# CHAPTER IV

# BIOASSAY-GUIDED ISOLATION OF TOPOISOMERASE I INHIBITORY ACTIVITY COMPOUNDS FROM *Grangea maderaspatana* (L.) Poir.

The present study, 6 of 27 ethanolic plant extracts; root and leaf of *Rhinacanthus nasutus*, whole plant of *Grangea maderaspatana*, caudex of *Stephania suberosa*, rhizome of *Curcuma longa* and rhizome of *Curcuma zedoaria*, demonstrated topoisomerase I inhibitory activity overall. Interesting, by the extract of *G. maderaspatana* has never been reported for their topoisomerase I inhibitory activity before. Therefore *G. maderaspatana* was then further selected for bioassay-guided fractionation by using chromatographic techniques.

This chapter, we isolated and elucidated topoisomerase I-targeted compounds from *G. maderaspatana*. The findings from this study may provide the rationale for cancer treatment of this plant in traditional medicine.

## 4.1 Materials and methods

### 4.1.1 Plant materials

The whole plants of *Grangea maderaspatana* (L.) Poir. were bought from Chaokromper drug store, Bangkok, Thailand in August 2008. It was identified by Associate Professor Thatree Phadungcharoen and Associate Professor Dr. Nijsiri Ruangrungsri at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand. Voucher specimen was deposited at the Museum of Natural Medicine, Chulalongkorn University, Bangkok, Thailand.

### 4.1.2 General techniques

#### 4.1.2.1 Analytical thin layer chromatography (TLC)

Technique: One dimension, ascending

Absorbent: Silica gel 60 $F_{254}$ (E. Merck) precoated plate

Layer thickness: 0.2 mm

Distance: 6 cm

Temperature: Laboratory temperature (30-35°C)

Detection: 1. Ultraviolet light at wavelengths of 254 and 365 nm

2. Anisaldehyde and heating at 105°C for 10 min.

4.1.2.2 Column chromatography

4.1.2.2.1 Vacuum liquid column chromatography

Absorbent: Silica gel 60 (No.7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck)

Packing method: Dry packing

Sample loading: The sample was dissolved in a small amount of organic solvents, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.

Detection: 1. Fractions were examined by TLC observing under UV light at the wavelength of 254 and 365 nm.

2. Fractions were examined by TLC observing after spray with anisaldehyde and heating at 105°C for 10 min.

4.1.2.2.2 Flash column chromatography

Absorbent: Silica gel 60 (No.9385) particle size 0.040-0.063 nm (70-230 mesh ASTM) (E. Merck)

Packing method: Wet packing

Sample loading: The sample was dissolved in a small amount eluent and then applied gently on top of the column.

Detection: Fractions were examined in the same way as described in section 4.1.2.2.1.

#### 4.1.2.2.3 Gel filtration chromatography

Absorbent: Sephadex LH 20 (Pharmacia)

Packing method: Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tigthtly.

Sample loading: The sample was dissolved in a small amount eluent and then applied gently on top of the column.

Detection: Fractions were examined in the same way as described in section 4.1.2.2.1.

### 4.1.2.3 Spectroscopic techniques

#### 4.1.2.3.1 Ultraviolet (UV) absorption spectra

UV (in methanol) spectra were obtained on a Shimadzu UV-160A UV/vis spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

#### 4.1.2.3.2 Infrared (IR) absorption spectra

Fourier Transform Infrared spectrometer, PerkinElmer spectrum one (Scientific and Technological Research Equipment Centre, Chulalongkorn University).

#### 4.1.2.3.3 Mass spectra (MS)

Mass spectra were recorded on a Micromass LCT spectrometer or a Thermo-Finnigan Polaris Q mass spectrometer (Department of Chemistry, Faculty of Sciences, Mahidol University).

#### 4.1.2.3.4 Proton and carbon-13 nuclear magnetic resonance ($^1$H and $^{13}$C-NMR) spectra

$^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

Solvents for NMR spectra were deuterated chloroform (chloroform-*d*). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

### 4.1.2.4 Physical property

#### 4.1.2.4.1 Optical rotations

Optical rotations were measured on a Perkin Elmer Polarimeter 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

