2 EXPERIMENTAL

2.1 Instruments and Chemicals

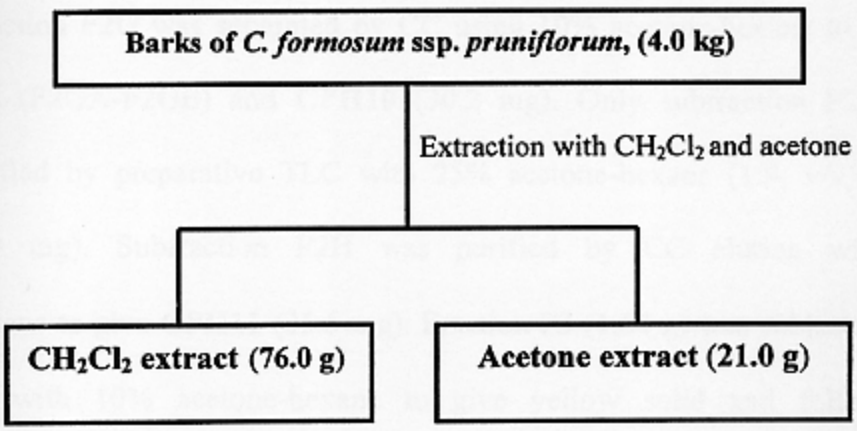
Melting points were determined on the Fisher-John melting point apparatus. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV-Vis and FT-IR spectra were recorded on SPECORD S 100 (Analytikjena) and Perkin-Elmer FTS FT-IR spectrophotometer, respectively. Single crystal X-ray diffraction measurements were collected using SMART 1-K CCD diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 Å) using ω-scan mode and SHELXTL for structure solution and refinement. The ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Varian UNITY INOVA and/or 300 MHz Bruker FT-NMR Ultra Shield^{TM} spectrometers in $CDCl_3$ or CD_3OD with TMS as the internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (J) are expressed in hertz. EI and HREI mass spectra were measured on a Kratos MS 25 RFA spectrometer. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except diethyl ether which was analytical grade reagent. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively.

2.2 Plant material

Barks and roots of *C. formosum* ssp. *pruniflorum* were collected in May 2004 from Nhong Khai Province in the northeastern part of Thailand. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. 0012677) was deposited at Prince of Songkla University Herbarium.

2.3 Extraction

Ground-dried barks (4.0 kg) were extracted with CH_2Cl_2 and acetone successively at room temperature (each 2 × 20 L, for 5 days). The crude extracts were evaporated under reduced pressure to afford brownish crude CH_2Cl_2 (76.0 g) and acetone (21.0 g) extracts. (see **Scheme 1**.)



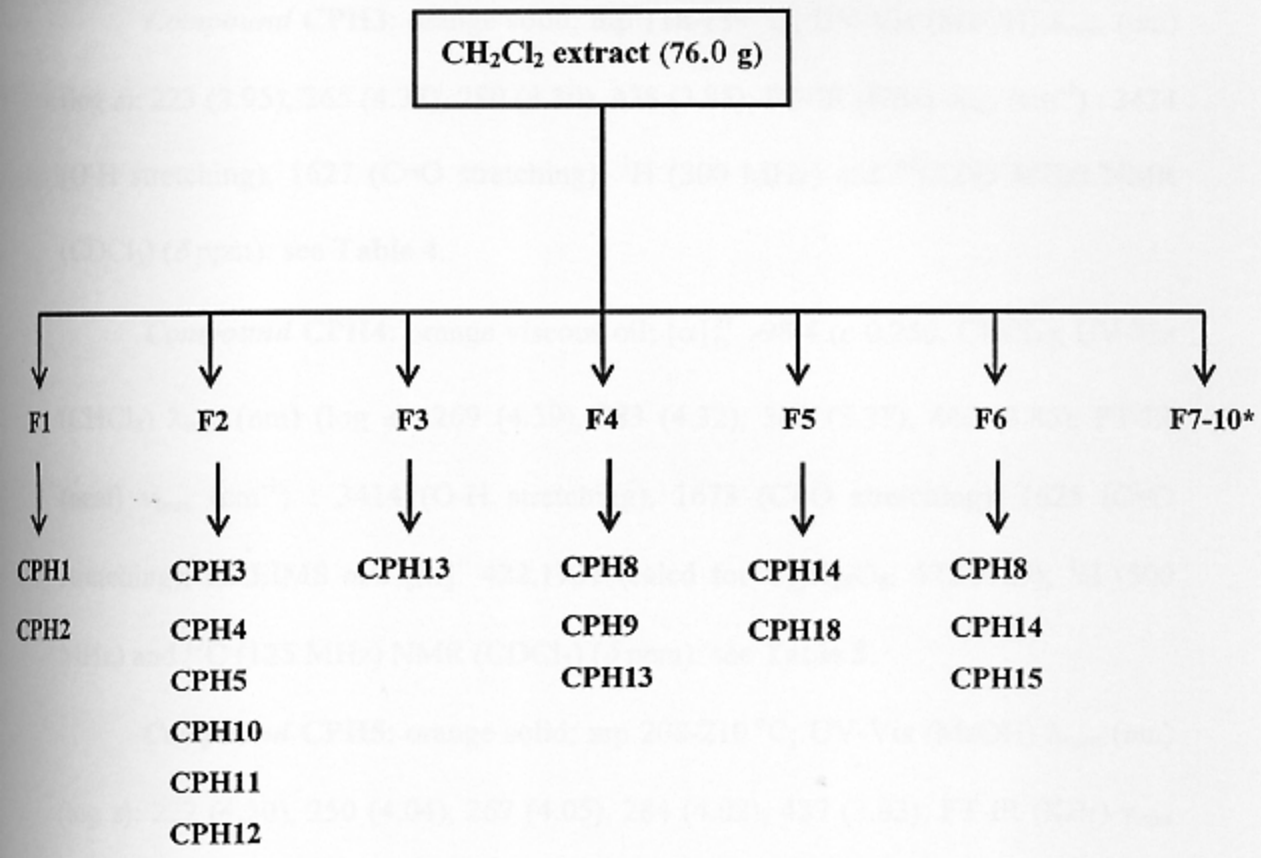
Scheme 1 Extraction of the barks of *C. formosum* ssp. *pruniflorum*

