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 ($\lambda_{\text{max}}$) 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$_3$OD. 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).

![Diagram of fractionation process](image)

* 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-dolab–4 (18),15–dien–3α–ol (1.2 mg), **TD14**: tagalsin E (4.1 mg), **TD13**: tagalsin F (50.2 mg) after crystallization from hexane/CH₂Cl₂, **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 CH2Cl2-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/CH2Cl2, 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: 15S-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 CH2Cl2-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 CH2Cl2-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/CH2Cl2, 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 CH2Cl2-acetone (8:2) as eluting solvent to afford TD7: ent-5α,15S,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,15S,3-oxodolabr-4(18)-ene-2,15,16-triol (4.6 mg), TD8: ent-5α,15S,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\textsubscript{2}Cl\textsubscript{2}, successively. Evaporation resulted in the crude extracts of hexane (32.9 g) and CH\textsubscript{2}Cl\textsubscript{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).

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.

* Not further investigated

**Scheme 8** Isolation of compounds TL7–TL8 and TL11
A portion of gummy residue from the CH₂Cl₂ 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).
Dried milled fruits of *C. tagal* (574.0 g) were extracted with hexane and CH$_2$Cl$_2$, successively. Evaporation resulted in the crude hexane (6.0 g) and CH$_2$Cl$_2$ (6.1 g) extracts. The combined crude hexane and CH$_2$Cl$_2$ 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$_3$) $\lambda_{\text{max}}$ nm (log ε): 243 (2.44), 295 (3.38); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3453 (O–H stretching), 1697 (C=O stretching), 1622 (C=C stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log ε): 234 (3.29), 294 (2.85); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3462 (O–H stretching), 1697, 1691 (C=O stretching), 1604 (C=C stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log ε): 285 (3.83); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3440 (O–H stretching), 1697 (C=O stretching), 1680 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log ε): 310 (3.64); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3430 (O–H stretching), 1710 (C=O stretching), 1684 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log ε): 284 (1.10); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3420 (O–H stretching), 1710 (C=O stretching), 1660 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ ppm) (75 MHz): see Table 5.

*Compound TD6*, ent-5α,15S,3-oxodolabr-4(18)-ene-2,15,16-triol: colorless oil; $[\alpha]_{D}^{27}$: -31.1° ($c = 1.15$, CHCl$_3$); UV (CHCl$_3$) $\lambda_{\text{max}}$ nm (log ε): 294 (4.21); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3402 (O–H stretching), 1697 (C=O stretching), 1616 (C=O stretching); $^1$H
NMR (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 6; HREIMS m/z [M]+ 336.2341 (calcd for C₂₀H₃₂O₄, 336.2301).

**Compound TD7**, ent-5α,15S,3-oxobolabr-1,4(18)-diene-2,15,16-triol: colorless oil; [α]²⁷° +120.0° (c = 0.33, CHCl₃); UV (CHCl₃) 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 (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 7; HREIMS m/z [M]+ 334.2133 (calcd for C₂₀H₃₀O₄, 334.2144).

**Compound TD8**, ent-5α,15S,2-oxodolabr-3-ene-3,15,16-triol: white solid, mp: 126–128°C; [α]²⁷°: -17.8° (c = 1.33, CHCl₃); UV (CHCl₃) 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 (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 8.

**Compound TD9**, ent-5α-dolabr-4(18)-ene-15,16-diol: colorless oil; [α]²⁷° +69.3° (c = 0.50, CHCl₃); UV (CHCl₃) 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 (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 9.

**Compound TD10**, ent-5α,18-oxodolabr-3,15-diene-2β-ol: colorless oil; [α]²⁷° +37.0° (c = 0.16, CHCl₃); UV (CHCl₃) λ max nm (log ε): 234 (3.56); IR (neat) ν max (cm⁻¹) 3406 (O–H stretching), 1690 (C=O stretching); ¹H NMR (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 10; HREIMS m/z [M]+ 302.2285 (calcd for C₂₀H₂₀O₂, 302.2246).

