CHAPTER 2
EXPERIMENTAL

2.1 Instruments and Chemicals

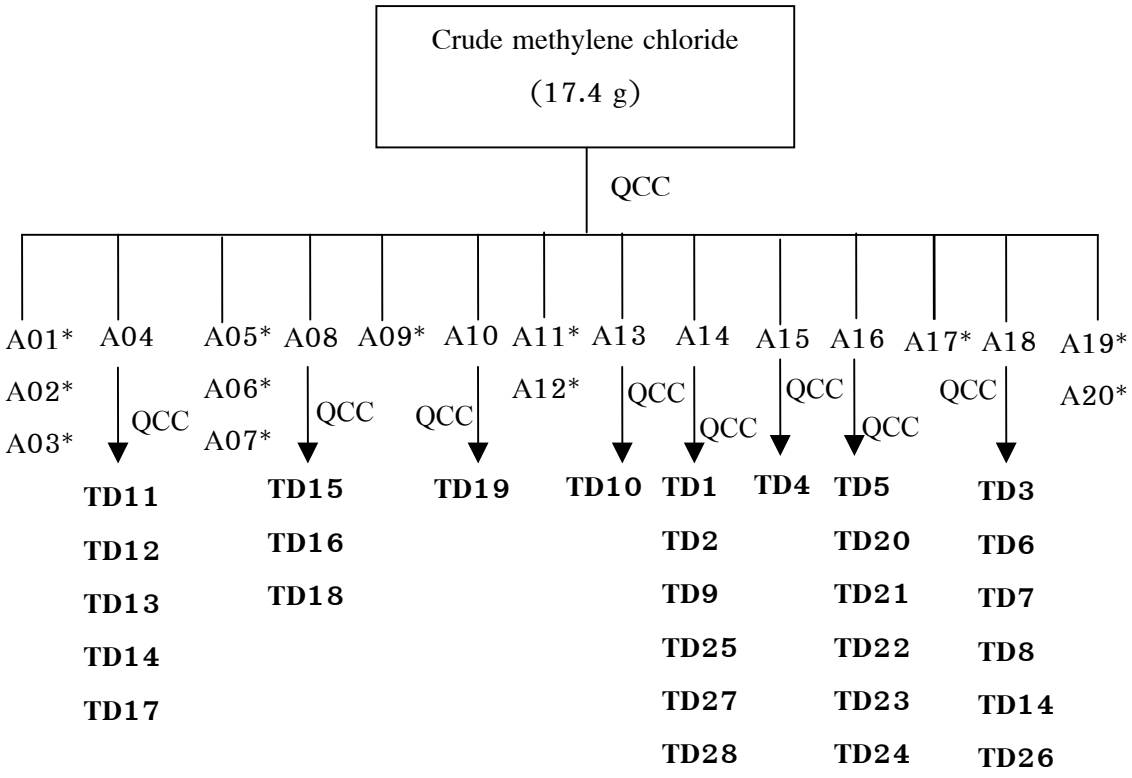
Melting points were determined on a Fisher-Johns melting point apparatus model 2572A and Electrothermal melting point apparatus model IA6301 and were uncorrected. UV spectra were measured with a Jasco polarimeter model P-1020 and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform or methanol solution. The IR spectra were measured with FTS FT-IR Perkin Elmer 2000 spectrophotometer and a Nicolet Magna IR 560 and major bands (ν) were recorded in wave number (cm^{-1}). 1D and 2D NMR spectra were recorded on a Bruker AV-300 and AV-500 spectrometer, operating at 300 and 500 MHz for proton and 75 and 125 MHz for carbon, respectively. Chemical shifts (δ) were expressed in ppm with reference to internal TMS in $CDCl_3$ and/or CD_3OD. Optical rotation $[\alpha]_D$ was measured in chloroform or methanol solution with Sodium D line (590 nm) on an AUTOPOL II and JASCO P-1020 polarimeter. The HREIMS were obtained on a MAT 95 XL mass spectrometer. The ESITOFMS were obtained on a Micromass LCT mass spectrometer. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except chloroform was analytical grade reagent. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 G (Merck). Precoated plates of silica gel 60 GF_{254} (Merck) were used for analytical purposes.

2.2 Plant material

The hypocotyls and fruits of *C. tagal* collecting at the Mangrove Research Station in Nakhon Si Thammarat province, in November 2002 (voucher specimen no. PSU 0012581) were provided by Assoc. Prof. Dr. Kan Chantrapromma. The bark of *C. tagal* were collected at Yaring Mangrove in Pattani province, in May 2005 (voucher specimen no. PSU 0012820). The plants were kindly identified by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University.

2.3 Extraction and isolation

The air-dried and crushed stem bark of *C. tagal* (4.8 kg) were extracted with methylene chloride and concentrated in vacuo to give residue (17.4 g) which was subjected to quick column chromatography over silica gel using solvents of increasing polarity from hexane through 50% acetone/hexane. The eluates were collected and combined based on TLC to give twenty fractions (A01-A20).



* Not further investigated

Scheme 7 Isolation of compounds **TD1-TD28**

Fraction A04 (1.22 g) was subjected to quick column chromatography using hexane-acetone (9.5:0.5) as eluting solvent to afford **TD17**: *ent*-5α-dolabr-4(18),15-diene-3α-ol (1.2 mg), **TD14**: tagalsin E (4.1 mg), **TD13**: tagalsin F (50.2 mg) after crystallization from hexane/CH_2Cl_2, **TD11**: tagalsin G (25.4 mg) and **TD12**: tagalsin C (20.0 mg).

