2 EXPERIMENTAL

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 UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform solution. Nuclear magnetic resonance spectra were recorded on a FTNMR Bruker Ultra Shield™ 300 MHz at Department of Chemistry, Faculty of Science, Prince of Songkla University. Spectra were recorded in deuterochloroform and DMSO-d6 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 distillated at their boiling poimt ranges prior to use except diethyl ether was analytical grade reagent. Quick column chromatography was perfromed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063 – 0.200).

2.2 Plant material

Seeds of *Xylocarpus granatum* were collected from The Mangrove Reseach Station at khanom district, Nakorn Si Thammarat province, Thailand. The plant was identified by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and the voucher specimen was deposited in the herbarium (collection number XG01).

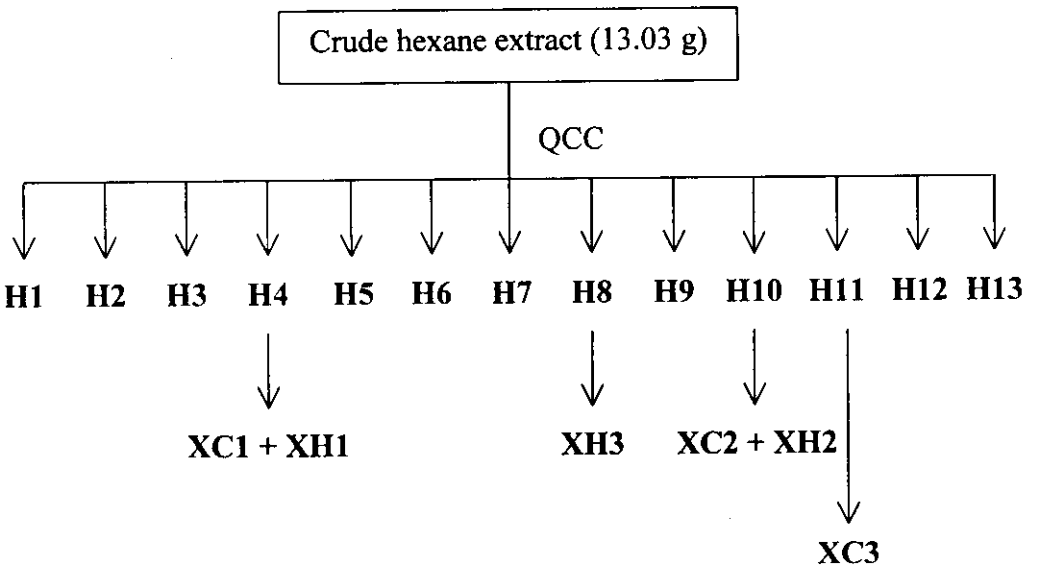
2.3 Extraction

Air-dried seeds (5.2 Kg) of *Xylocarpus granatum* were extracted with hexane, CH_2Cl_2 and MeOH, successively, at room temperature. The mixture was filtered and concentrated under reduced pressure to give the crude hexane (13.03 g), dichloromethane (68.67 g) and methanol extracts (299.98 g)

2.4 Isolation and Chemical Investigation

2.4.1 Investigation of the crude hexane extract from the seeds of *X. granatum*

The pale yellow viscous oil from the hexane extract (13.03 g) was purified by quick column chromatography (QCC) and eluted with gradient elution of hexane, EtOAc and MeOH which afforded twelve fractions (H1-H12).



Scheme 4 Isolation of compounds **XC1**, **XC2**, **XC3**, **XH1**, **XH2** and **XH3**

Fraction H4, (0.3981 g) as pale yellow viscous oil, was subjected to repeated chromatography (CC) 10% EtOAc : hexane to give compounds **XC1** (0.1222 g) and **XH1**(0.1214 g).

Fraction H8, (0.1201 g) as pale yellow viscous oil, was subjected to repeated chromatography (CC) 15% EtOAc : hexane to give compound **XH3** (0.0307 g).

Fraction H10, (2.3270 g) as pale yellow viscous oil, was subjected to repeated chromatography (CC) 30% EtOAc : hexane to give compounds **XC2** (1.5802 g) and **XH2** (0.0138 g).

Fraction H11, (1.2430 g) as pale yellow viscous oil, was subjected to repeated chromatography (CC) 30% EtOAc : hexane to give compound **XC3** (0.9580 g).