**Compound TD11**, tagalsin G: white solid, mp: 90–91°C; [α]²⁷°: -17.6° (c = 2.38, CHCl₃); UV (CHCl₃) 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 (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 11.

**Compound TD12**, tagalsin C,: colorless oil; [α]²⁷° +92.3° (c = 0.05, CHCl₃); UV (CHCl₃) λ max nm (log ε): 312 (3.24); IR (neat) ν max (cm⁻¹) 3426 (O–H stretching), 1716 (C=O stretching); ¹H NMR (CDCl₃) (δ ppm) (300 MHz) and ¹³C NMR (CDCl₃) (δ ppm) (75 MHz): see Table 12.
Compound TD13, tagalsin F: white solid, mp: 101–102°C; $[\alpha]_{D}^{27} = -37.8^\circ$ (c = 2.05, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 295 (3.15); IR (neat) $\nu_{max}$ (cm⁻¹) 3450 (O–H stretching), 1693 (C=O stretching), 1624 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (75 MHz): see Table 13.

Compound TD14, tagalsin E: colorless oil; $[\alpha]_{D}^{27} = -8.4^\circ$ (c = 1.00, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 236 (3.18), 290 (2.66); IR (neat) $\nu_{max}$ (cm⁻¹) 3458 (O–H stretching), 1701 (C=O stretching), 1640 (C=C stretching), 1623 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (75 MHz): see Table 14.

Compound TD15, tagalsin A: white solid, mp: 68–69°C; $[\alpha]_{D}^{27} = +120.0^\circ$ (c = 0.75, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 288 (4.12); IR (neat) $\nu_{max}$ (cm⁻¹) 3416 (O–H stretching), 1668 (C=O stretching), 1651 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (75 MHz): see Table 15.

Compound TD16, tagalsin B: white solid, mp: 83–84°C; $[\alpha]_{D}^{27} = +165.0^\circ$ (c = 2.25, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 286 (3.84); IR (neat) $\nu_{max}$ (cm⁻¹) 3438 (O–H stretching), 1692 (C=O stretching), 1638 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (75 MHz): see Table 16.

Compound TD17, ent-5α-dolabr-4(18),15-diene-3α-ol: colorless oil; $[\alpha]_{D}^{27} = +40.8^\circ$ (c = 0.25, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 241 (3.24), 293 (2.97); IR (neat) $\nu_{max}$ (cm⁻¹) 3402 (O–H stretching), 1635 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (75 MHz): see Table 17.

Compound TD18, tagalsin D: white solid, mp: 87–88°C; $[\alpha]_{D}^{27} = +38.5^\circ$ (c = 2.64, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 243 (2.56), 273 (2.65); IR (neat) $\nu_{max}$ (cm⁻¹) 3417 (O–H stretching), 1635 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (75 MHz): see Table 18.

Compound TD19, tagalsin H: white solid, mp: 87–88°C; $[\alpha]_{D}^{27} = -4.5^\circ$ (c = 1.50, CHCl₃); UV (CHCl₃) $\lambda_{max}$ nm (log $\varepsilon$): 240 (2.53); IR (neat) $\nu_{max}$ (cm⁻¹) 1701 (C=O stretching), 1636 (C=C stretching); $^1$H NMR (CDCl₃) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl₃) ($\delta$ ppm) (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$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 238 (3.60); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3406 (O-H stretching), 1667 (C=O stretching), 1596 (C=C stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 245 (3.51); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3350 (O-H stretching), 1671 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (500 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 244 (3.42); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3437 (O-H stretching), 1678 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ ppm) (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$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 243 (2.70); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3436 (O-H stretching), 1682 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (300 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ 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$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 252 (3.46), 294 (3.66); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3450 (O-H stretching), 1680 (C=O stretching); $^1$H NMR (CDCl$_3$) ($\delta$ ppm) (500 MHz) and $^{13}$C NMR (CDCl$_3$) ($\delta$ ppm) (125 MHz): see Table 24; HREIMS m/z [M]$^+$ 292.2051 (calcd for C$_{18}$H$_{26}$O$_3$, 2922038).