Fraction A08 (1.01 g) was subjected to quick column chromatography using hexane–acetone mixtures with increasing polarity as eluting solvent (9.4:0.6–8.8:1.2) to afford **TD15**: tagalsin A (30.1 mg), **D16**: tagalsin B (38.2 mg) and **TD18**: tagalsin D (30.3 mg).

Fraction A10 (0.20 g) was subjected to quick column chromatography using hexane–acetone (8.8:2.0) as eluting solvent to afford **TD19**: tagalsin H (6.1 mg).

Fraction A13 (0.12 g) was subjected to quick column chromatography using hexane–acetone (7:3) as eluting solvent to afford **TD10**: *ent*-5α,18-oxodolabr-3,15-diene-2β-ol (4.2 mg).

Fraction A14 (1.75 g) was subjected to quick column chromatography using CH_2Cl_2–acetone (9:1) as eluting solvent to afford **TD1**: *ent*-5α,3,15-dioxodolabr-4(18)-ene-16,18-diol (30.4 mg) after crystallization from hexane/CH_2Cl_2, **TD2**: *ent*-5α,3,15-dioxodolabr-4(18)-ene-16-ol (33.2 mg), **TD9**: *ent*-5α-dolabr-4(18)-ene-15,16-diol (6.0 mg), **TD25**: 15*S*-isoprima-8(14)-15,16-diol (30.1 mg), **TD27**: *ent*-kauran-16α,17-diol (8.3 mg) after crystallization from acetone and **TD28**: *ent*-kauran-16β,17-diol (4.6 mg).

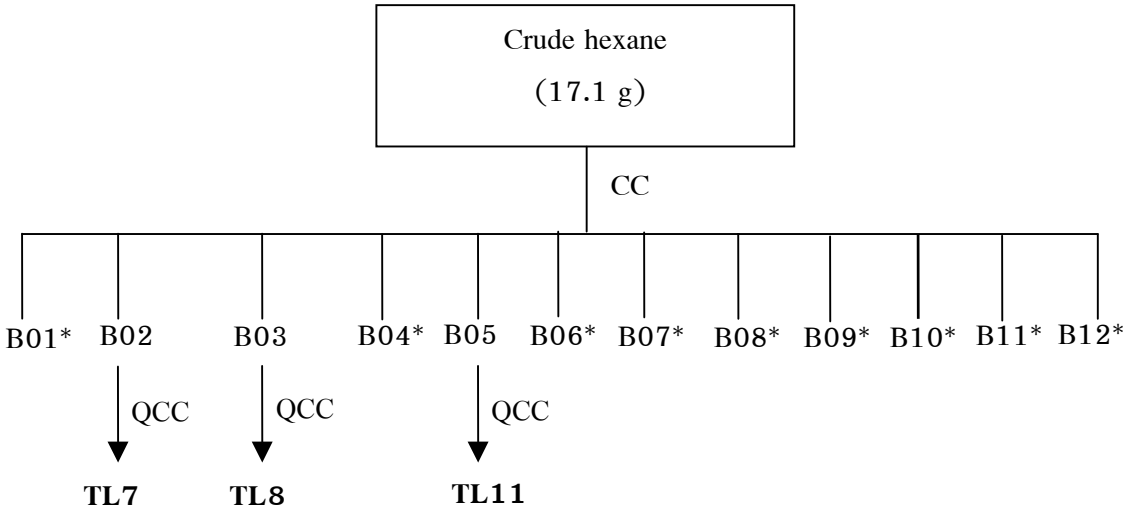
Fraction A15 (0.25 g) was subjected to quick column chromatography using CH_2Cl_2–acetone (8.5:1.5) as eluting solvent to afford **TD4**: *ent*-5α,3,15-dioxodolabr-1,4(18)-diene-2,16-diol (40.8 mg).

Fraction A16 (1.72 g) was subjected to quick column chromatography using CH_2Cl_2–acetone (8.5:1.5) as eluting solvent to afford **TD5**: *ent*-5α,2,15-dioxodolabr-3-ene-3,16-diol (16.5 mg) after crystallization from hexane/CH_2Cl_2, **TD20**: *ent*-5α,3-oxo-15,16-nordolabr-1,4(18)-diene-13-ol (15.0 mg), **TD22**: *ent*-5α,18β,3-oxo-15,16-nordolabr-4,18-epoxy-1-ene-13-ol (1.2 mg), **TD21**: *ent*-5α,18α,3-oxo-15,16-nordolabr-4,18-epoxy-1-ene-13-ol (3.3 mg), **TD23**: *ent*-5α,18β,3-oxo-15,16-nordolabr-1-ene-13-ol (15.0 mg) and **TD24**: *ent*-5α,3-oxo-15,16-nordolabr-4(18)-ene-13,18-diol (1.2 mg).

Fraction A18 (1.60 g) was subjected to quick column chromatography using CH_2Cl_2–acetone (8:2) as eluting solvent to afford **TD7**: *ent*-5α,15*S*,3-oxobolabr-1,4(18)-diene-2,15,16-triol (4.1 mg), **TD3**: *ent*-5α,18β,3,15-dioxodolabr-4,18-epoxy-1-ene-2,16-diol (5.0 mg), **TD6**: *ent*-5α,15*S*,3-oxodolabr-4(18)-ene-2,15,16-triol (4.6 mg), **TD8**: *ent*-5α,15*S*,2-oxodolabr-3-ene-3,15,16-triol (10.2

mg) and **TD26**: isoprima-8(14)-ene-15,16,19-triol (5.1 mg) after crystallization from acetone.