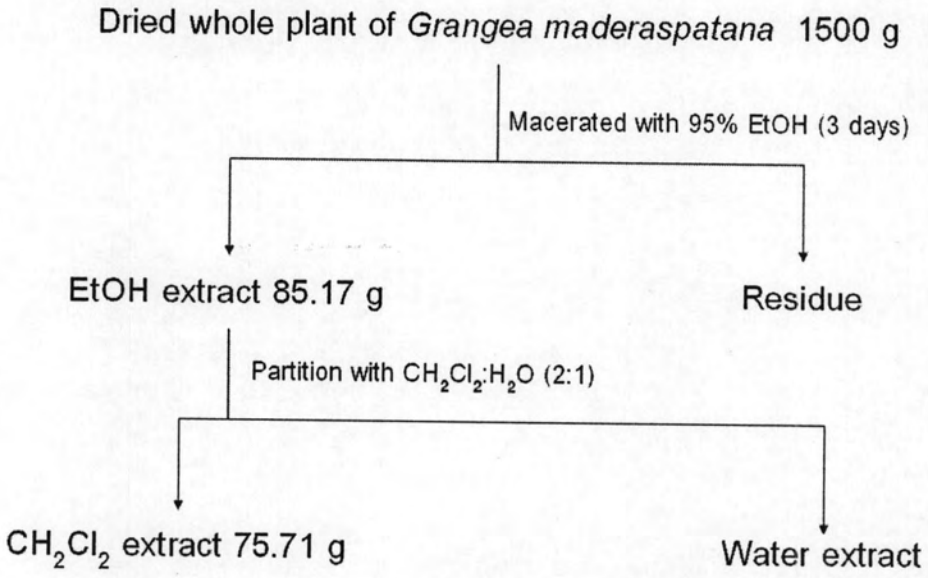
#### 4.1.2.4.2 Solubility

All organic solvents employed throughout this work were of commercial grade and were redistilled prior to use.

### 4.1.3 Extraction and isolation

#### 4.1.3.1 Extraction methods

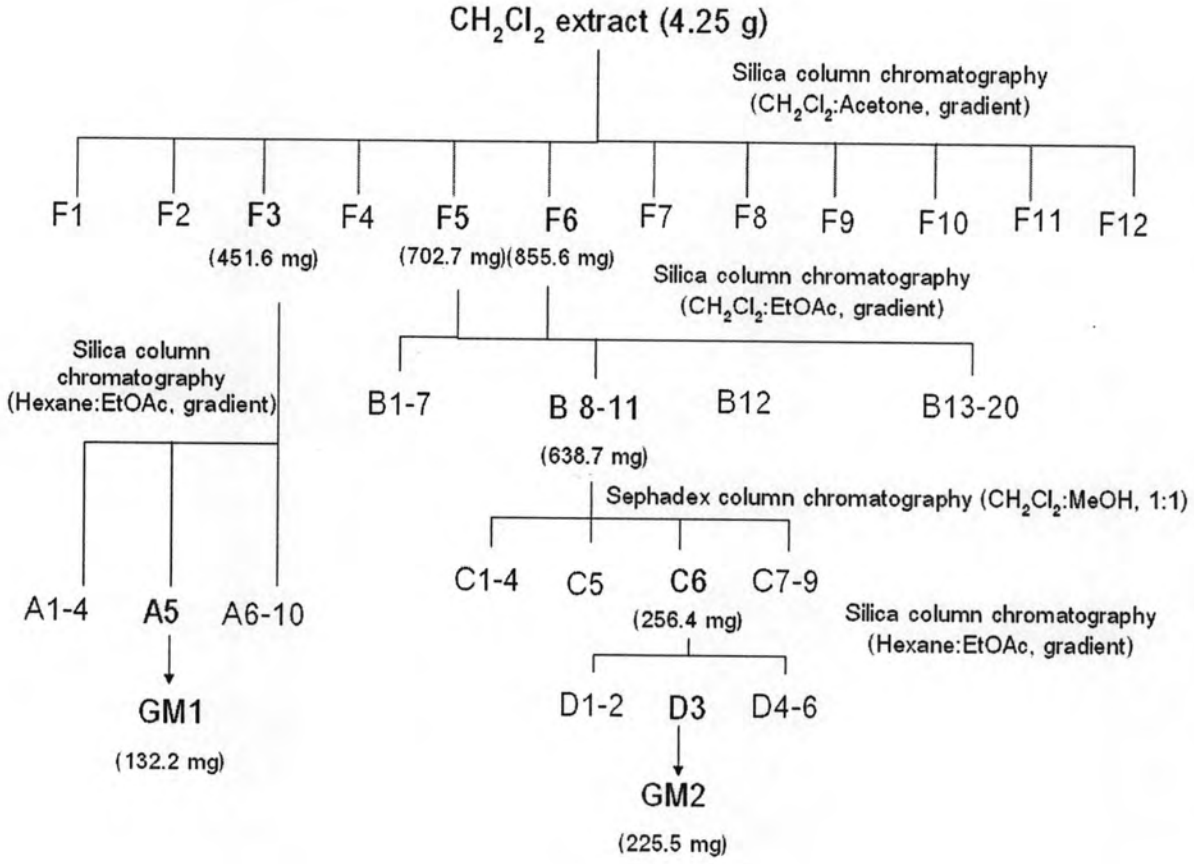
Plant materials were cut into small pieces and dried in a hot air oven at 55°C. Dried plant material was ground to a coarse powder using a hammer mill and stored at an ambient temperature prior to extraction. The powdered plant material (1500 g) was macerated with 95% ethanol (EtOH) (8 L) for 3 days and filtered. The filtrate was evaporated under reduced pressure until dryness. The ethanolic extract (85.17 g) was partitioned with dichloromethane ($CH_2Cl_2$:$H_2O$, 2:1) (6x300 ml), to give $CH_2Cl_2$ extract (75.71 g) (Scheme 4.1).