**Compound TD25**, ent-15S-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$_3$) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 246 (2.59); IR (neat) $\nu_{\text{max}}$ (cm$^{-1}$) 3386 (O-H stretching), 1656 (C=C stretching);
\(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) 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-*15S-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\(_3\)) \(\lambda_{max}\) nm (log \(\varepsilon\)): 244 (2.56); IR (neat) \(\nu_{max}\) (cm\(^{-1}\)) 3420 (O-H stretching), 1663 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) ppm) (75 MHz): see Table 26.

**Compound** TD27, *ent-*kauran-16\(\alpha\),17-diol: white solid, mp: 174–175°C; [\(\alpha\)]\(_D\)\(^{27}\) -9.2° (c = 2.25, CHCl\(_3\)); IR (neat) \(\nu_{max}\) (cm\(^{-1}\)) 3387 (O-H stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) ppm) (75 MHz): see Table 27.

**Compound** TD28, *ent-*kauran-16\(\beta\),17-diol: colorless plate crystals from acetone, mp: 134–135°C; [\(\alpha\)]\(_D\)\(^{27}\) -37.5° (c = 0.30, CHCl\(_3\)); IR (neat) \(\nu_{max}\) (cm\(^{-1}\)) 3402 (O-H stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) 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) \(\nu_{max}\) (cm\(^{-1}\)): 3343 (O-H stretching), 2945 (C-H stretching), 1638 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) 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) \(\nu_{max}\) (cm\(^{-1}\)): 3382 (O-H stretching), 2942 (C-H stretching), 1645 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) 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) \(\nu_{max}\) (cm\(^{-1}\)): 3415 (O-H stretching), 2942 (C-H stretching), 1686 (C=O stretching), 1645 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) 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) \(\nu_{max}\) (cm\(^{-1}\)): 3436 (O-H stretching), 2947 (C-H stretching), 1704 (C=O stretching), 1643 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta\) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta\) ppm) (75 MHz): see Table 32.
Compound TL5, betulonic acid: white solid, mp: 250–254°C; \([\alpha]^2_D = +32.0^\circ (c = 0.37,\) MeOH); IR (KBr) \(\nu_{\text{max}} (\text{cm}^{-1}): 2914 \) (C–H stretching), 1704 (C=O stretching), 1642 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta \) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta \) ppm) (75 MHz): see Table 33.

Compound TL6, 3\(\beta\)-E-coumaroyllupeol: white solid, mp: 166–167°C; \([\alpha]^2_D = +20.0^\circ (c = 0.05,\) MeOH); UV (MeOH) \(\lambda_{\text{max}} (\text{nm}) (\log \varepsilon): 227 (4.10), 313 (4.38);\) IR (KBr) \(\nu_{\text{max}} (\text{cm}^{-1}): 3397 \) (O–H stretching), 1726 (C=O stretching), 1602 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta \) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta \) ppm) (75 MHz): see Table 34.

Compound TL7, 3\(\beta\)-E-feruloyllupeol: white solid, mp: 167–169°C; \([\alpha]^2_D = +140.0^\circ (c = 0.03,\) MeOH); UV (MeOH) \(\lambda_{\text{max}} (\text{nm}) (\log \varepsilon): 234 (4.02), 298 (4.06), 325 (4.20);\) IR (KBr) \(\nu_{\text{max}} (\text{cm}^{-1}): 3534 \) (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\(_4\): 601.4260); \(^1\)H NMR (CDCl\(_3\)) (\(\delta \) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta \) ppm) (75 MHz): see Table 35.

Compound TL8, 3\(\beta\)-Z-feruloyllupeol: white solid, mp: 195–197°C; \([\alpha]^2_D = +41.7^\circ (c = 0.06,\) MeOH); UV (MeOH) \(\lambda_{\text{max}} (\text{nm}) (\log \varepsilon): 235 (3.57), 296 (3.56), 325 (3.71);\) IR (KBr) \(\nu_{\text{max}} (\text{cm}^{-1}): 3538 \) (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); \(^1\)H NMR (CDCl\(_3\)) (\(\delta \) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta \) ppm) (75 MHz): see Table 36.