Dried milled hypocotyls of *C. tagal* (5.3 kg) were extracted with hexane and CH_2Cl_2, successively. Evaporation resulted in the crude extracts of hexane (32.9 g) and CH_2Cl_2 (128.6 g), respectively. A portion of the hexane extract (17.1 g) was subjected to column chromatography using gradient elution of hexane and ethyl acetate (10:0-7:3) to afford twelve fractions (B01-B12).



* Not further investigated

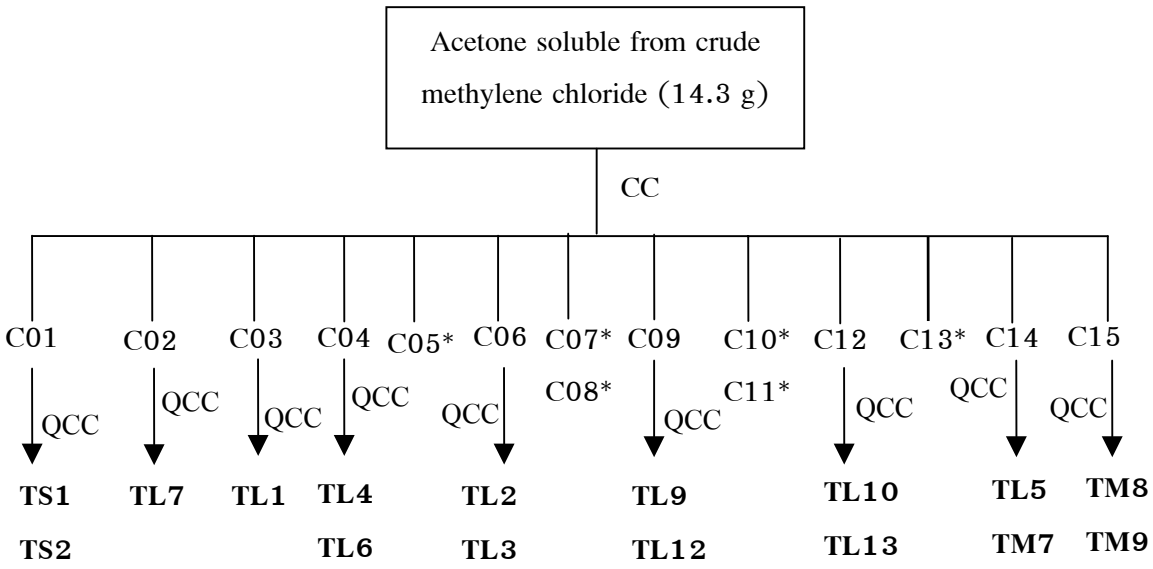
Scheme 8 Isolation of compounds **TL7**-**TL8** and **TL11**

Fraction B02 (1.43 g) was subjected to quick column chromatography using hexane and ethyl acetate mixtures with increasing polarity as eluting solvent (10:0-9:1) to afford **TL7**: 3β-*E*-feruloyllupeol (160.8 mg) after crystallization from acetone.

Fraction B03 (0.15 g) was subjected to quick column chromatography using hexane-ethyl acetate (9.5:0.5) as eluting solvent to afford **TL8**: 3β-*Z*-feruloyllupeol (12.2 mg).

Fraction B05 (0.52 g) was subjected to quick column chromatography using hexane-ethyl acetate (9:1) as eluting solvent to give **TL11**: 3β-acetylbetulinic acid (38.4 mg) after crystallization from acetone.

A portion of gummy residue from the CH_2Cl_2 extract (25.0 g) was treated with acetone to give acetone-soluble and -insoluble fractions. The acetone-soluble fraction was concentrated to afford gummy residue (14.3 g) which was subjected to column chromatography. The column was eluted with gradient elution of hexane and ethyl acetate (10:0-7:3). The eluates were combined on the basis of TLC to give fifteen fractions (C01-C15).



* Not further investigated

Scheme 9 Isolation of compounds **TS1-TS2, TL1-TL7, TL9-TL10, TL12-TL13** and **TM7-TM9**

A mixture of **TS1**:β-sitosterol and **TS2**: stigmasterol were obtained (50 mg) by crystallization from acetone from fraction C01 (0.21 g).

TL7: 3β-*E*-feruloyllupeol (40.4 mg) was isolated from fraction C02 (0.12 g) by crystallization from acetone.

Fraction C03 (0.22 g) was subjected to quick column chromatography using hexane-ethyl acetate (9:1) as eluting solvent to afford **TL1**: lupeol (14.6 mg)

Fraction C04 (0.85 g) was subjected to quick column chromatography using hexane–ethyl acetate (9:1) as eluting solvent to afford **TL6**: 3β-*E*-coumaroyllupeol (5.1 mg) and **TL4**: 3-*epi*-betulinic acid (15.2 mg).

