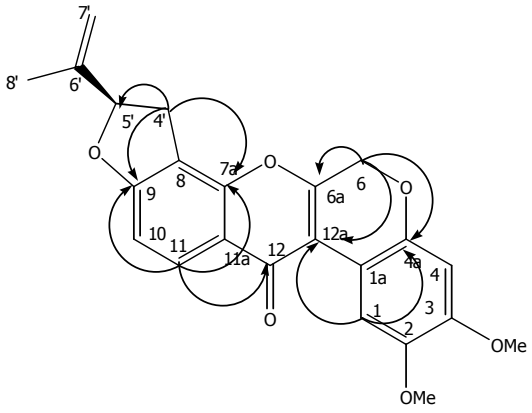
Compound **STC4** was isolated as a yellow solid, mp : 209-211°C, $[\alpha]_D^{27}$: +22.72° (*c* = 0.022, $CHCl_3$). In the UV spectrum, strong absorptions at 245, 280 and 310 nm were detected. The presence of a conjugated carbonyl group at 1635 cm^{-1} was suggested in the IR spectrum.

The ^1H NMR spectrum (**Table 69**), revealed the presence of the oxymethylene protons at δ 4.99 (2H, *s*, H-6). Signals of four aromatic protons were discernible at δ 8.14 and 6.93 (each 1H, *d*, *J* = 8.7 Hz, H-11, H-10) and δ 8.46 and 6.55 (each 1H, *s*, H-1, H-4). Compound **STC4** also contained two phenolic methyl ethers which appeared as two *singlets* at δ 3.96 and 3.87 (each 3H). Terminal olefinic protons at δ 5.14 (1H, *br s*) and 4.99 (1H, *m*) were assigned to be signal of H-7′. Two *doublet of doublet* signals at δ 3.55 (1H, *J* = 15.9, 9.3 Hz), 3.20 (1H, *J* = 15.9, 9.3 Hz) and a *triplet* signal at δ 5.41 (1H, *J* = 9.3 Hz) were suggested to be signals of H-4′α, H-4′β and H-5′β, respectively. A methyl group at δ 1.80 (3H, *s*, H-8′) was apparent. The ^{13}C NMR and the DEPT spectral data (**Table 69**) indicated the existence of three methyl carbons (δ 56.26, 55.85 and 17.09), three methylene carbons (δ 112.91, 64.76 and 31.38), five methine carbons (δ 127.75, 110.01, 108.64, 100.33 and 87.91), eleven quaternary

carbons (δ 164.78, 156.09, 152.20, 148.87, 146.22, 143.94, 142.84, 118.85, 113.00, 111.51 and 110.52) and a carbonyl carbon (δ 174.23).

In the HMBC experiments, correlation of H-11 (δ 8.14) to C-7a (δ 152.20), C-9 (δ 164.78) and C-12 (δ 174.23) and of H-4′ (δ 3.55) to C-8 (δ 113.00), C-7a (δ 152.20), C-9 (δ 164.78) and C-6′ (δ 142.84) suggested that the furan ring was connected to the 8, 9-position. The correlation of H-6 (δ 4.99) to C-4a (δ 146.22), C-6a (δ 156.09) and C-12a (δ 111.51) enabled us to deduce the location of the double bond to be at C-6a and C-12a position.

Comparison of ^{1}H NMR spectral data (**Table 70**) of compound **STC4** and **STH6** revealed close structural similarity. Difference in the spectrum of compound **STC4** and **STH6** was shown as an extra double bond between C-6a and C-12a position in **STC4** but not in **STH6**. Thus, compound **STC4** was identified as 6a,12a-dehydro-α-rotenone (Carlson, *et.al*. 1973).



Selected HMBC correlation of **STC4**

Table 69 ^{1}H, ^{13}C and HMBC spectral data of compound **STC4**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
1a	110.52	C	-	-
1	110.01	CH	8.46 (1H, *s*)	C-2, C-3, C-4a and C-12a
2	143.94	C	-	-
3	148.87	C	-	-
4	100.33	CH	6.55 (1H, *s*)	C-1a, C-2, C-3 and C-4a
4a	146.22	C	-	-
6	64.76	CH_2	4.99 (2H, *s*)	C-4a, C-6a and C-12a
6a	156.09	C	-	-
7a	152.20	C	-	-
8	113.00	C	-	-
9	164.78	C	-	-
10	108.64	CH	6.93 (1H, *d*, *J* = 8.7 Hz)	C-8, C-9 and C-11a
11	127.75	CH	8.14 (1H, *d*, *J* = 8.7 Hz)	C-7a, C-9 and C-12
11a	118.85	C	-	-
12	174.23	C	-	-
12a	111.51	C	-	-
4′α	31.38	CH_2	3.55 (1H, *dd*, *J* = 15.9, 9.3 Hz)	} C-8, C-7a, C-9, C-5′ and C-6′
β			3.20 (1H, *dd*, *J* = 15.9, 9.3 Hz)	
5′	87.91	CH	5.41 (1H, *t*, *J* = 9.3 Hz)	C-8, C-9, C-6′, C-7′ and C-8′
6′	142.84	C	-	-
7′	112.91	CH_2	5.14 (1H, *br s*)	} C-5′ and C-8′
			4.99 (1H, *m*)	
8′	17.09	CH_3	1.80 (3H, *s*)	C-4′, C-5′, C-6′, and C-7′
2-OMe	56.26	CH_3	3.96 (3H, *s*)	C-2
3-OMe	55.85	CH_3	3.87 (3H, *s*)	C-3

[#] Carbon type deduced from DEPT experiment.