Compound XC1 (Colorless solid, mp: 263-265 °C)

$[\alpha]_D^{26}$: -41° (c = 1.22, $CHCl_3$)

EIMS *m/z*: 438 (9) $[M]^+$, 316 (94), 315 (100)

UV ($CHCl_3$) λ_{max} (nm): 225

IR (NaCl) ν (cm^{-1}): 1741 (C=O stretching of lactone), 1709 (C=O stretching of ketone), 1670 (C=O stretching of α,β-unsaturated carbonyl group)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 7**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 7**

DEPT ($CDCl_3$): see **Table 4**

Compound XC2 (Colorless solid, mp: 228-230 °C)

$[\alpha]_D^{26}$: -40° (c = 1.31, $CHCl_3$)

EIMS *m/z*: 486 (21) $[M]^+$, 349 (24), 348 (100), 330 (27), 149 (30), 135 (9), 121 (28)

UV ($CHCl_3$) λ_{max} (nm): 212

IR (NaCl) ν(cm^{-1}): 3452 cm^{-1} (O-H stretching) and 1716 cm^{-1} (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 13**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 13**

DEPT ($CDCl_3$): see **Table 10**

Compound XC3 (colorless oil)

$[\alpha]_D^{26}$: -46° (c = 0.96, $CHCl_3$)

UV ($CHCl_3$) λ_{max} (nm): 210

IR (NaCl) ν(cm^{-1}): 3460 cm^{-1} (O-H stretching) and 1732 cm^{-1} (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 19**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 19**

DEPT-135° ($CDCl_3$): see **Table 16**

Compound XH1 (colorless oil)

$[\alpha]_D^{26}$: -44 ° (c = 0.02, $CHCl_3$)

CIMS *m/z*: 471 (81) $[M]^+$, 439 (100), 411 (26), 375 (22), 357(15), 343 (15)

UV ($CHCl_3$) λ_{max} (nm): 209

IR (NaCl) ν (cm^{-1}): 1733 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 37**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 37**

DEPT-135° ($CDCl_3$): see **Table 34**

Compound XH2 (colorless oil)

$[\alpha]_D^{26}$: -40 ° (c = 0.01, acetone)

HRESIMS *m/z*: 781.2325 $[M+Na]^+$, 759.2474 $[M+H]^+$

UV ($CHCl_3$) λ_{max} (nm): 216

IR (NaCl) ν (cm^{-1}): 3555 cm^{-1} (O-H stretching) and 1733 cm^{-1} (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 43**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 43**

DEPT-135° ($CDCl_3$): see **Table 40**

Compound XH3 (colorless oil)

$[\alpha]_D^{26}$: -35 ° (c = 0.02, acetone)

CIMS *m/z*: 701 $[M]^+$

UV ($CHCl_3$) λ_{max} (nm): 217

IR (NaCl) ν (cm^{-1}): 3481 cm^{-1} (O-H stretching) and 1736 cm^{-1} (C=O stretching)

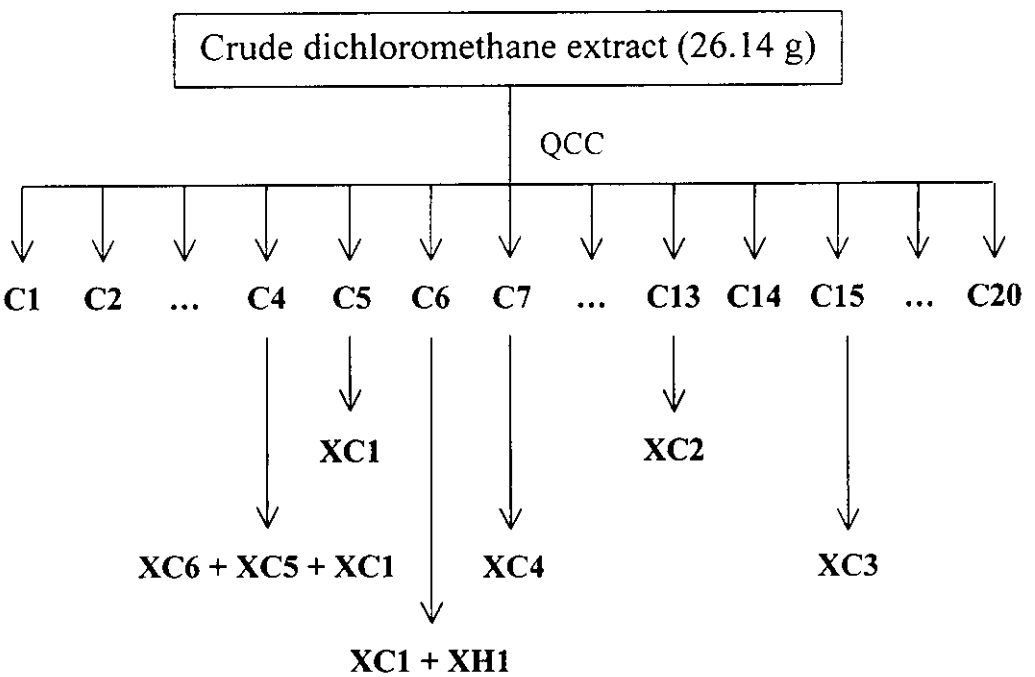
^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 49**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 49**