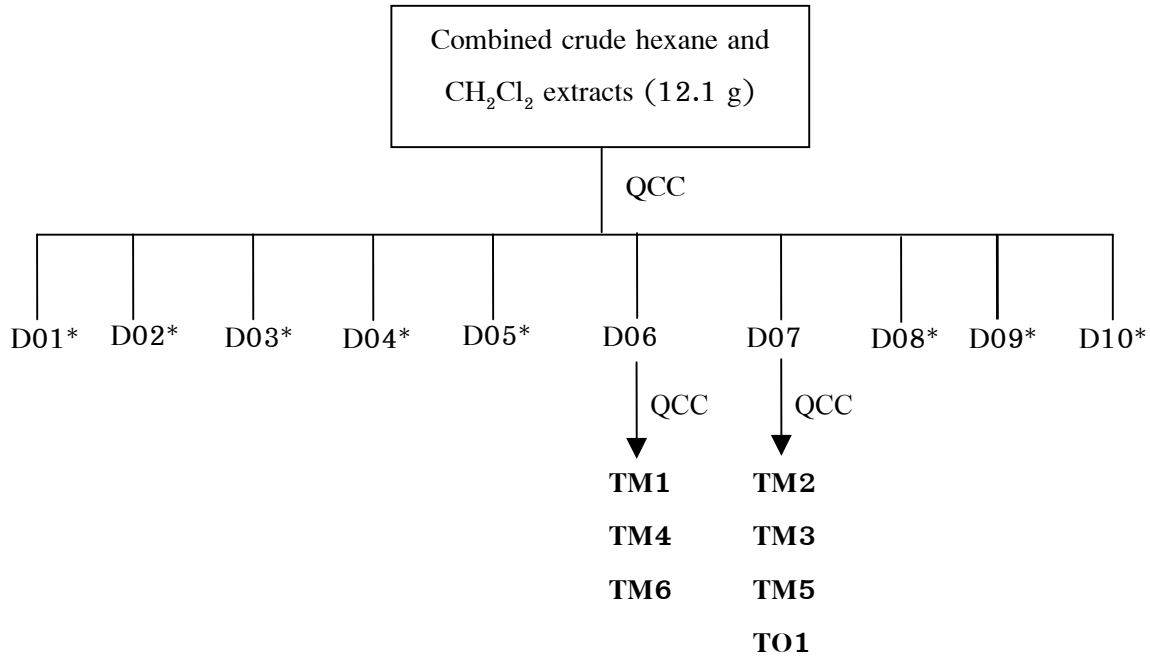
Fraction C06 (0.09 g) was subjected to quick column chromatography using hexane–ethyl acetate (8:2) as eluting solvent to afford **TL2**: betulin (7.2 mg) and **TL3**: betulinic acid (9.3 mg).

Fraction C09 (0.19 g) was subjected to quick column chromatography using hexane–ethyl acetate (8:2) as eluting solvent to afford **TL12**: 3β-*E*-feruloylbetulinic acid (97.5 mg) and **TL9**: 3β-*E*-feruloylbetulin (64.3 mg).

Fraction C12 (0.32 g) was subjected to quick column chromatography using hexane–ethyl acetate (9:1–8:2) as eluting solvent to afford **TL13**: 3β-*E*-caffeoylbetulinic acid (8.1 mg) and **TL10**: 3β-*E*-caffeoylbetulin (7.2 mg).

Fraction C14 (0.53 g) was subjected to quick column chromatography using hexane–acetone (8:2) as eluting solvent to afford **TL5**: betulonic acid (25.2 mg) and **TM7**: 20(*S*)-3β,20,25,28-tetrahydroxydammar-23-ene (5.0 mg).

Fraction C15 (0.35 g) was subjected to quick column chromatography using hexane–acetone (8:2) as eluting solvent to afford **TM8**: 20(*S*)-3β,20,28-trihydroxydammar-24-ene (11.2 mg) and **TM9**: 20(*S*)-3β,20,24,28-tetrahydroxydammar-25-ene (4.1 mg).



* Not further investigated

Scheme 10 Isolation of compounds **TM1**–**TM6** and **TO1**

Dried milled fruits of *C. tagal* (574.0 g) were extracted with hexane and CH_2Cl_2, successively. Evaporation resulted in the crude hexane (6.0 g) and CH_2Cl_2 (6.1 g) extracts. The combined crude hexane and CH_2Cl_2 extracts (12.1 g) was subjected to quick column chromatography using gradient elution of hexane and ethyl acetate (10:0–7:3) to afford ten fractions (D01–D10) on the basis of TLC analysis.

Fraction D06 (0.44 g) was subjected to quick column chromatography using hexane–ethyl acetate (9:1) as eluting solvent, to afford **TM1**: dammarenediol II (31.0 mg) and **TM4**: 20(*S*)-3β,20-dihydroxy-24-perhydroxydammar-25-ene (40.0 mg), the latter after crystallization from acetone and **TM6**: 20(S)-3β, 20-dihydroxydammar-23, 25-diene (5.0 mg).

Fraction D07 (0.72 g) was subjected to quick column chromatography using gradient elution of hexane and acetone mixture with increasing polarity (9:1–8:2) to afford **TO1**: oleanolic acid (30.4 mg), **TM5**: ocotillol II (6.1 mg), **TM3**: fouquierol (12.4 mg), and **TM2**: isofouquierol (6.2 mg).

2.4 Physical properties of isolates

Compound ***TD1,*** *ent*-5α,3,15-dioxodolabr-4(18)-ene-16,18-diol: colorless plate crystals from methylene chloride/hexane, mp: 122-123°C; $[\alpha]_D^{27}$: -24.0° (*c* = 1.96, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 243 (2.44), 295 (3.38); IR (neat) ν_{max} (cm^{-1}) 3453 (O-H stretching), 1697 (C=O stretching), 1622 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 1**; HREIMS *m/z* $[M]^+$ 334.2159 (calcd for $C_{20}H_{30}O_4$, 334.2144).

Compound ***TD2,*** *ent*-5α,3,15-dioxodolabr-4(18)-ene-16-ol: colorless oil; $[\alpha]_D^{27}$: +2.0° (*c* = 2.58, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 234 (3.29), 294 (2.85); IR (neat) ν_{max} (cm^{-1}) 3462 (O-H stretching), 1697, 1691 (C=O stretching), 1604 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 2**; HREIMS *m/z* $[M]^+$ 318.2154 (calcd for $C_{20}H_{30}O_3$, 318.2195).