Table 70 Comparison of ^{1}H NMR spectral data between compound **STC4** and **STH6**

Position	Compound STC4 δ_H (ppm) (recorded in $CDCl_3$)	Compound STH6 δ_H (ppm) (recorded in $CDCl_3$)
1	8.46 (1H, *s*)	6.77 (1H, *d*, *J* = 0.6 Hz)
4	6.55 (1H, *s*)	6.46 (1H, *s*)
6α	} 4.99 (2H, *s*)	4.61 (1H, *dd*, *J* = 12, 3.3 Hz)
β		4.18 (1H, *d*, *J* = 12 Hz)
6a	-	4.92 (1H, *m*)
10	6.93 (1H, *d*, *J* = 8.7 Hz)	6.51 (1H, *d*, *J* = 8.4 Hz)
11	8.14 (1H, *d*, *J* = 8.7 Hz)	7.84 (1H, *d*, *J* = 8.4 Hz)
12a	-	3.84 (1H, *d*, *J* = 3 Hz)
4′α	3.55 (1H, *dd*, *J* = 15.9, 9.3 Hz)	3.32 (1H, *dd*, *J* = 15.9, 9 Hz)
β	3.20 (1H, *dd*, *J* = 15.9, 9.3 Hz)	2.96 (1H, *dd*, *J* = 15.9, 9 Hz)
5′	5.41 (1H, *t*, *J* = 9.3 Hz)	5.24 (1H, *t*, *J* = 9 Hz)
7′	5.14 (1H, *br s*)	5.07 (1H, *s*)
	4.99 (1H, *m*)	4.93 (1H, *m*)
8′	1.80 (3H, *s*)	1.77 (3H, *s*)
2-OMe	3.96 (3H, *s*)	3.76 (3H, *s*)
3-OMe	3.87 (3H, *s*)	3.81 (3H, *s*)

Table 71 Comparison of ^{13}C NMR spectral data between compounds **STC4** and **STH6**

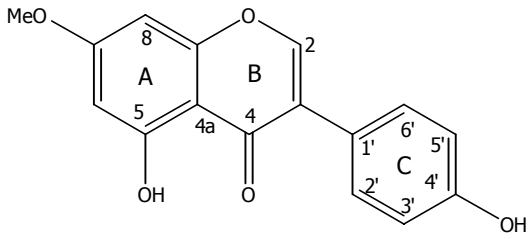
Position	Compound STC4 δ_C (ppm) (recorded in $CDCl_3$)	Compound STH6 δ_C (ppm) (recorded in $CDCl_3$)
1a	110.52	104.76
1	110.01	110.27
2	143.94	143.84 [a]
3	148.87	149.44 [a]
4	100.33	100.86
4a	146.22	147.33
6	64.76	66.25
6a	156.09	72.18
7a	152.20	157.93
8	113.00	112.95
9	164.78	167.36
10	108.64	104.88
11	127.75	129.97
11a	118.85	113.30
12	174.23	188.97
12a	111.51	44.56
4′	31.38	31.24
5′	87.91	87.83
6′	142.84	143.00
7′	112.91	112.57
8′	17.09	17.11
2-OMe	56.26	56.36
3- OMe	55.85	56.88

[a] Interchangeable values in each column.

Table 72 Comparison of ^{1}H NMR spectral data between compound **STC4** and 6a,12a-dehydro-α-rotenone

Position	**Compound STC4 δ_H (ppm) (recorded in $CDCl_3$)**	**6a,12a-Dehydro-α-rotenone δ_H (ppm) (recorded in $CDCl_3$)**
1	8.46 (1H, *s*)	8.44 (1H, *s*)
4	6.55 (1H, *s*)	6.52 (1H, *s*)
6	4.99 (2H, *s*)	4.97 (2H, *s*)
10	6.93 (1H, *d*, *J* = 8.7 Hz)	6.89 (1H, *d*, *J* = 8.6 Hz)
11	8.14 (1H, *d*, *J* = 8.7 Hz)	8.11(1H, *d*, *J* = 8.6 Hz)
4′α	3.55 (1H, *dd*, *J* = 15.9, 9.3 Hz)	3.52 (1H, *dd*, *J* = 16, 9.6 Hz)
β	3.20 (1H, *dd*, *J* = 15.9, 9.3 Hz)	3.15 (1H, *dd*, *J* = 16, 9.6 Hz)
5′	5.41 (1H, *t*, *J* = 9.3 Hz)	5.39 (1H, *t*, *J* = 8.2 Hz)
7′	5.14 (1H, *br s*)	5.12 (1H, *s*)
	4.99 (1H, *m*)	4.97 (1H, *s*)
8′	1.80 (3H, *s*)	1.80 (3H, *s*)
2-OMe	3.96 (3H, *s*)	3.94 (3H, *s*)
3-OMe	3.87 (3H, *s*)	3.84 (3H, *s*)

2.3.1.19 Compound STC5

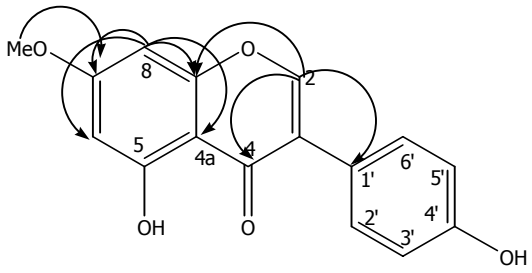


Compound **STC5** was isolated as yellow viscous oil. In the UV spectrum, strong absorptions at 211, 249, 260, 290 and 342 nm were detected. The presence of a conjugated carbonyl group at 1732 cm^{-1} and a hydroxyl group at 3421 cm^{-1} were suggested in the IR spectrum.

The ^{1}H NMR spectral data (**Table 73**), revealed the presence of a sharp *singlet* signal of a chelated hydroxyl group at δ 12.90. A typical *singlet* signal of vinylic proton H-2 of isoflavone was observed at δ 7.94. A *meta*-coupled signal of aromatic protons was present at δ 6.36 and 6.43 and were deduced to be signals of H-6 and H-8. Signals of AA′BB′ type were assigned to H-2′/6′ and H-3′/5′ of the C ring, at δ 7.40 (2H) and 6.94 (2H), respectively. A *singlet* signal of methoxy group appeared at δ 3.89. The ^{13}C NMR and the DEPT spectral data (**Table 73**) indicated the existence of a methyl carbon (δ 55.80), five methine carbons (δ 152.68, 130.33, 115.53, 98.21 and 92.43), seven quaternary carbons (δ 165.55, 162.71, 157.75, 154.54, 125.58, 123.60 and 114.75) and a carbonyl carbon (δ 182.00).