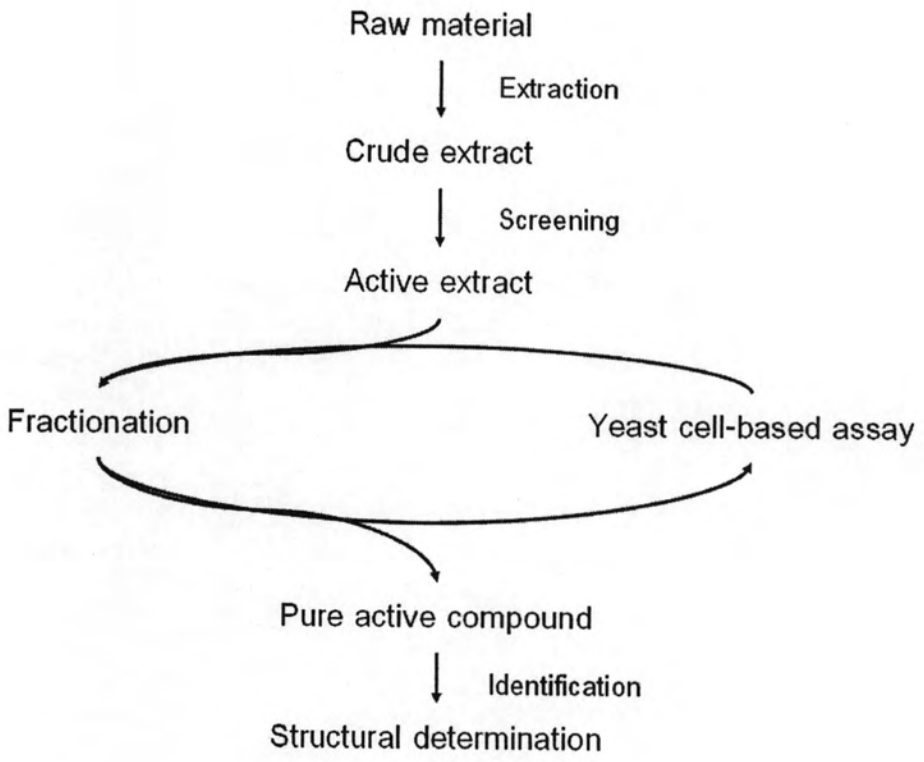

**Scheme 4.1** Extraction scheme of whole plant of *G. maderaspatana*.

#### 4.1.3.2 Bioassay-guided fractionation process

Crude $CH_2Cl_2$ extract of *G. maderaspatana* was fractionated by vacuum liquid column chromatography. Then, all fractions were tested against topoisomerase I inhibitory activity by yeast-cell based assay. The most active fractions were then used for further fractionation step. This procedure finally led to isolate of pure compounds. The procedure from the $CH_2Cl_2$ extract to the isolation of pure compounds is shown in Scheme 4.2. The isolation and identification of the active ingredients which were tested at serial dilution concentrations in yeast cell-based assay. The bioassay-guided fractionation process showed in Scheme 4.3.




**Scheme 4.2** Separation of the $CH_2Cl_2$ extract of *G. maderaspatana*.




Scheme 4.3 Bioassay-guided fractionation process.

#### 4.1.3.3 Separation and isolation

##### 4.1.3.3.1 Isolation of $CH_2Cl_2$ extract

The $CH_2Cl_2$ extract (4.25 g) was separated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No.7734, 200 g). The $CH_2Cl_2$ extract was dissolved in a small amount of $CH_2Cl_2$, triturated with silica gel (No. 7734) and dried under vacuum. Elution was performed in a polarity gradient manner with mixture of $CH_2Cl_2$ and acetone (10:0 to 0:10). The eluants were collected 60 ml per fraction and examine by TLC (silica gel, $CH_2Cl_2$-acetone 9:1) to yield 112 fractions. Fractions with similar chromatographic pattern were combined to yield 12 fractions: F1 (107.1 mg), F2 (16.4 mg), F3 (451.6 mg), F4 (439.0 mg), F5 (702.7 mg), F6 (855.6 mg), F7 (91.8 mg), F8 (408.8 mg), F9 (149.7 mg), F10 (135.5 mg), F11 (438.7 mg), F12 (432.1 mg).

##### 4.1.3.3.2 Isolation of compound GM1 ((-)-frullanolide)

Fraction F3 (451.6 mg) was purified on silica gel column chromatography (silica gel No. 9385, gradient mixture of Hexane-Ethyl acetate (EtOAc) 10:0 to 0:10) to give 132.2 mg of compound GM1 as white needles ($R_f$ 0.85, silica gel $CH_2Cl_2$-Acetone 9:1). It was identified as (-)-frullanolide.

##### 4.1.3.3.3 Isolation of compound GM2 ((-)-7α-hydroxyfrullanolide)

Fraction F5 (702.7 mg) and F6 (855.6 mg) were combined and fractionated on silica gel column chromatography (No. 7734). Elution was performed in polarity gradient manner for mixture $CH_2Cl_2$ and EtOAc (10:0 to 0:10). Fractions (103 fractions) showing similar chromatographic pattern were combined (TLC, silica gel, $CH_2Cl_2$-Acetone 9:1) to yield 20 fractions: B1 (13.7 mg), B2 (2.6 mg), B3 (31.3 mg), B4 (10.9 mg), B5 (10.9 mg), B6 (67.1 mg), B7 (85.2 mg), B8 (147.6 mg), B9 (397.8 mg), B10 (49.7 mg), B11 (43.8 mg), B12 (98.3 mg), B13 (87.1 mg), B14 (92.6 mg), B15 (127.2 mg), B16 (79.4 mg), B17 (44.5 mg), B18 (48.0 mg), B19 (66.4 mg), B20 (28.4mg).