Compound ***TD3,*** fouquierol: colorless oil; $[\alpha]_D^{27}$ +109.0° (*c* = 0.98, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 285 (3.83); IR (neat) ν_{max} (cm^{-1}) 3440 (O-H stretching), 1697 (C=O stretching), 1680 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 3**; HREIMS *m/z* $[M]^+$ 348.1983 (calcd for $C_{20}H_{28}O_5$, 348.1937).

Compound ***TD4,*** *ent*-5α,3,15-dioxodolabr-1,4(18)-diene-2,16-diol: colorless oil; $[\alpha]_D^{27}$ +235.4° (*c* = 4.58, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 310 (3.64); IR (neat) ν_{max} (cm^{-1}) 3430 (O-H stretching), 1710 (C=O stretching), 1684 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 4.**

Compound ***TD5,*** *ent*-5α,2,15-dioxodolabr-3-ene-3,16-diol: colorless plate crystals ($CHCl_3$), mp 153-154°C, $[\alpha]_D^{27}$: +66.4° (*c* = 2.29, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 284 (1.10); IR (neat) ν_{max} (cm^{-1}) 3420 (O-H stretching), 1705 (C=O stretching), 1660 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 5.**

Compound ***TD6,*** *ent*-5α,15*S*,3-oxodolabr-4(18)-ene-2,15,16-triol: colorless oil; $[\alpha]_D^{27}$ -31.1° (*c* = 1.15, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 294 (4.21); IR (neat) ν_{max} (cm^{-1}) 3402 (O-H stretching), 1697 (C=O stretching), 1616 (C=C stretching); ^1H

NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 6**; HREIMS *m/z* $[M]^+$ 336.2341 (calcd for $C_{20}H_{32}O_4$, 336.2301).

Compound ***TD7,*** *ent*-5α,15*S*,3-oxobolabr-1,4(18)-diene-2,15,16-triol: colorless oil; $[\alpha]_D^{27}$ +120.0° (*c* = 0.33, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 306 (3.58); IR (neat) ν_{max} (cm^{-1}) 3412 (O-H stretching), 1711 (C=O stretching), 1602 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 7**; HREIMS *m/z* $[M]^+$ 334.2133 (calcd for $C_{20}H_{30}O_4$, 334.2144).

Compound ***TD8,*** *ent*-5α,15*S*,2-oxodolabr-3-ene-3,15,16-triol: white solid, mp: 126-128°C; $[\alpha]_D^{27}$: -17.8° (*c* = 1.33, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 285 (1.20); IR (neat) ν_{max} (cm^{-1}) 3435 (O-H stretching), 1710 (C=O stretching), 1600 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 8.**

Compound ***TD9,*** *ent*-5α-dolabr-4(18)-ene-15,16-diol: colorless oil; $[\alpha]_D^{27}$ +69.3° (*c* = 0.50, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 246 (3.27), 282 (2.93); IR (neat) ν_{max} (cm^{-1}) 3416 (O-H stretching), 1642 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 9.**

Compound ***TD10,*** *ent*-5α,18-oxodolabr-3,15-diene-2β-ol: colorless oil; $[\alpha]_D^{27}$ +37.0° (*c* = 0.16, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 234 (3.56); IR (neat) ν_{max} (cm^{-1}) 3406 (O-H stretching), 1690 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 10**; HREIMS *m/z* $[M]^+$ 302.2285 (calcd for $C_{20}H_{30}O_2$, 302.2246).

Compound ***TD11,*** tagalsin G: white solid, mp: 90-91°C; $[\alpha]_D^{27}$: -17.6° (*c* = 2.38, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 284 (1.27); IR (neat) ν_{max} (cm^{-1}) 3433 (O-H stretching), 1714 (C=O stretching), 1658 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 11.**

Compound ***TD12,*** tagalsin C,: colorless oil; $[\alpha]_D^{27}$ +92.3° (*c* = 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 312 (3.24); IR (neat) ν_{max} (cm^{-1}) 3426 (O-H stretching), 1716 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 12.**

Compound ***TD13,*** tagalsin F: white solid, mp: 101-102°C; $[\alpha]_D^{27}$: -37.8° (*c* = 2.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 295 (3.15); IR (neat) ν_{max} (cm^{-1}) 3450 (O-H stretching), 1693 (C=O stretching), 1624 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 13.**

Compound ***TD14,*** tagalsin E: colorless oil; $[\alpha]_D^{27}$: -8.4° (*c* = 1.00, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 236 (3.18), 290 (2.66); IR (neat) ν_{max} (cm^{-1}) 3458 (O-H stretching), 1701 (C=O stretching), 1640 (C=C stretching), 1623 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 14.**

Compound ***TD15,*** tagalsin A: white solid, mp: 68-69°C; $[\alpha]_D^{27}$: +120.0° (*c* = 0.75, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 288 (4.12); IR (neat) ν_{max} (cm^{-1}) 3416 (O-H stretching), 1668 (C=O stretching), 1651 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 15.**

Compound ***TD16,*** tagalsin B: white solid, mp: 83-84°C; $[\alpha]_D^{27}$: +165.0° (*c* = 2.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 286 (3.84); IR (neat) ν_{max} (cm^{-1}) 3438 (O-H stretching), 1692 (C=O stretching), 1638 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 16.**