2.4 Isolation and Chemical Investigation

2.4.1. From the CH_2Cl_2 extract of the barks of *C. formosum* ssp. *pruniflorum*

The crude CH_2Cl_2 extract was subjected to QCC eluting with increasing polarities of EtOAc and acetone in hexane to afford 10 fractions (F1-F10). Fraction F1 (2.01 g) was separated by CC with 5% acetone–hexane to afford 3 subfractions (F1A-F1C). Subfraction F1B was further purified by CC with 10% EtOAc–hexane to give **CPH1** (3.0 mg) and **CPH2** (2.3 mg). Fraction F2 (58.06 g) was further separated by CC using a gradient of hexane with EtOAc to afford 10 subfractions (F2A-F2J). Subfraction F2C was separated by CC with 20% EtOAc-hexane to yield **CPH3** (68.2 mg) and **CPH4** (5.2 mg). Subfraction F2D was subjected to CC eluting with 60% CH_2Cl_2–hexane to give 3 fractions (F2DA-F2DC). Only subfraction F2DB was further purified by preparative TLC with 30% CH_2Cl_2–hexane to give **CPH12** (1.5 mg). Subfraction F2G was separated by CC using 10% acetone-hexane to afford 5 subfractions (F2GA-F2GE) and **CPH10** (30.2 mg). Only subfraction F2GB was further purified by preparative TLC with 25% acetone-hexane (1:9, v/v) to give **CPH5** (5.0 mg). Subfraction F2H was purified by CC eluting with 40% CH_2Cl_2–hexane to give **CPH11** (35.5 mg). Fraction F3 (1.54 g) was subjected to CC and eluted with 10% acetone-hexane to give yellow solid and followed by recrystallization from CH_3OH-$CHCl_3$ (1:9, *v/v*) to yield **CPH13** (122.3 mg). Fraction F4 (2.02 g) was subjected to CC using 30% EtOAc-hexane to give 7 subfractions (F4A-F4G), **CPH13** (27.7 mg) and **CPH8** (2.4 mg). Subfraction F4F was further

purified by preparative TLC using 15% EtOAc-hexane to give **CPH9** (15.2 mg). Fraction F5 was subjected to CC and eluted with 10% acetone-hexane to afford 5 subfractions (F5A-F5E). Subfraction F5B was separated by CC using 20% acetone-hexane to give **CPH18** (4.5 mg). Subfraction F5D was separated by CC with 15% acetone-hexane to give **CPH14** (14.2 mg). Fraction F6 was subjected to CC with 15% acetone-hexane to afford 7 subfractions (F6A-F6G). Subfraction F6B was purified by CC with 30% EtOAc-hexane to yield **CPH15** (8.0 mg). Subfraction F6C was separated by CC with 15% acetone-hexane to afford 3 subfractions (F6CA-F6CC). Subfraction F6CB was further purified by preparative TLC eluting with 15% acetone-hexane to give **CPH8** (5.0 mg). Subfraction F6F was separated by CC 20% acetone-hexane to give **CPH14** (10.8 mg). (see **Scheme 2**.)