The location of the methoxy group was deduced to be at C-7 by the result of the 2D HMBC correlation and NOE experiments. The NOE studies displayed enhancement of the H-6 (δ 6.36) and H-8 (δ 6.43) signals upon irradiation of the methoxy proton at C-7, it thus confirmed the position of O<u>Me</u> of A ring. Thus,

compound **STC5** was identified as 4′-hydroxy-7-methoxyisoflavone (Lin, *et.al*., 1991).



Selected HMBC correlation of **STC5**

Table 73 ^{1}H, ^{13}C and HMBC spectral data of compound **STC5**

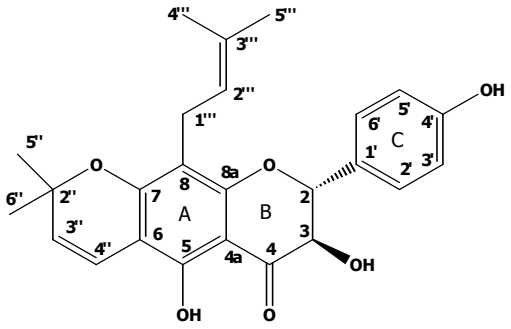
Position	δ_C [#] **(ppm)**		δ_H **(ppm)**	**HMBC**
2	152.68	CH	7.94 (1H, *s*)	C-3, C-4, C-8a and C-1′
3	125.58	C	-	-
4	182.00	C	-	-
4a	114.75	C	-	-
5-OH	162.71	C	12.90 (1H, *s*)	-
6	98.21	CH	6.36 (1H, *d*, *J* = 2.4 Hz)	C-4a and C-8
7	165.55	C	-	-
8	92.43	CH	6.43 (1H, *d*, *J* = 2.4 Hz)	C-4a, C-6, C-7 and C-8a
8a	157.75	C	-	-
1′	123.60	C	-	-
2′/ 6′	130.33	CH	7.40 (1H, *d*, *J* = 8.7 Hz)	C-3, C-4′ and C-6′/ 2′
3′/ 5′	115.53	CH	6.94 (1H, *d*, *J* = 8.7 Hz)	C-1′, C-4′ and C-5′/ 3′
4′	154.54	C	-	-
7-OMe	55.80	CH_3	3.89 (3H, *s*)	C-7

[#] Carbon type deduced from DEPT experiment.

Table 74 Comparison of ^{1}H NMR spectral data between compound **STC5** and prunetin

Position	Compound STC5 δ_H (ppm) (recorded in $CDCl_3$)	Prunetin δ_H (ppm) (recorded in DMSO-d_6)
2	7.94 (1H, *s*)	8.04 (1H, *s*)
5-OH	12.90 (1H, *s*)	12.82 (1H, *s*)
6	6.36 (1H, *d*, *J* = 2.4 Hz)	6.26 (1H, *d*, *J* = 2.1 Hz)
8	6.43 (1H, *d*, *J* = 2.4 Hz)	6.40 (1H, *d*, *J* = 2.1 Hz)
2′/ 6′	7.40 (1H, *d*, *J* = 8.7 Hz)	7.34 (1H, *d*, *J* = 8.5 Hz)
3′/ 5′	6.94 (1H, *d*, *J* = 8.7 Hz)	6.78 (1H, *d*, *J* = 8.5 Hz)
7-OMe	3.89 (3H, *s*)	3.80 (3H, *s*)

2.3.1.20 Compound STC6

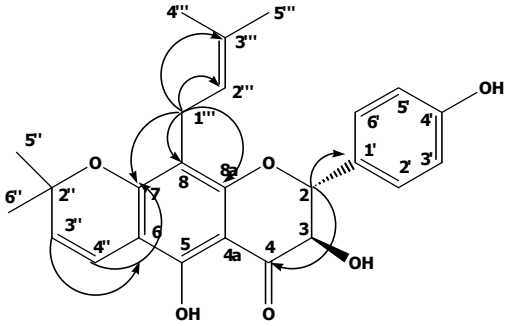


Compound **STC6** was obtained as a yellow viscous oil, $[\alpha]_D^{27}$: +90.91° (*c* = 0.011, $CHCl_3$). The IR spectrum showed absorption bands at 3420 and 1627 cm^{-1} corresponding to a hydroxyl group and conjugated carbonyl group, respectively. The UV spectrum showed maxima at 203, 224, 267, 274, 300, 314 and 369 nm, which are typical absorptions of flavanone.

The ^{1}H NMR spectrum (**Table 75**) showed a sharp *singlet* signal of a chelated hydroxyl group 5-OH at δ 11.35. The *doublet* signals at δ 4.98 (1H, *d, J* = 12 Hz) and 4.49 (1H, *d, J* = 12 Hz) are characteristic of the antiperiplanar conformation of

the C-2 and C-3 protons of a 3-hydroxyflavanone. The *doublet* resonanecs at δ 5.53 (1H, *J* = 10 Hz) and 6.64 (1H, *J* = 10 Hz), each equivalent to one proton, and the two *singlet* resonances at δ 1.44 (3H) and 1.45 (3H), were characteristic of the *cis* double bond and *gem*-dimethyl group of a 2,2-dimethylchromene moiety, respectively. Two *ortho*-coupled *doublets* centered at δ 7.42 (2H, *J* = 8.4 Hz) and 6.89 (2H, *J* = 8.4 Hz) were assigned to the protons of a *para*-disubstituted benzene ring (C ring). The presence of an isoprenyl group was inferred from two *singlets* at δ 1.60 and 1.64 (each 3H, *s*, Me-4′′′ (Me-5′′′)), a *broad doublet* at δ 3.18 (2H, *J* = 7.5 Hz, H-1′′′) and a *multiplet* at δ 5.11 (1H, H-2′′′). The ^{13}C NMR (**Table 75**) and the DEPT spectral data indicated the existence of four methyl carbons (δ 28.38, 28.35, 25.79 and 17.77), a methylene carbon (δ 21.25), seven methine carbons (δ 128.95, 126.28, 122.08, 115.44, 115.39, 82.76 and 72.47), ten quaternary carbons (δ 161.00, 159.30, 156.21, 155.92, 131.35, 128.78, 109.23, 103.12, 100.23 and 78.44) and a carbonyl carbon (δ 196.16).