Fraction B8-B11 (638.7 mg) was separated on Sephadex LH20 column ($CH_2Cl_2$:MeOH, 1:1). Thirty fractions were combined according to their TLC pattern (silica

gel, $CH_2Cl_2$-Acetone 9:1) to yield 9 fractions: C1 (10.4 mg), C2 (14.8 mg), C3 (36.7 mg), C4 (89.5 mg), C5 (158.2 mg), C6 (256.4 mg), C7 (22.1 mg), C8 (20.3 mg), C9 (14.4 mg).

Fraction C6 (256.4 mg) was purified by repeated column chromatography (silica gel No. 9385, gradient mixture of Hexane-EtOAc 10:0 to 0:10) to give compound GM2 as yellow oil (225.5 mg, $R_f$ 0.74, silica gel, $CH_2Cl_2$-Acetone 9:1). It was identified as (-)-7α-hydroxyfrullanolide.

#### 4.1.4 Topoisomerase I inhibitory activity assay

The compounds were prepared by solubilized in dimethylsulfoxide (DMSO), sonicated, and filtered sterilized using 0.25 µm millipored filter. The compounds were further diluted in S.C. ura$^-$ media to produce 6 concentrations of 5, 10, 25, 50, 100 and 200 µg/ml of each agent. The media were prepared under two different conditions for carbon source; glucose or galactose. Positive controls were 2.5, 5, and 10 µg/ml camptothecin. And vehicle controls were 125, 250, and 500 µg/ml DMSO alone.

The transformant yeasts were grown overnight at 30°C in a shaking incubator set at 200 rpm in liquid S.C. ura$^-$ media containing glucose. The cultures were adjusted to and $OD_{600}$ of 0.3 and serially diluted 10-fold and 5 µl aliquots spotted onto selective plates supplemented with 2% glucose or galactose and individual test exact. Incubated the culture plates at 30°C for 3-4 days, and cell viability of yeast in the presence of plant extracts was observed. The yeast survival was determined by comparison of the viability of colonies in the vehicle control culture (DMSO plate) with those in the positive control culture (CPT plate) under glucose or galactose agar medium.

#### 4.1.5 *In vitro* cytotoxicity assay

Cytotoxicity against primate cell line (Vero) and cancer cell growth inhibitory activities were tested by National Center for Genetic Engineering and Biotechnology (BIOTECH), National Science and Technology Development Agency (NSTDA). The bioassay laboratory protocol for all testing was described in Appendix B.

## 4.2 Results

### 4.2.1 Bioassay-guided fractionation of *Grangea maderaspatana* extract

Bioassay-guided fractionation was performed on the $CH_2Cl_2$ extract in order to isolate the bioactive compounds from *G. maderaspatana*. In fractionation process, the fractions were tested against topoisomerase I inhibitory activity by yeast cell-based assay. The growing cultures of transformant yeasts on selective medium containing various concentration of crude EtOH, crude $CH_2Cl_2$, and 12 fractions of *G. maderaspatana* extracts are shown in Figure 4.1.

The growing cultures of transformant yeasts on selective medium containing 100 µg/ml, fixing cancentration, of 12 fractions of crude $CH_2Cl_2$ showed five active fractions (Figure 4.2). The five fractions exhibited topoisomerase I inhibitory activities were F2, F3, F4, F5, and F6. Fraction F5 showed the highest topoisomerase I inhibitory activity among five fractions. The most active fractions were then used for further fractionation step. This procedure finally led to isolate of two pure compounds, GM1 ((-)-frullanolide) and GM2 ((-)-7α-hydroxyfrullanolide). Both GM1 and GM2 demonstrated topoisomerase I inhibitory activities (Figure 4.3). The procedure from crude extract to the isolation of pure compounds is shown in Scheme 4.1 - 4.3.