* No further investigation

Scheme 2 Isolation of compounds **CPH1, CPH2, CPH3, CPH4, CPH5, CPH8, CPH9, CPH10, CPH11, CPH12, CPH13, CPH14, CPH15** and **CPH18**

***Compound* CPH1**: red-orange solid; mp 157-159 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 221 (4.30), 253 (4.04), 265 (4.04), 286 (4.02), 480 (3.82); FT-IR (KBr) ν_{max} (cm^{-1}) : 3338 (O-H stretching), 1628 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 2**.

***Compound* CPH2**: red-orange solid; mp 201-203 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 220 (3.59), 278 (3.39), 425 (3.04); FT-IR (KBr) ν_{max} (cm^{-1}) : 3425 (O-H stretching), 1624 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 3**.

***Compound* CPH3**: orange solid; mp 118-119 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 223 (3.95), 265 (4.37), 280 (4.30), 438 (3.85); FT-IR (KBr) ν_{max} (cm^{-1}) : 3424 (O-H stretching), 1627 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 4**.

***Compound* CPH4**: orange viscous oil; $[\alpha]_D^{27}$ -98.4 (*c* 0.250, $CHCl_3$); UV-Vis ($CHCl_3$) λ_{max} (nm) (log ε): 269 (4.39), 283 (4.32), 366 (3.37), 440 (3.86); FT-IR (neat) ν_{max} (cm^{-1}) : 3414 (O-H stretching), 1673 (C=O stretching), 1625 (C=O stretching); HREIMS *m/z* $[M]^+$ 422.1737 (calcd for $C_{25}H_{26}O_6$, 422.1729); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 5**.

***Compound* CPH5**: orange solid; mp 208-210 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 222 (4.30), 250 (4.04), 267 (4.05), 284 (4.02), 437 (3.83); FT-IR (KBr) ν_{max} (cm^{-1}) : 3350 (O-H stretching), 1646 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 6**.

***Compound* CPH8**: greenish brown viscous oil; UV-Vis ($CHCl_3$) λ_{max} (nm) (log ε): 242 (3.48), 274 (3.65), 405 (2.97); FT-IR (KBr) ν_{max} (cm^{-1}) : 3414 (O-H stretching), 1632 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 9**.

***Compound* CPH9**: greenish brown viscous oil; UV-Vis ($CHCl_3$) λ_{max} (nm) (log ε): 241 (4.08), 278 (4.42), 3.18 (3.68), 335 (3.59), 405 (3.70); FT-IR (KBr) ν_{max} (cm^{-1}) : 3400 (O-H stretching), 1632 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 10**.

***Compound* CPH10**: yellow-green solid; mp 114-116 °C; $[\alpha]_D^{27}$ 0 (*c* 2.1650, $CHCl_3$); UV-Vis (MeOH) λ_{max} (nm) (log ε): 207 (4.66), 279 (4.17), 361 (4.29); FT-IR

(neat) ν_{max} (cm^{-1}) : 3446 (O-H stretching), 1613 (C=O stretching); HREIMS *m/z* $[M]^+$ 782.3797 (calcd for $C_{50}H_{54}O_8$, 782.3819), EIMS *m/z* 406 (35) $[M]^+$, 392 (2), 352 (35), 309 (100), 270 (79), 56 (69); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 11**.

***Compound* CPH11**: yellow-green solid; mp 208-209 °C; $[\alpha]_D^{27}$ 0 (*c* 0.6500, $CHCl_3$); UV-Vis (MeOH) λ_{max} (nm) (log ε): 210 (3.90), 219 (3.91), 273 (3.91), 360 (3.59); FT-IR (KBr) ν_{max} (cm^{-1}) : 3446 (O-H stretching), 1631 (C=O stretching); HREIMS *m/z* $[M]^+$ 674.2864 (calcd for $C_{42}H_{42}O_8$, 674.2880), EIMS *m/z* 338 (50) $[M]^+$, 295 (100), 283 (55); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 12**.

***Compound* CPH12**: yellow solid; mp144-146 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 205 (4.26), 223 (4.18), 253 (4.35), 327 (3.88), 369 (3.46); FT-IR (neat) ν_{max} (cm^{-1}) : 3424 (O-H stretching), 1642 (C=O stretching); ^1H (300 MHz) and ^{13}C (125 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 13**.