Compound ***TD17,*** *ent*-5α-dolabr-4(18),15-diene-3α-ol: colorless oil; $[\alpha]_D^{27}$ +40.8° (*c* = 0.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 241 (3.24), 293 (2.97); IR (neat) ν_{max} (cm^{-1}) 3402 (O-H stretching), 1635 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 17.**

Compound ***TD18,*** tagalsin D: white solid, mp: 87-88°C; $[\alpha]_D^{27}$: +38.5° (*c* = 2.64, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 243 (2.56), 273 (2.65); IR (neat) ν_{max} (cm^{-1}) 3417 (O-H stretching), 1635 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 18.**

Compound ***TD19,*** tagalsin H: white solid, mp: 87-88°C; $[\alpha]_D^{27}$: -4.5° (*c* = 1.50, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 240 (2.53); IR (neat) ν_{max} (cm^{-1}) 1701 (C=O stretching), 1636 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 19.**

Compound ***TD20,*** *ent*-5α,3-oxo-15,16-nordolabr-1,4(18)-diene-13-ol: white solid, mp: 152-154°C; $[\alpha]_D^{27}$ +235.0° (*c* = 0.33, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 238 (3.60); IR (neat) ν_{max} (cm^{-1}) 3406 (O-H stretching), 1667 (C=O stretching), 1596 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 20**; HREIMS *m/z* $[M]^+$ 274.1954 (calcd for $C_{18}H_{26}O_2$, 274.1933).

Compound ***TD21,*** *ent*-5α,18α,3-oxo-15,16-nordolabr-4,18-epoxy-1-ene-13-ol: white solid, mp: 174-175°C; $[\alpha]_D^{27}$ +130.2° (*c* = 0.18, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 245 (3.51); IR (neat) ν_{max} (cm^{-1}) 3350 (O-H stretching), 1671 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (500 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 21**; HREIMS *m/z* $[M]^+$ 290.1846 (calcd for $C_{18}H_{26}O_3$, 290.1882).

Compound ***TD22,*** *ent*-5α,18β,3-oxo-15,16-nordolabr-4,18-epoxy-1-ene-13-ol: white solid, mp: 180-181°C; $[\alpha]_D^{27}$ +104.7° (*c* = 0.75, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 244 (3.42); IR (neat) ν_{max} (cm^{-1}) 3437 (O-H stretching), 1678 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δppm) (75 MHz): see **Table 22**; HREIMS *m/z* $[M]^+$ 290.1858 (calcd for $C_{18}H_{26}O_3$, 290.1882).

Compound ***TD23,*** *ent*-5α,18β,3-oxo-15,16-nordolabr-1-ene-13-ol: white solid, mp 164-165°C; $[\alpha]_D^{27}$ +100.7° (c = 1.48, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 243 (2.70); IR (neat) ν_{max} (cm^{-1}) 3436 (O-H stretching), 1682 (C=O stretching) ; ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 23**; HREIMS *m/z* $[M]^+$ 276. (calcd for $C_{18}H_{26}O_3$, 276.2089).

Compound ***TD24,*** *ent*-5α,3-oxo-15,16-nordolabr-4(18)-ene-13,18-diol: white solid, mp: 147-148°C; $[\alpha]_D^{27}$ -56.3° (*c* = 0.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 252 (3.46), 294 (3.66); IR (neat) ν_{max} (cm^{-1}) 3450 (O-H stretching), 1680 (C=O stretching); ^1H NMR ($CDCl_3$) (δ ppm) (500 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 24**; HREIMS *m/z* $[M]^+$ 292.2051 (calcd for $C_{18}H_{26}O_3$, 2922038).

Compound ***TD25,*** *ent*-15*S*-isoprima-8(14)-15,16-diol: colorless plate crystals from acetone, mp: 104-105°C; $[\alpha]_D^{27}$: -17.7° (*c* = 1.93, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 246 (2.59); IR (neat) ν_{max} (cm^{-1}) 3386 (O-H stretching), 1656 (C=C stretching);

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 25**; HREIMS *m/z* $[M]^+$ 306.2573 (calcd for $C_{20}H_{34}O_2$, 306.2559).

Compound ***TD26,*** *ent*-15*S*-isoprima-8(14)-ene-15,16,19-triol: white solid, mp: 119-120°C; $[\alpha]_D^{27}$ -17.8° (*c* = 1.33, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 244 (2.56); IR (neat) ν_{max} (cm^{-1}) 3420 (O-H stretching), 1663 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 26.**

Compound ***TD27,*** *ent*-kauran-16α,17-diol: white solid,mp: 174-175°C; $[\alpha]_D^{27}$ -9.2° (*c* = 2.25, $CHCl_3$); IR (neat) ν_{max} (cm^{-1}) 3387 (O-H stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 27.**

Compound ***TD28,*** *ent*-kauran-16β,17-diol: colorless plate crystals from acetone, mp: 134-135°C; $[\alpha]_D^{27}$ -37.5° (*c* = 0.30, $CHCl_3$); IR (neat) ν_{max} (cm^{-1}) 3402 (O-H stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 28.**

Compound ***TL1,*** lupeol: white solid, mp: 193-194°C; $[\alpha]_D^{28}$: +25.0° (*c* = 0.20, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3343 (O-H stretching), 2945 (C-H stretching), 1638 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 29.**

Compound ***TL2,*** betulin: white solid, mp: 230-231°C; $[\alpha]_D^{28}$: +16.7° (*c* = 0.15, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3382 (O-H stretching), 2942 (C-H stretching), 1645 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 30.**