The location of an isoprenyl unit was deduced to be at C-8 by the result of the 2D HMBC correlations of H-1′′′ to C-7 (δ 161.00), C-8 (δ 109.23), C-8a (δ 159.30), C-2′′′ (δ 122.08) and C-3′′′ (δ 131.35), of H-4′′ (δ 6.64) to C-7 (δ 161.00) and C-2′′ (δ 78.44), and of H-3′′ (δ 5.53) to C-6 (δ 103.12) and C-2′′ (δ 78.44), respectively. These correlations confirmed the presence of dimethylchromene ring and suggested that this unit fused to aromatic nucleus at C-6 and C-7.



Selected HMBC correlation of **STC6**

Table 75 ^{1}H, ^{13}C and HMBC spectral data of compound **STC6**

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
2	82.76	CH	4.98 (1H, *d*, *J* = 12 Hz)	C-4 and C-1′
3	72.47	CH	4.49 (1H, *d*, *J* = 12 Hz)	C-2 and C-1′
4	196.16	C	-	-
4a	100.23	C	-	-
5	156.21	C	11.35 (OH, *s*)	C-5, C-6 and C-4a
6	103.12	C	-	-
7	161.00	C	-	-
8	109.23	C	-	-
8a	159.30	C	-	-
1′	128.78	C	-	-
2′/ 6′	128.95	CH	7.42 (2H, *d*, *J* = 8.4 Hz)	C-2, C-4′ and C-6′/2′
3′/ 5′	115.44[a]	CH	6.89 (2H, *d*, *J* = 8.4 Hz)	C-1′, C-5′/3′and C-4′
4′	155.92	C	-	-
2″	78.44	C	-	-
3″	126.28	CH	5.53 (1H, *d*, *J* = 10 Hz)	C-6 and C-2″
4″	115.39[a]	CH	6.64 (1H, *d*, *J* = 10 Hz)	C-7 and C-2″
5″	28.35[b]	CH_3	1.44[*] (3H, *s*)	C-2″, C-3″and C-6″
6″	28.38[b]	CH_3	1.45[*] (3H, *s*)	C-2″, C-3″ and C-5″
1‴	21.25	CH_2	3.18 (2H, *br d*, *J* = 7.5 Hz)	C-7, C-8, C-8a, C-2‴ and C-3‴
2‴	122.08	CH	5.11 (1H, *m*)	C-4‴and C-5‴
3‴	131.35	C	-	-
4‴	17.77[c]	CH_3	1.60[†] (3H, *s*)	C-2‴, C-3‴ and C-5‴
5‴	25.79[c]	CH_3	1.64[†] (3H, *s*)	C-2‴, C-3‴ and C-4‴

[a,b,c,*,] [†]Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment.

Comparison of ^{1}H NMR spectral data (**Table 76**) and ^{13}C NMR spectral data (**Table 77**) between compound **STC6** and **STH1**, showed similarity except **STC6** has an extra hydroxyl at C-3. The proton at C-3 was shown as a downfield doublet signal at δ 4.49 in ^{1}H NMR and signal of oxymethine carbon at δ 72.47. Thus compound **STC6** was identified as lupinifolinol (Smalberger, *et.al*., 1974).

Table 76 Comparison of ^{1}H NMR spectral data between compound **STC6** and **STH1**

Position	**Compound STC6 δ_H (ppm) (recorded in $CDCl_3$)**	**Compound STH1 δ_H (ppm) (recorded in $CDCl_3$)**
2	4.98 (1H, *d*, *J* = 12 Hz)	5.33 (1H, *dd*, *J* = 13, 3 Hz)
3α	4.49 (1H, *d*, *J* = 12 Hz)	3.04 (1H, *dd*, *J* = 17.5, 13 Hz)
β		2.80 (1H, *dd*, *J* = 17.5, 3 Hz)
5-OH	11.35 (OH, *s*)	12.24 (OH, *s*)
2′, 6′	7.42 (2H, *d*, *J* = 8.4 Hz)	7.31 (2H, *d*, *J* = 8.5 Hz)
3′, 5′	6.89 (2H, *d*, *J* = 8.4 Hz)	6.87 (2H, *d*, *J* = 8.5 Hz)
3′′	5.53 (1H, *d*, *J* = 10 Hz)	5.50 (1H, *d*, *J* = 10 Hz)
4′′	6.64 (1H, *d*, *J* = 10 Hz)	6.63 (1H, *d*, *J* = 10 Hz)
5′′/ 6′′	1.44/1.45 (each 3H, *s*)	1.43/1.45 (each 3H, *s*)
1′′′	3.18 (2H, *br d*, *J* = 7.5 Hz)	3.21 (2H, *br d*, *J* = 7 Hz)
2′′′	5.11 (1H, *m*)	5.14 (1H, *triplet of guintet*, *J* = 9, 1.5 Hz)
4′′′/ 5′′′	1.64/1.60 (each 3H, *s*)	1.65 (6H, *s*)

Table 77 Comparison of ^{13}C NMR spectral data between compound **STC6** and **STH1**