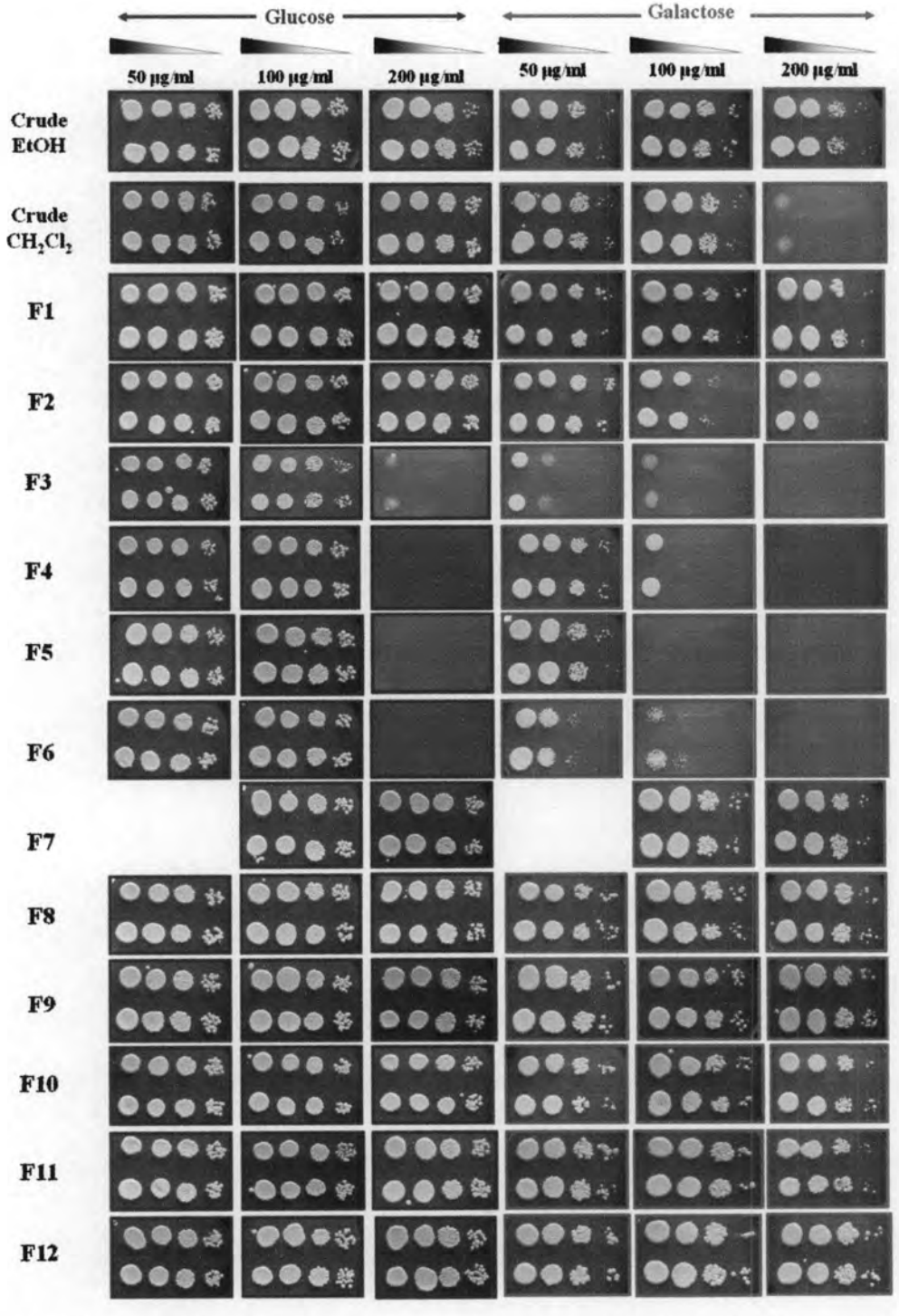



**Figure 4.1** Yeast cell-based assay of fractions (F) from *G. maderaspatana* whole plant extracts with exponentially growing of *S. cerevisiae* expressing topoisomerase I. Five microliters of ten-fold serial dilutions were spotted on plates containing various concentrations of crude EtOH, crude $CH_2Cl_2$, and 12 fractions of *G. maderaspatana* extracts. Plates were incubated at 30°C for 48 hr. The expression of exogenous topoisomerase I was repressed by glucose (3 left panels) and induced by galactose (3 right panels).