Compound TL9, 3\(\beta\)-E-feruloylbetulin: white solid, mp: 152–154°C; \([\alpha]^2_D = +16.2^\circ (c = 0.40,\) MeOH); UV (MeOH) \(\lambda_{\text{max}} (\text{nm}) (\log \varepsilon): 325 (4.50), 297 (4.41), 234 (4.53), 215 (4.63);\) IR (neat) \(\nu_{\text{max}} (\text{cm}^{-1}): 3360 \) (O–H stretching), 1685 (C=O stretching), 1590 (C=C stretching); \(^1\)H NMR (CDCl\(_3\)) (\(\delta \) ppm) (300 MHz) and \(^{13}\)C NMR (CDCl\(_3\)) (\(\delta \) ppm) (75 MHz): see Table 37.

Compound TL10, 3\(\beta\)-E-caffeoylbetulin: white solid, mp: 160–163°C; \([\alpha]^2_D = +47.0^\circ (c = 1.00,\) MeOH); UV (MeOH) \(\lambda_{\text{max}} (\text{nm}) (\log \varepsilon): 318 (4.21), 285 (4.10), 231 (4.03);\) IR (neat) \(\nu_{\text{max}} (\text{cm}^{-1}): 3413 \) (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; [α]$^\text{D}_{28}$: +8.0° (c = 0.05, MeOH); IR (neat) ν$_{\text{max}}$ (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; [α]$^\text{D}_{28}$: +7.8° (c = 0.76, MeOH); UV (MeOH) λ$_{\text{max}}$ (nm) (log ε): 325 (4.11), 299 (4.23); IR (neat) ν$_{\text{max}}$ (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; [α]$^\text{D}_{28}$: +10.6° (c = 0.05, MeOH); UV (MeOH) λ$_{\text{max}}$ (nm) (log ε): 327 (4.10), 301 (4.00), 327 (4.11); IR (neat) ν$_{\text{max}}$ (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$_{\text{max}}$ (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; [α]$^\text{D}_{28}$: +31.8° (c = 0.30, MeOH); IR (neat) ν$_{\text{max}}$ (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; [α]$^\text{D}_{28}$: +24.0° (c = 0.20, MeOH); IR (neat) ν$_{\text{max}}$ (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; [α]$^\text{D}_{28}$: +38.4° (c = 0.10, MeOH); IR (neat) ν$_{\text{max}}$ (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; [α]$^\text{D}_{28}$: +54.1° (c = 0.04, MeOH); IR (neat) ν$_\text{max}$ (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; [α]$^\text{D}_{28}$: +19.3° (c = 0.05, MeOH); IR (neat) ν$_\text{max}$ (cm$^{-1}$): 3452 (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; [α]$^\text{D}_{28}$: +62.5° (c = 0.03, MeOH); IR (neat) ν$_\text{max}$ (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; [α]$^\text{D}_{28}$: +55.6° (c = 0.02, MeOH); IR (neat) ν$_\text{max}$ (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; [α]$^\text{D}_{28}$: +50.0° (c = 0.02, MeOH); IR (neat) ν$_\text{max}$ (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; [α]$^\text{D}_{28}$: +52.6° (c = 0.02, MeOH); IR (neat) ν$_\text{max}$ (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; [α]$^\text{D}_{28}$: +82.0° (c = 0.10, MeOH); IR (neat) ν$_\text{max}$ (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 logarithmic growth phase were harvested and diluted to 10⁵ 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 µL) 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 IC₅₀ value. Plates were incubated at 37°C under 5% CO₂ 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 IC₅₀ 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-N²-2-ethanesulfonic acid), 32 mM NaHCO₃ and
10% heat activated human serum with 3% erythrocytes and incubated at 37 °C in an incubator with 3% CO₂. 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 [³H] 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 (IC₅₀) represents the concentration which indicates 50% reduction in parasite growth. The positive control compound was artemisinin (IC₅₀ 3.3–3.9 nM).