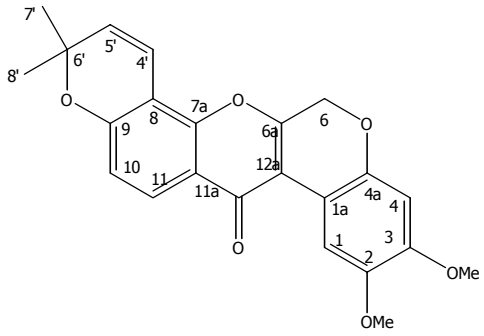
Position	Compound STC6 δ_C (ppm) (recorded in $CDCl_3$)	Compound STH1 δ_C (ppm) (recorded in $CDCl_3$)
2	82.76	78.49
3	72.47	43.14
4	196.16	196.57
4a	100.23	102.62
5	156.21	156.53
6	103.12	102.78
7	161.00[b]	159.92[a]
8	109.23[c]	108.63
8a	159.30	159.36[a]
1′	128.78	130.84
2′, 6′	128.95	127.67
3′, 5′	115.44[a]	115.50
4′	155.92	155.96
2″	78.44	78.12
3″	126.28	125.98
4″	115.39[a]	115.59
5″/6″	28.35/28.38	28.26/28.36
1‴	21.25	21.43
2‴	122.08	122.43
3‴	131.35	131.09
4‴/5‴	25.79/17.77	25.78/17.79

[a,b,c]Assignment with the same superscripts may be interchanged.

Table 78 Comparison of ^{1}H NMR spectral data between compound **STC6** and lupinifolinol

Position	**Compound STC6 δ_H (ppm) (recorded in $CDCl_3$)**	**Lupinifolinol δ_H (ppm) (recorded in $CDCl_3$)**
2	4.98 (1H, *d*, *J* = 12 Hz)	4.95 (1H, *d*, *J* = 12 Hz)
3	4.49 (1H, *d*, *J* = 12 Hz)	4.50 (1H, *d*, *J* = 12 Hz)
3-OH	-	3.80
5-OH	11.35 (OH, *s*)	12.38 (OH, *s*)
2′, 6′	7.42 (2H, *d*, *J* = 8.4 Hz)	7.45 (2H, *d*, *J* = 8.5 Hz)
3′, 5′	6.89 (2H, *d*, *J* = 8.4 Hz)	6.77 (2H, *d*, *J* = 8.5 Hz)
4′-OH	-	6.10
3″	5.53 (1H, *d*, *J* = 10 Hz)	5.50 (1H, *d*, *J* = 10 Hz)
4″	6.64 (1H, *d*, *J* = 10 Hz)	6.63 (1H, *d*, *J* = 10 Hz)
5″/ 6″	1.44/1.45 (each 3H, *s*)	1.45 (6H, *s*)
1‴	3.18 (2H, *br d*, *J* = 7.5 Hz)	3.16 (2H, *t*, *J* = 7.0 Hz)
2‴	5.11 (1H, *m*)	5.11 (1H, *t*, *J* = 7.0 Hz)
4‴/ 5‴	1.64/1.60 (each 3H, *s*)	1.64/1.59 (each 3H, *s*)

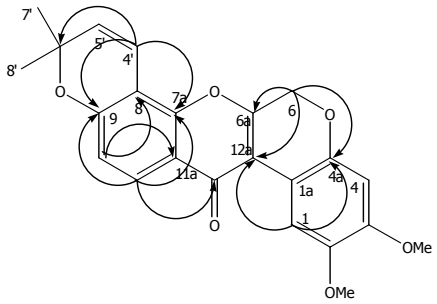
2.3.1.21 Compound STC7



Compound **STC7** was obtained as a yellow viscous oil. Comparison of the ^{1}H NMR spectral data (**Table 80**) of **STC7** and **STH5** revealed close structural similarity. Difference in the spectrum of compound **STC7** and **STH5** was that ^{1}H NMR signals of **STH5** at δ 4.91 (1H, *m*, H-6aβ) and 3.83 (1H, *dd*, *J* = 3.9 Hz, H-12aβ) were not observed in **STC7**. The signals of two olefinic protons at δ 6.79 (1H, *dd*, *J* = 9.6, 0.6 Hz) and 5.74 (1H, *d*, *J* = 9.6 Hz) and a *singlet* of two methyl groups at δ 1.50 (6H) which corresponded to a part of dimethylchromene ring were detected. Two methoxy groups resonated as two *singlets* at δ 3.88 (3-OMe) and 3.96 (2-OMe). Two *singlet* signals at δ 6.56 and 8.46 were assigned as H-4 and H-1, respectively. It also exhibited a *singlet* signal due to protons on carbon atoms bearing oxygen atoms at δ 5.03 (2H, *s*, H-6). An *ortho*-coupled *doublet* centered at δ 8.06 (1H, *J* = 9 Hz) and δ 6.89 (1H, *J* = 9, 0.6 Hz) were assigned to the protons of a tetra-substituted benzene ring.

In the HMBC correlations (**Table 79**), the carbon signals at C-7a (δ 151.5) and C-9 (δ 157.0) showed correlation to H-4′ (δ 6.79) and C-7a (δ 151.5), C-9 (δ 157.0) and C-12 (δ 174.0) to H-11 (δ 8.06). These correlations confirmed the presence of a dimethylchromene ring and suggested that this unit fused to aromatic nucleus at C-8 and C-9. The H-6 (δ 5.03) showed correlation to C-4a (δ 146.0), C-6a (δ 156.5)

and C-12a (δ 113.0) which enabled us to deduce the location of the double bond to be at the C-6a and C-12a positions. Thus, compound **STC7** was identified to be 6a,12a-dehydrodeguelin (Andrei, *et.al.* 1997).