Compound ***TL3,*** betulinic acid: white solid, mp: 279-280°C; $[\alpha]_D^{28}$: +15.0° (*c* = 0.10, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3415 (O-H stretching), 2942 (C-H stretching), 1686 (C=O stretching), 1645 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 31.**

Compound ***TL4,*** 3-*epi*-betulinic acid: white solid, mp: 257-259°C, $[\alpha]_D^{28}$: -10.0° (*c* = 0.05, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3436 (O-H stretching), 2947 (C-H stretching), 1704 (C=O stretching), 1643 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 32.**

Compound ***TL5,*** betulonic acid: white solid, mp: 250-254°C; $[\alpha]_D^{28}$: +32.0° (*c* = 0.37, MeOH); IR (KBr) ν_{max} (cm^{-1}): 2914 (C-H stretching), 1704 (C=O stretching), 1642 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 33.**

Compound ***TL6,*** 3β-*E*-coumaroyllupeol: white solid, mp: 166-167°C; $[\alpha]_D^{28}$: +20.0° (*c* = 0.05, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 227 (4.10), 313 (4.38); IR (KBr) ν_{max} (cm^{-1}): 3397 (O-H stretching), 1726 (C=O stretching), 1602 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 34.**

Compound ***TL7,*** 3β-*E*-feruloyllupeol: white solid, mp: 167-169°C; $[\alpha]_D^{27}$: +140.0° (*c* = 0.03, MeOH); UV (MeOH) λ_{max} (nm) (log ε) : 234 (4.02), 298 (4.06), 325 (4.20); IR (KBr) ν_{max} (cm^{-1}): 3534 (O-H stretching), 1703 (C=O stretching), 1635, 1604 (C=C stretching); ESITOFMS ($[M-H]^-$) m/z 601.4244 (calcd. For $C_{40}H_{57}O_4$: 601.4256); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 35.**

Compound ***TL8,*** 3β-*Z*-feruloyllupeol: white solid, mp: 195-197°C; $[\alpha]_D^{27}$: +41.7° (*c* = 0.06, MeOH), UV (MeOH) λ_{max} (nm) (log ε) : 235 (3.57), 296 (3.56), 325 (3.71); IR (KBr) ν_{max} (cm^{-1}): 3538 (O-H stretching), 1708 (C=O stretching), 1595 (C=C stretching); ESITOFMS ($[M-H]^-$) m/z 601.4260 (calcd. For $C_{40}H_{57}O_4$: 601.4260); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 36.**

Compound ***TL9***, 3β-*E*-feruloylbetulin: white solid, mp: 152-154°C; $[\alpha]_D^{28}$: +16.2° (*c* = 0.40, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 325 (4.50), 297 (4.41), 234 (4.53), 215 (4.63); IR (neat) ν_{max} (cm^{-1}): 3360 (O-H stretching), 1685 (C=O stretching), 1590 (C=C stretching); ^1H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 37.**

Compound ***TL10,*** 3β-*E*-caffeoylbetulin: white solid, mp: 160-163°C; $[\alpha]_D^{28}$: +47.0° (*c* = 1.00, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 318 (4.21), 285 (4.10), 231 (4.03); IR (neat) ν_{max} (cm^{-1}): 3413 (O-H stretching), 1726 (C=O stretching), 1605

(C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 38.**

Compound ***TL11,*** 3β-acetylbetulinic acid: white solid, mp: 269-271°C; $[\alpha]_D^{28}$: +8.0° (*c* = 0.05, MeOH); IR (neat) ν_{max} (cm^{-1}): 1740 (C=O stretching), 1704 (C=O stretching), 1637 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 39.**

Compound ***TL12,*** 3β-*E*-feruloylbetulinic acid: white solid, mp: 224-225°C; $[\alpha]_D^{28}$: +7.8° (*c* = 0.76, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 325 (4.11), 299 (4.23); IR (neat) ν_{max} (cm^{-1}): 3650 (O-H stretching), 1700 (C=O stretching), 1604 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 40.**

Compound ***TL13,*** 3β-*E*-caffeoylbetulinic acid: white solid, mp: 254-256°C; $[\alpha]_D^{28}$: +10.6° (*c* = 0.05, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 327 (4.10), 301 (4.00), 327 (4.11); IR (neat) ν_{max} (cm^{-1}): 3426 (O-H stretching), 1723 (C=O stretching), 1607 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 41.**

Compound ***TS1***, β-sitosterol and ***TS2,*** stigmasterol: white solid, mp: 131-132°C; IR (neat) ν_{max} (cm^{-1}): 3425 (O-H stretching), 1642 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) see **Figure 93.**

Compound ***TM1,*** dammarenediol II: colorless oil; $[\alpha]_D^{28}$: +31.8° (*c* = 0.30, MeOH); IR (neat) ν_{max} (cm^{-1}): 3440 (O-H stretching), 1642 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 42.**

Compound ***TM2,*** isofouquierol: white solid, mp: 128-129°C; $[\alpha]_D^{28}$: +24.0° (*c* = 0.20, MeOH); IR (neat) ν_{max} (cm^{-1}): 3404 (O-H stretching), 1643 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 43.**

Compound ***TM3,*** fouquierol: white solid, mp: 156-158°C; $[\alpha]_D^{28}$: +38.4° (*c* = 0.10, MeOH); IR (neat) ν_{max} (cm^{-1}): 3414 (O-H stretching), 1610 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 44.**

Compound ***TM4,*** 20(*S*)-3β,20-dihydroxy-24-perhydroxydammar-25-ene: white solid, mp: 183-185°C; $[\alpha]_D^{28}$: +54.1° (*c* = 0.04, MeOH); IR (neat) ν_{max} (cm^{-1}): 3450 (O-H stretching), 1665 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 45**; ESITOFMS m/z 499.3754 (calcd for $C_{30}H_{52}O_4$+Na, 499.3763).