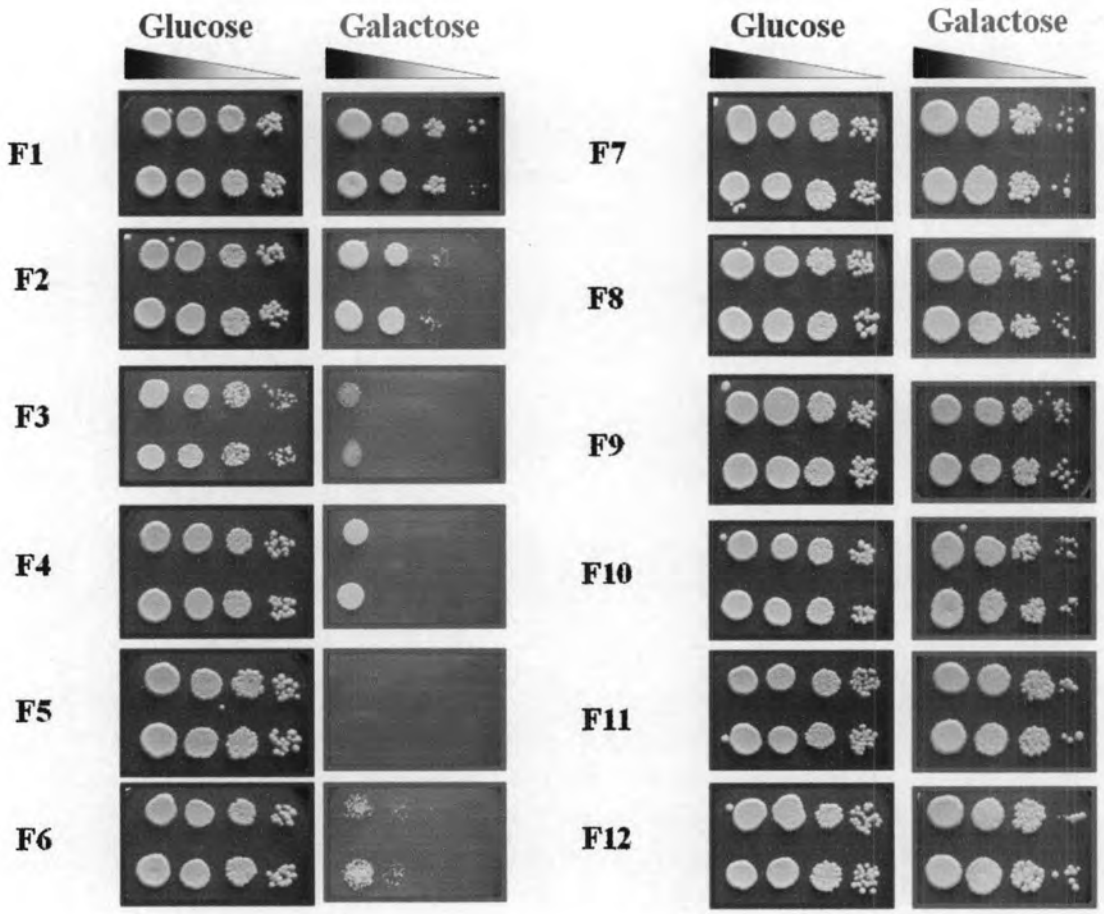



**Figure 4.2** Yeast cell-based assay at 100 µg/ml of fractions (F) from *G. maderaspatana* whole plant extracts with exponentially growing of *S. cerevisiae* expressing topoisomerase I. Five microliters of ten-fold serial dilutions were spotted on plates which containing 100 µg/ml of fraction 1-12. Plates were incubated at 30°C for 48 hr. The expression of exogenous topoisomerase I was repressed by glucose (left panels) and induced by galactose (right panels).

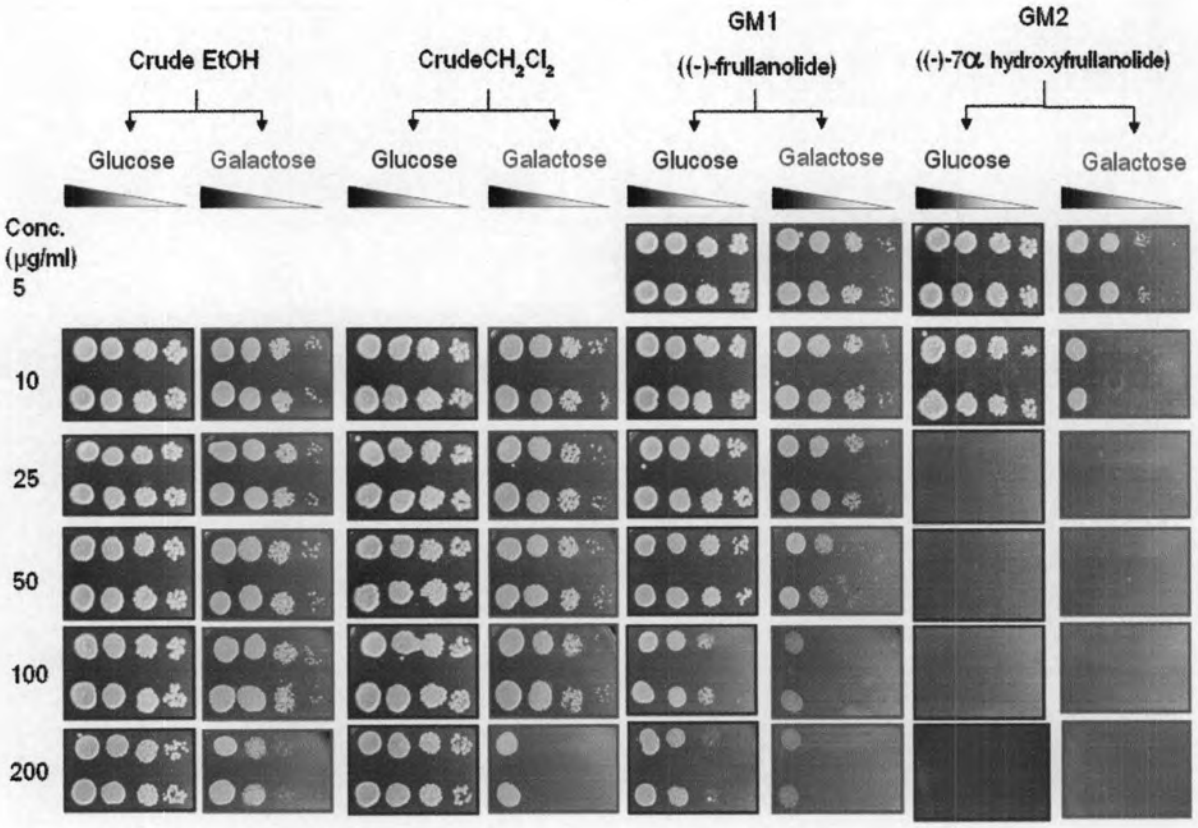



**Figure 4.3** Yeast cell-based assay of *G. maderaspatana* with exponentially growing of *S. cerevisiae* expressing topoisomerase I. Five microliters of ten-fold serial dilutions were spotted on plates containing various concentrations of crude EtOH and crude $CH_2Cl_2$ of *G. maderaspatana* extract, and compound GM1 and GM2. Plates were incubated at 30°C for 48 hr. The expression of exogenous topoisomerase I was repressed by glucose (left panels) and induced by galactose (right panels)