Selected HMBC correlation of **STC7**

Table 79 ^{1}H, ^{13}C and HMBC spectral data of compound **STC7**

Position	$\delta_C^{\#}$**(ppm)**	δ_H **(ppm)**	**HMBC Correlation**
1a	111.0[a]	-	-
1	110.0	8.46 (1H, *s*)	C-2, C-3, C-4a and C-12a
2	144.0	-	-
3	149.0	-	-
4	101.0	6.56 (1H, *s*)	C-1a, C-3 and C-4a
4a	146.0	-	-
6	66.0	5.03 (2H, *s*)	C-4a, C-6a and C-12a
6a	156.5[b]	-	-
7a	151.5	-	-
8	109.0[a]	-	-
9	157.0[b]	-	-
10	116.0[c]	6.89 (1H, *dd*, *J* = 9, 0.6 Hz)	C-8 and C-11a
11	128.0	8.06 (1H, *d*, *J* = 9 Hz)	C-7a, C-9 and C-12
11a	118.0	-	-
12	174.0	-	-

Table 79 (Continued)

Position	δ_C[#](ppm)	δ_H (ppm)	HMBC Correlation
12a	113.0	-	-
4′	115.0[c]	6.79 (1H, *dd*, *J* = 9.6, 0.6 Hz)	C-7a, C-9 and C-6′
5′	131.0	5.74 (1H, *d*, *J* = 9.6 Hz)	C-8, C-6′ and C-7′/8′
6′	78.0	-	-
7′/8′	28.0	1.50 (6H, *s*)	C-5′, C-6′ and C-8′/7′
2-OMe	56.0	3.96 (3H, *s*)	C-2
3-OMe	57.0	3.88 (3H, *s*)	C-3

[a,b,c]Assignment with the same superscripts may be interchanged, [#] Carbon position deduced from HMBC correlation and comparison with previous report.

Table 80 Comparison of ^{1}H NMR spectral data between compound **STC7** and **STH5**

Position	Compound STC7 δ_H (ppm) (recorded in $CDCl_3$)	Compound STH5 δ_H (ppm) (recorded in $CDCl_3$)
1	8.46 (1H, *s*)	6.78 (1H, *d*, *J* = 0.6 Hz)
4	6.56 (1H, *s*)	6.46 (1H, *s*)
6_{ax}	} 5.03 (2H, *s*)	4.64 (1H, *dd*, *J* = 12, 3.3 Hz)
6_{eq}		4.19 (1H, *d*, *J* = 12 Hz)
6a	-	4.91 (1H, *m*)
10	6.89 (1H, *dd*, *J* = 8.7, 0.6 Hz)	6.46 (1H, *dd*, *J* = 8.7, 0.6 Hz)
11	8.06 (1H, *d*, *J* = 8.7 Hz)	7.74 (1H, *d*, *J* = 8.7 Hz)
12a	-	3.83 (1H, *d*, *J* = 3.9 Hz)
4′	6.79 (1H, *dd*, *J* = 9.9, 0.6 Hz)	6.65 (1H, *dd*, *J* = 9.9, 0.6 Hz)
5′	5.74 (1H, *d*, *J* = 9.9 Hz)	5.55 (1H, *d*, *J* = 9.9 Hz)
7′/8′	1.50 (6H, *s*)	1.38/1.45 (3H, *s*)
2-OMe	3.96 (3H, *s*)	3.77 (3H, *s*)
3-OMe	3.88 (3H, *s*)	3.80 (3H, *s*)

Table 81 Comparison of ^{1}H NMR spectral data between compound **STC7** and 6a,12a-dehydrodeguelin

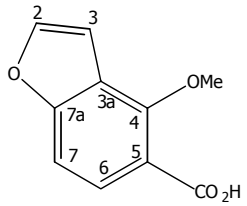
Position	**Compound STC7 δ_H (ppm) (recorded in $CDCl_3$)**	**6a,12a-Dehydrodeguelin δ_H ppm) (recorded in $CDCl_3$)**
1	8.46 (1H, *s*)	8.45 (1H, *s*)
4	6.56 (1H, *s*)	6.56 (1H, *s*)
6	5.03 (2H, *s*)	5.02 (2H, *s*)
10	6.89 (1H, *dd*, *J* = 9, 0.6 Hz)	6.87 (1H, *d*, *J* = 8.8 Hz)
11	8.06 (1H, *d*, *J* = 9 Hz)	8.04 (1H, *d*, *J* = 8.8 Hz)
4′	6.79 (1H, *dd*, *J* = 9.6, 0.6 Hz)	6.77 (1H, *d*, *J* = 10 Hz)
5′	5.74 (1H, *d*, *J* = 9.6 Hz)	5.76 (1H, *d*, *J* = 10 Hz)
7′/8′	1.50 (6H, *s*)	1.50 (6H, *s*)
2-OMe	3.96 (3H, *s*)	3.96 (3H, *s*)
3- OMe	3.88 (3H, *s*)	3.87 (3H, *s*)

Table 82 Comparison of ^{13}C NMR spectral data between compound **STC7** and 6a,12a-dehydrodeguelin

Position	**Compound STC7 δ_C (ppm) (recorded in $CDCl_3$)**	**6a,12a-Dehydrodeguelin δ_C (ppm) (recorded in $CDCl_3$)**
1a	111.0[a]	109.1[a]
1	110.0	110.0
2	144.0	144.1
3	149.0	149.0
4	101.0	100.4
4a	146.0	146.3
6	66.0	64.8
6a	156.5[b]	156.2[b]
7a	151.5	151.1
8	109.0[a]	110.5[a]
9	157.0[b]	157.2[b]
10	116.0[c]	114.7[c]
11	128.0	130.6
11a	118.0	118.5
12	174.0	174.4
12a	113.0	111.7
4′	115.0[c]	115.4[c]
5′	131.0	126.5
6′	78.0	77.8

[a,b,c]Interchangeable values in each column.

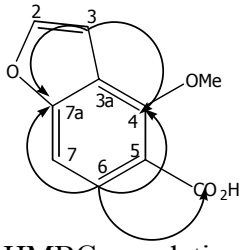
2.3.1.22 Compound STC8



Compound **STC8** was obtained as a yellow solid, mp : 132-134°C;. The IR spectrum showed absorption bands at 3447 and 1652 cm^{-1} corresponding to a hydroxyl group and a conjugated carbonyl group, respectively. The UV spectra showed maxima at 221, 246, 254 and 293 nm, which are typical absorptions of benzofuran.