DEPT-135° ($CDCl_3$): see **Table 46**

2.4.2 Investigation of the crude dichloromethane extract from the seeds of X. granatum

The pale yellow viscous oil from the dichloromethane extract (26.14 g) was purified by quick column chromatography (QCC) and eluted with gradient elution of hexane, EtOAc and MeOH which afforded twenty fractions (H1-H20).



Scheme 5 Isolation of compounds **XH1**, **XC1**, **XC2**, **XC3**, **XC4**, **XC5** and **XC6**

Fraction C4, (0.6145 g) as a pale yellow viscous oil was subjected to repeated chromatography (CC) 20% acetone : hexane increasing polarity with acetone to give compound **XC6** (0.0644), **XC5** (0.0591 g) and **XC1** (0.1217 g)

Fraction C5, (0.1010 g) as a yellow viscous oil, upon crystallization from hexane : CH_2Cl_2 : EtOAc give **XC1** (0.0805 g)

Fraction C6, (0.4127 g) as a yellow viscous oil was subjected to repeated chromatography (CC) 10% EtOAc : hexane to gives **XC1** (0.0207 g) and **XH1** (0.0102 g)

Fraction C7, (1.5460 g) as a pale yellow viscous oil was subjected to repeated chromatography (CC) 10% acetone : hexane, upon crystallization from acetone : CH_2Cl_2 give **XC4** (0.0418 g)

Fraction C13, (6.9976 g) as a yellow viscous oil, upon crystallization from hexane : EtOAc give **XC2** (5.6657 g)

Fraction C15, (3.4981 g) as pale yellow viscous oil, was subjected to repeated chromatography (CC) 30% EtOAc : hexane to give compound **XC3** (2.5740 g)

Compound XC4 (Colorless solid, mp: 246-248 °C)

$[\alpha]_D^{26}$: -47 ° (c = 0.068, acetone)

IR (NaCl) ν(cm^{-1}): 3321 cm^{-1} (O-H stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 25**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 25**

DEPT-135° ($CDCl_3$): see **Table 22**

Compound XC5 (Colorless oil)

$[\alpha]_D^{26}$: -75 ° (c = 0.035, $CHCl_3$)

FABMS *m/z*: 473.2 (24) $[M+1]^+$, 313.2 (3), 185.1 (51), 131.1 (10), 93.0 (100)

IR (NaCl) ν(cm^{-1}): 3426 cm^{-1} (O-H stretching) and 1704 cm^{-1} (C=O stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): see **Table 31**

^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 31**

DEPT-135° ($CDCl_3$): see **Table 28**

Compound XC6 (white solid, mp: 138-140 °C)

IR (NaCl) ν(cm^{-1}): 3442 (O-H stretching)

^{1}H NMR ($CDCl_3$) (δppm) (300 MHz): 5.36 (2H, *d*, *J* = 5.4 Hz), 5.16 (1H, *dd*, *J* = 15.3, 8.4 Hz), 5.02 (1H, *dd*, *J* = 15.3, 8.4 Hz), 3.53 (2H, *m*), 2.35-2.15 (*m*), 2.07-1.89 (*m*), 1.89-1.74 (*m*), 1.70-1.40 (*m*), 1.35-1.02 (m), 1.01 (*s*), 0.93 (*s*), 0.91 (*s*), 0.87-0.76 (*m*), 0.70 (*s*), 0.68 (*s*)