### 4.2.2 Physical and spectral data of the isolate compounds

#### 4.2.2.1 Compound GM1 ((-)-frullanolide)

Compound GM1 was obtained as white needle, soluble in $CH_2Cl_2$ (132.2 mg, 0.16 % base on dried weight of whole plant).

UV: $\lambda$max nm (log $\varepsilon$), in methanol; Figure C1

221.0 (15.11)

IR: $\nu$max ($cm^{-1}$), in $CCl_4$; Figure C2

3099, 2928, 1757, 1668, 1645, 1266, 1146 and 911

EIMS: m/z (% relative intensity); Figure C3

232 (12), 218 (17), 217 (100), 172 (12), 171 (82), 161 (15) and 91 (19)

$^1$H NMR: $\delta$ ppm, 300 MHz, in $CDCl_3$; see Table 4.1, Figure C4

$^{13}$C NMR: $\delta$ ppm, 75 MHz, in $CDCl_3$; see Table 4.1, Figure C5 and Figure C6

$[\alpha]^{28}D$: $-72.2^{\circ}$ (*C* 0.00083; MeOH)

#### 4.2.2.2 Compound GM2 ((-)-7$\alpha$-hydroxyfrullanolide)

Compound GM2 was obtained as yellow oil, soluble in $CH_2Cl_2$ (225.5 mg, 0.27% base on dried weight of whole plant).

UV: $\lambda$max nm (log $\varepsilon$), in methanol; Figure C7

221.0 (14.38)

IR: $\nu$max ($cm^{-1}$), in $CCl_4$; Figure C8

3429, 3103, 2931, 1746, 1667, 1285, 1164 and 945

EIMS: m/z (% relative intensity); Figure C9

248 (9), 233 (100), 230 (13), 197 (54), 187 (30) and 169 (80)

$^1$H NMR: $\delta$ ppm, 300 MHz, in $CDCl_3$; see Table 4.2, Figure C10 and Figure C11

$^{13}$C NMR: $\delta$ ppm, 75 MHz, in $CDCl_3$; see Table 4.2, Figure C12 and Figure C13

$[\alpha]^{28}D$: $-74.7^{\circ}$ (*C* 0.000174 ; MeOH)

### 4.2.3 Structure determination of isolated compounds

#### 4.2.3.1 Structure determination of compound GM1 ((-)-frullanolide)

Compound GM1 was obtained as white needles. The EI mass spectrum (Figure C3) showed a molecular ion [$M^+$] at m/z 232, coresponding to $C_{15}H_{20}O_2$.

The IR spectrum exhibited signal at 3099 $cm^{-1}$ (=C-H-), 2928 $cm^{-1}$ (C-H), 1757 $cm^{-1}$ (C=O, $\gamma$-lactone) and 911 $cm^{-1}$ (unsaturated alkene). (Figure C2)

The $^1H$ NMR spectrum (Figure C4 and Table 4.1) of compound GM1 showed the signal at $\delta$ 6.17 (1H, s) which was due to Hb-13, 5.59 (1H, s) for Ha-13, 5.27 (1H, d, $J$ = 5.7 Hz) for H-6; 2.95 (1H, dt, $J$ = 16.2, 7.2 Hz) for H-7; 1.77 (3H, s) for H-15 and 1.09 (3H, s) for H-14. The signal at 6.17 and 5.59 were represented for methylene protons of C-13.

The $^{13}C$ NMR, DEPT 90 and DEPT 135 spectrum (Figure C5, Figure C6 and Table 4.1) exhibited 15 carbon signals, corresponding to 2 methyls, 6 methylenes, 2 methines and 5 quaternary carbons. Through comparison of its $^1H$ and $^{13}C$ NMR data, IR data and MS data with previously published data, compound GM1 was identified as (-)-frullanolide (Ruangrungsi *et al.*, 1989).