The ^{1}H NMR spectra of **STC8** (**Table 83)** exhibited the characteristic signals of two furanic protons at δ 7.69 (1H, *d*, *J* = 2.0 Hz) and δ 7.01 (1H, *dd*, *J* = 2.0, 1.0 Hz). Two *ortho*-coupled *doublets* centered at δ 8.14 (1H, *J* = 9.0 Hz) and δ 7.35 (1H, *J* = 9.0, 1.0 Hz) were assigned to the protons of a tetra-substituted benzene ring. A *singlet* signal at δ 4.34 (3H, *s*) was assigned to the methoxyl group of benzene ring at C-4. The ^{13}C NMR spectral data (**Table 83**) showed a total of 10 carbons with one carbonyl group at δ 164.48 and DEPT experiment showed a methyl (δ 61.50), four methine (δ 145.53, 129.34, 107.86 and 105.12) and four quaternary carbons (δ 159.72, 153.27, 117.68 and 113.84). The assignments of all carbons were achieved by ^{13}C, HMQC and HMBC experiments.

The location of furan ring was assigned to be fused on to a neighboring ring at the C-3a (δ 113.84) and C-7a (δ 159.72) position based on the HMBC correlation. Thus, the structure of the compound **STC8** was elucidated as 4-methoxy-1-benzofuran-5-carboxylic acid.



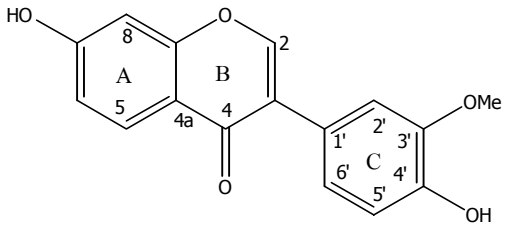
Selected HMBC correlation of **STC8**

Table 83 ^{1}H, ^{13}C and HMBC spectral data of compound **STC8**

Position	$\delta_C^{\#}$**(ppm)**	δ_H **(ppm)**	**HMBC Correlation**
2	145.53 (CH)	7.69 (1H, *d*, *J* = 2.0 Hz)	C-3, C-3a and C-7a
3	105.12 (CH)	7.01 (1H, *dd*, *J* = 2.0, 1.0 Hz)	C-2, C-4 and C-7a
3a	113.84 (C)	-	-
4	153.27 (C)	-	-
5	117.68 (C)	-	-
6	129.34 (CH)	8.14 (1H, *d*, *J* = 9.0 Hz)	C-4, 5-C=O and C-7a
7	107.86 (CH)	7.35 (1H, *dd*, *J* = 9.0, 1.0 Hz)	C-3a, C-5, C-7a
7a	159.72 (C)	-	-
4-OMe	61.50 (CH_3)	4.34 (3H, *s*)	C-4
5-C=O	164.48 (C)	-	-

$^{\#}$ Carbon position deduced from DEPT.

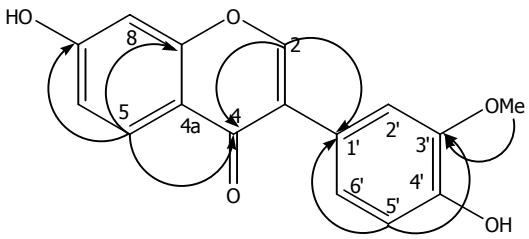
2.3.1.23 Compound STC9



Compound **STC9** was isolated as a yellow solid, mp : 132-133.5°C. In the UV spectrum, strong absorptions at 221, 248, 254 and 288 nm were detected. The presence of a conjugated carbonyl group at 1631 cm^{-1} and a hydroxyl group at 3461 cm^{-1} were suggested in the IR spectrum.

The ^{1}H NMR spectral data (**Table 84**), revealed the presence of a typical *singlet* signal of vinylic proton H-2 of isoflavone which was observed at δ 7.97. Two ABX type signals of aromatic protons were present at δ 8.12 (1H, *d*, *J* = 9 Hz, H-5), 6.97 (1H, *dd*, *J* = 9, 2.1 Hz, H-6) and 6.88 (1H, *d*, *J* = 2.1 Hz, H-8). Another sets of signals was deduced to be signals of H-2′, H-6′ and H-5′ of the C ring at δ 7.12 (1H, *d*, *J* = 2.1 Hz), 7.06 (1H, *dd*, *J* = 8.4, 2.1 Hz) and 6.93 (1H, *d*, *J* = 8.4 Hz), respectively. A *singlet* signal of methoxy group appeared at δ 3.90. The ^{13}C NMR and the DEPT spectral data (**Table 84**) indicated the existence of a methyl carbon (δ 55.82), seven methine carbons (δ 152.58, 127.64, 120.52, 115.57, 115.10, 110.97 and 102.31), seven quaternary carbons (δ 162.41, 158.05, 147.10, 145.57, 124.81, 124.41 and 117.25) and a carbonyl carbon (δ 176.47).

The location of the methoxy group was deduced to be at C-3′ by the result of the 2D HMBC correlation and NOE experiments. The NOE studies displayed enhancement of the H-2′ (δ 7.12) signal upon irradiation of the methoxy proton at C-3′, it thus confirmed the position of OMe of C ring. Thus, compound **STC9** was identified as 7, 4′-dihydroxy-3′-methoxyisoflavone (Yahara, *et.al*., 1989).



Selected HMBC correlation of **STC9**

Table 84 ^{1}H , ^{13}C and HMBC spectral data of compound **STC9**

Position	δ_C [#] **(ppm)**		δ_H **(ppm)**	**HMBC**
2	152.58	CH	7.97 (1H, *s*)	C-3, C-4 and C-8a
3	124.41	C	-	-
4	176.47	C	-	-
4a	117.25	C	-	-
5	127.64	CH	8.12 (1H, *d*, *J* = 9 Hz)	C-4, C-7 and C-8a
6	115.10	CH	6.97 (1H, *dd*, *J* = 9, 2.1 Hz)	C-4a and C-8
7	162.41	C	-	-
8	102.31	CH	6.88 (1H, *d*, *J* = 2.1 Hz)	C-4a, C-6, C-7 and C-8a
8a	158.05	C	-	-
1′	124.81	C	-	-
2′	115.57	CH	7.12 (1H, *d*, *J* = 2.1Hz)	C-3, C-4′ and C-6′
3′	147.10	C	-	-
4′	145.57	C	-	-
5′	110.97	CH	6.93 (1H, *d*, *J* = 8.4 Hz)	C-1′, C-3′ and C-4′
6′	120.52	CH	7.06 (1H, *dd*, *J* = 8.4, 2.1 Hz)	C-3, C-1′, C-2′ and C-3′
3′-OMe	55.82	CH_3	3.90 (3H, *s*)	C-4′
7-OH	-	-	9.44 (1H, *s*)	C-6, C-7 and C-8

[#] Carbon type deduced from DEPT experiment.