***Compound* CPH13**: brown-yellow solid; mp183-184 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 240 (4.28), 283 (4.62), 338 (4.25); FT-IR (KBr) ν_{max} (cm^{-1}) : 3446 (O-H stretching), 1649 (C=O stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 14**.

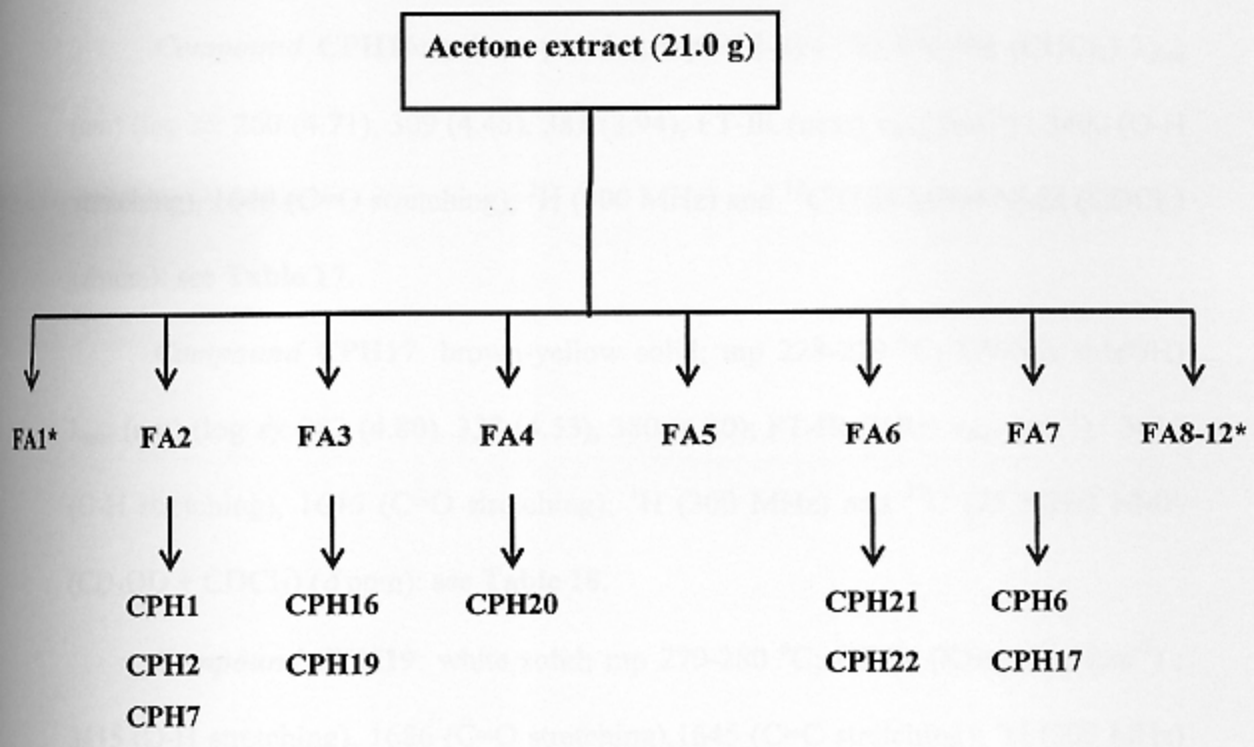
***Compound* CPH14**: yellow solid; mp180-181 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 203 (4.26), 253 (4.42), 287 (3.92), 328 (4.09); FT-IR (KBr) ν_{max} (cm^{-1}) : 3380 (O-H stretching), 1621 (C=O stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 15**.

***Compound* CPH15**: yellow solid; mp 218-219 °C; UV-Vis ($CHCl_3$) λ_{max} (nm) (log ε): 245 (4.00), 297 (3.76), 337 (3.42); FT-IR (KBr) ν_{max} (cm^{-1}) : 3345 (O-H stretching), 1635 (C=O stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δppm): see **Table 16**.

***Compound* CPH18**: white solid; mp 193-194 °C; FT-IR (KBr) ν_{max} (cm^{-1}) : 3416 (O-H stretching), 1638 (C=C stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δppm): see **Table 19**.