Compound ***TM5,*** ocotillol II: white solid, mp: 205-207°C; $[\alpha]_D^{28}$: +19.3° (*c* = 0.05, MeOH); IR (neat) ν_{max} (cm^{-1}): 3402 (O-H stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 46.**

Compound ***TM6,*** 20(S)-3β, 20-dihydroxydammar-23, 25-diene: colorless oil; $[\alpha]_D^{28}$: +62.5° (*c* = 0.03, MeOH); IR (neat) ν_{max} (cm^{-1}): 3430 (O-H stretching), 1630 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 47.**

Compound ***TM7,*** 20(*S*)-3β,20,25,28-tetrahydroxydammar-23-ene: colorless oil; $[\alpha]_D^{28}$: +55.6° (*c* = 0.02, MeOH); IR (neat) ν_{max} (cm^{-1}): 3480 (O-H stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 48**; ESITOFMS m/z 499.3754 (calcd for $C_{30}H_{52}O_4$+Na, 499.3763).

Compound ***TM8,*** 20(*S*)-3β,20,28-trihydroxydammar-24-ene: colorless oil; $[\alpha]_D^{28}$: +50.0° (*c* = 0.02, MeOH); IR (neat) ν_{max} (cm^{-1}): 3390 (O-H stretching), 1655 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 49.**

Compound ***TM9,*** 20(*S*)-3β,20,24,28-tetrahydroxydammar-25-ene: colorless oil; $[\alpha]_D^{28}$: +52.6° (*c* = 0.02, MeOH); IR (neat) ν_{max} (cm^{-1}): 3390 (O-H stretching), 1660 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 50**; ESITOFMS m/z 499.3767 (calcd for $C_{30}H_{52}O_4$+Na, 499.3763).

Compound ***TO1,*** oleanolic acid: white solid, mp: 275-276°C; $[\alpha]_D^{28}$: +82.0° (*c* = 0.10, MeOH); IR (neat) ν_{max} (cm^{-1}): 3456 (O-H stretching), 1690 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz): see **Table 51.**

2.5 Bioassay

Cytotoxicity; Oral human epidermal carcinoma (KB), breast cancer (BC) and Human, small cell lung cancer (NCI-H187).

Antimalarial assay; *Plasmodium falciparum*, K1 strain.

Cytotoxicity

The cytotoxic assay employed the colorimetric method reported by Skehan et al. (1990). KB (human epidermoid carcinoma of cavity, ATCC CCL-17) and BC (breast cancer cell line) were determined by colorimetric cytotoxicity assay that measured cell growth from cellular protein content according to Skehan et al. (1990). Elliptine was used as positive control. DMSO (10%) was used as negative control. Briefly, cells at a logarithimic growth phase were harvested and diluted to 10^5 cells/mL with fresh medium and gently mixed. Testing compound was dissolved in DMSO (concentration at 20 mg/mL), and this solution was then diluted with distilled water to obtain a stock solution at 0.4 mg/mL (with 10% DMSO). The stock solution (10 μL) and cell suspension (190 μ L) were transferred into microtiter plates (concentration at 20 μg/mL with 0.05% DMSO). If the compound is active at 20 μg/mL, a series of solutions were prepared by twofold dilution of the stock solution (diluted with 10% DMSO solution), and exposed to cells as mentioned above, in order to obtain IC_{50} value. Plates were incubated at 37°C under 5% CO_2 atmosphere for 72 h. After incubation period, cells were fixed by 50% trichloroacetic acid. The plates were incubated at 4°C for 30 min, washed with water, and air-dried at room temperature. The plates were stained with 0.05% sulforhodamine B (SRB) dissolved in 1% acetic for 30 min. After staining period, SRB was removed with 1% acetic acid. Plated were air-dried before bound dye was solubilized with 10 mM Tris base for 5 min on shaker. Optical density was read in a microtiter plate reader at wavelength 510 nm. Ellipticine, the reference substance, exhibited activity toward BC and KB cell lines, both with the IC_{50} of 0.3 μg/mL.

Antimalarial activity

Plasmodium falciparum (K1, multi drug resistant strain) was cultivated in *vitro* according to Trager and Jensen, 1976 in RPMI 1640 medium containing 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 32 mM $NaHCO_3$ and

10% heat activated human serum with 3% erythrocytes and incubated at 37 ^{o}C in an incubator with 3% CO_2. Cultures were diluted with fresh medium and erythrocytes every day according to cell growth. Quantitative assessment of antimalarial activity in *vitro* was determined by microculture radioisotope techniques based upon the methods described by Desjardins et al., 1979. Briefly, a mixture of 200 μL of 1.5% erythrocytes with 1% parasitemia at early ring stage was pre-exposed to 25 μL of the medium containing a test sample dissolved in 1% DMSO (0.1% final concentration) for 24 hr employing the incubation conditions described above. Subsequently, 25 μL of [^{3}H] hypoxanthine (Amersham, USA) in culture medium (10 μCi) was added to each well and plates were incubated for an additional 24 hr. Levels of incorporated radioactively labeled hypoxanthine indicating parasite growth were determined using the TopCount microplate scintillation counter (Packard, USA). Inhibition concentration (IC_{50}) represents the concentration which indicates 50% reduction in parasite growth. The positive control compound was artemisinin (IC_{50} 3.3-3.9 nM).