Table 85 Comparison of ^{1}H NMR spectral data between compound **STC9** and 7, 4′-dihydroxy-3′-methoxyisoflavone

Position	**Compound STC9 δ_H (ppm) (recorded in $CDCl_3$+CD_3OD)**	**7, 4′-dihydroxy-3′-methoxy-isoflavone δ_H (ppm) (recorded in Acetone-d_6)**
2	7.97 (1H, *s*)	8.26 (1H, *s*)
5	8.12 (1H, *d*, *J* = 9 Hz)	8.00 (1H, *d*, *J* = 8 Hz)
6	6.97 (1H, *dd*, *J* = 9, 2.1 Hz)	
8	6.88 (1H, *d*, *J* = 2.1 Hz)	
2′	7.12 (1H, *d*, *J* = 2.1Hz)	
5′	6.93 (1H, *d*, *J* = 8.4 Hz)	6.80-7.18 (total 5H, *m*)
6′	7.06 (1H, *dd*, *J* = 8.4, 2.1 Hz)	
3′-OMe	3.90 (3H, *s*)	3.81 (3H, *s*)

2.3.2 Biological activities of the pure compounds from C. manghas and D. trifoliata

The biological activities of the pure compounds (**SM1-SM6**) from *Cerbera manghas* were tested against KB, BC and NCI-H187 cell lines. Compounds **SM1**, **SM2, SM3** and **SM5** exhibited significant cytotoxic effect with ED_{50} values in the general range of 2.30-0.0017 μg/ml, whereas compound **SM4** was strongly active against BC but moderately active against KB and NCI-H187 cell lines. Compound **SM6** exhibited strong activity against KB and BC but moderate activity against NCI-H187 cell lines. The crude extract of the stems of *Derris trifoliata* also showed cytotoxic activity against KB, BC and NCI-H187 cell lines. Compounds **STH5-STH11** exhibited significant cytotoxic effect with ED_{50} values in the general range of 4.17-0.02 μg/ml, whereas compound **STH1**, **STH3** and **STC3** exhibited no activity. Compound **STH2** exhibited moderate activity against BC cell lines, whereas both compound **STH12** and **STC6** were weakly active against BC cell lines. Compound **STC1** and **STC4** exhibited strong activity against NCI-H187 but inactive against KB and BC cell lines. Compound **STH4** exhibited weak activity against KB, moderate activity against BC and strong activity against NCI-H187 cell line. The activities of all compounds are summarized in **Table 86**.

2.3.3 Conclusions

Chemical investigation of constituents from the seeds of *C. manghas* and the stems of *D. trifoliata* led to isolation of four new compounds (**SM6**, **STH4**, **STH10** and **STH11**), together with twenty-seven known compounds. Eleven pure compounds (**SM1**-**SM3**, **SM5**, **STH5**-**STH11**) exhibited significant cytotoxic activity against KB, BC and NCI-H187 cell lines, so they have potential to be developed into anti-cancer drug.

Table 86 Biological activities of crude extract and pure compounds from *C. manghas* and *D. trifoliata*

Sample	KB [a]		BC [b]		NCI-H187 [c]	
	Cytotoxicity	ED_{50}	Cytotoxicity	ED_{50}	Cytotoxicity	IC_{50}
STH	Weak	13.83	Strong	2.50	Strong	0.25
STC	Moderate	5.18	Strong	0.81	Strong	0.05
SM1	Strong	0.017	Strong	0.048	Strong	0..076
SM2	Strong	0.05	Strong	1.48	Strong	0.10
SM3	Strong	1.29	Strong	0.77	Strong	2.30
SM4	Moderate	7.56	Strong	4.62	Moderate	7.42
SM5	Strong	1.92	Strong	1.63	Strong	1.24
SM6	Strong	1.75	Strong	0.0006	Moderate	16.7
STH1	Inactive	-	Inactive	-	Inactive	-
STH2	Inactive	-	Moderate	9.68	Inactive	-
STH3	Inactive	-	Inactive	-	Inactive	-
STH4	Weak	12.51	Moderate	5.66	Strong	1.70
STH5	Strong	0.625	Strong	0.62	Strong	0.04
STH6	Strong	0.625	Strong	0.07	Strong	0.15
STH7	Strong	0.625	Strong	0.06	Strong	0.02
STH8	Strong	0.90	Strong	3.65	Strong	0.14
STH9	Strong	0.625	Strong	0.10	Strong	0.12
STH10	Strong	2.12	Strong	4.17	Strong	0.79
STH11	Strong	1.51	Strong	1.36	Strong	1.10
STH12	Inactive	-	Weak	10.73	Inactive	-
STC1	Inactive	-	Inactive	-	Strong	1.17
STC2	Weak	10.34	Weak	12.77	Weak	12.19
STC3	Inactive	-	Inactive	-	Inactive	-
STC4	Inactive	-	Inactive	-	Strong	1.31
STC5	Moderate	8.06	Inactive	-	Moderate	5.28
STC6	Inactive	-	Weak	16.00	Inactive	-

[a] Oral human epidermoid carcinoma, [b] Human breast cancer cells, [c] Human small cells lung cancer, **STH** = The hexane extract of the stems of *D. trifoliata*, **STC** = The methylene chloride extract of stems of *D. trifoliata*. $\mathbf{ED_{50}}$ and IC_{50} = Effective Dose, **MIC** = Minimum Inhibitory Concentration.

APPENDIX