2.4.2. From the acetone extract of the barks of *C. formosum* ssp. *pruniflorum*

The acetone extract was subjected to QCC eluting with a gradient of hexane-acetone to afford 12 fractions (FA1-FA12). Fraction FA2 (1.98 g) was subjected to CC with 3% acetone-hexane to afford 6 subfractions (FA2A-FA2F). Subfraction FA2B was further separated by CC with 5% EtOAc-hexane to give 4 subfractions (FA2B1-FA2B4) and **CPH7** (3.0 mg). Only subfraction FA2B1 was further purified by CC with 5% acetone-hexane to yield **CPH1** (2.6 mg) and **CPH2** (1.0 mg). Fraction FA3 was separated by CC and eluted with 10% EtOAc-hexane to give **CPH16** (4.0 mg) and **CPH19** (15.7 mg). Fraction FA4 was recrystallized from CH_3OH-$CHCl_3$ (1:9, *v/v*) to yield **CPH20** (61.5 mg). Fraction FA6 was recrystallized from CH_3OH-$CHCl_3$ (1:9, *v/v*) to give a mixture of **CHP21** and **CPH22** (34.2 mg). Fraction FA7 was subjected to CC eluting with 20% acetone-hexane to give **CPH6** (3.1 mg) and **CPH17** (5.0 mg). (see **Scheme 3**.)



* No further investigation

Scheme 3 Isolation of compounds **CPH1, CPH2, CPH6, CPH7, CPH16, CPH17, CPH19, CPH20, CPH21, CPH22**

***Compound* CPH6**: orange solid; mp 254-256 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 220 (4.29), 251 (4.04), 266 (4.05), 285 (4.02), 441 (3.85); FT-IR (neat) ν_{max} (cm^{-1}) : 3400 (O-H stretching), 1642 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR (DMSO) (δ ppm): see **Table 7**.

***Compound* CPH7**: orange solid; mp 224-226 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 208 (3.05), 224 (3.59), 265 (3.37), 285 (3.39), 424 (3.04); FT-IR (KBr) ν_{max} (cm^{-1}) : 3446 (O-H stretching), 1646 (C=O stretching); ^{1}H (300 MHz) and ^{13}C (125 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 8**.

***Compound* CPH16**: yellow powder; mp 212-214 °C; UV-Vis ($CHCl_3$) λ_{max} (nm) (log ε): 260 (4.71), 309 (4.45), 381 (3.94); FT-IR (neat) ν_{max} (cm^{-1}) : 3400 (O-H stretching), 1640 (C=O stretching); ^1H (300 MHz) and ^{13}C (125 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 17**.

***Compound* CPH17**: brown-yellow solid; mp 228-229 °C; UV-Vis (MeOH) λ_{max} (nm) (log ε): 282 (4.80), 338 (4.53), 380 (4.30); FT-IR (KBr) ν_{max} (cm^{-1}) : 3415 (O-H stretching), 1646 (C=O stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR (CD_3OD + $CDCl_3$) (δ ppm): see **Table 18**.

***Compound* CPH19**: white solid; mp 279-280 °C; FT-IR (KBr) ν_{max} (cm^{-1}) : 3415 (O-H stretching), 1686 (C=O stretching),1645 (C=C stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 20**.

***Compound* CPH20**: colorless crystal; mp 245-247 °C; FT-IR (KBr) ν_{max} (cm^{-1}) : 1715 (C=O stretching); ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm): see **Table 21**.

***Compounds* CPH21** and **CPH22**: colorless crystal; ^1H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) (δ ppm).

2.5 Bioassays

2.5.1 Antibacterial assay

The isolated compounds from the barks of *C. formosum* ssp. *pruniflorum* were tested for antibacterial activities against, *Bacillus substilis* (obtained from Department

of Industrial Biotechnology, Faculty of Agroindustrial, PSU), *Staphylococcus aureus* TISTR517 (obtained from Microbial Resources Center (MIRCEN), Bangkok, Thailand), *Streptococcus faecalis*, *Salmonella typhi*, *Shigella sonei* and *Pseudomonas aeruginosa*. The last four microrganism were obtained from Department of Pharmacognosy and Botany, Faculty of Pharmacy, PSU. The antibacterial assay employed was the same as described in Boonsri *et al.* (Boonsri *et al.*, 2006). Vancomycin which was used as a standard showed antibacterial activity of 75 μg/mL.

2.5.2 Cytotoxic assay

The procedure for cytotoxic assay was performed by the sulphorhodamine B (SRB) assay as described by Skehan *et al.* (Skehan *et al.*, 1990). In this study, four cancer cell lines obtained from National Cancer Institute, Bangkok, Thailand, were used including MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (Human cervical cancer) and HT-29 (colon cancer). Camptothecin which was used as a standard showed cytotoxic activity in the range of 0.2-2.0 μg/mL.