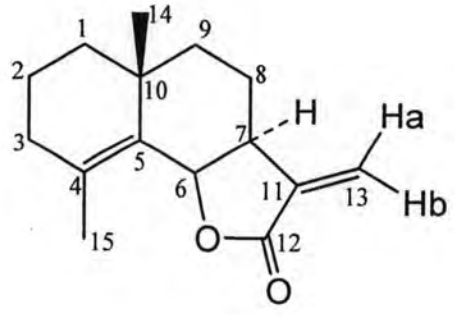



**Figure 4.4** Chemical structure of GM1, **(-)-Frullanolide.**

Table 4.1 NMR spectral data of compound GM1 and (-)-frullanolide ($CDCl_3$).

| Position | $^1H$ (mult, *J* in Hz) | | $^{13}C$ (mult) | |
|---|---|---|---|---|
| | (-) Frullanolide | GM1 | (-)-Frullanolide | GM1 |
| 1α | 1.35 td (13.2, 3.3) | 1.30 td (13.5, 3.3) | 39.1 t | 39.1 t |
| 1β | 1.43 dt (13.2, 3.5) | 1.43 dt (12.6, 3.9) | | |
| 2α | 1.62 m | 1.63 m | 18.2 t | 18.1 t |
| 2β | 1.82 m | 1.83 m | | |
| 3α | 2.09 br d | 2.10 br d | 33.1 t | 33.1 t |
| 3β | 2.11 m | 2.12 m | | |
| 4 | - | - | 138.5 s | 138.4 s |
| 5 | - | - | 128.5 s | 128.5 s |
| 6 | 5.27 d (5.9) | 5.27 d (5.7) | 75.9 d | 75.9 d |
| 7 | 2.95 dt (10.0, 5.9) | 2.96 dt (16.2, 7.2) | 41.2 d | 41.2 d |
| 8α | 1.70 m | 1.71 m | 25.0 t | 25.0 t |
| 8β | 1.65 m | 1.65 m | | |
| 9α | 1.27 ddd (13.2, 12.3, 4.4) | 1.27 ddd (11.7, 10.8, 5.4) | 37.9 t | 37.8 t |
| 9β | 1.48 dt (13.2, 3.9) | 1.46 t (12.6, 3.9) | | |
| 10 | - | - | 32.6 s | 32.6 s |
| 11 | - | - | 142.3 s | 142.3 s |
| 12 | - | - | 170.9 s | 170.9 s |
| 13a | 5.58 d (1.0) | 5.59 s | 120.1 t | 120.0 t |
| 13b | 6.16 d (1.0) | 6.17 s | | |
| 14 | 1.08 s | 1.09 s | 25.8 q | 25.8 q |
| 15 | 1.76 s | 1.77 s | 19.3 q | 19.3 q |

#### 4.2.3.2 Structure determination of compound GM2 ((-)-7α-hydroxyfrullanolide)

Compound GM2 was obtained as yellow oil. The EI mass spectrum (Figure C9) showed a molecular ion [$M^+$] at m/z 248, corresponding to $C_{15}H_{20}O_3$.

The IR spectrum showed signals at 3438 $cm^{-1}$ (O-H), 3103 $cm^{-1}$ (=C-H-), 2931 $cm^{-1}$ (C-H), 1746 $cm^{-1}$ (C=O, γ-lactone) and 945 $cm^{-1}$ (unsaturated alkene) (Figure C8).

The $^1H$ NMR spectrum (Figure C10; Figure C11 and Table 4.2) of compound GM2 showed the signal at δ 6.15 (1H, s) which was due to Hb-13, 5.80 (1H, s) for Ha-13, 5.04 (1H, s) for H-6; 5.27 (1H, s) for OH; 1.71 (3H, s) for H-15 and 1.01 (3H, s) for H-14. The signal at 6.15 and 5.80 were represented for methylene protons of C-13. The signal at 5.27 was represented for hydroxyl proton because this signal was lower integration when adding $D_2O$ (Figure C11).

The $^{13}C$ NMR, DEPT 90 and DEPT 135 spectrum (Figure C12, Figure C13 and Table 4.2) exhibited 15 carbon signals, corresponding to 2 methyls, 6 methylenes, 1 methine and 6 quaternary carbons. Through comparison of its $^1H$ and $^{13}C$ NMR data, IR data and MS data with previously published data, compound GM1 was identified as (-)-7α-hydroxyfrullanolide (Ruangrungsi *et al.*, 1989).

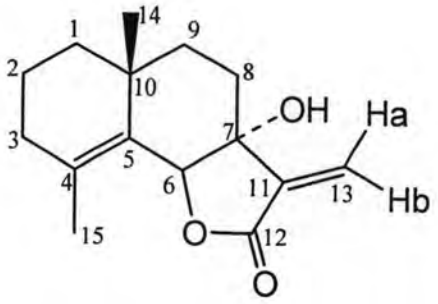



**Figure 4.5** Chemical structure of GM2, (-)-7α-Hydroxyfrullanolide.

Table 4.2 NMR spectral data of compound GM2 and (-)-7α-hydroxyfrullanolide ($CDCl_3$).

| Position | $^1H$ (mult, *J* in Hz) | | $^{13}C$ (mult) | |
|---|---|---|---|---|
| | (-)-7α-Hydroxy frullanolide | GM2 | (-)-7αHydroxy frullanolide | GM2 |
| 1α | 1.40 m | 1.38 m | 38.8 t | 38.6 t |
| 1β | 1.45 m | 1.49 m | | |
| 2α | 1.65 m | 1.61 m | 18.2 t | 18.0 t |
| 2β | 1.83 m | 1.83 m | | |
| 3α | 2.12 m | 2.06 m | 33.1 t | 33.0 t |
| 3β | 2.12 m | 2.04 m | | |
| 4 | - | - | 140.5 s | 140.2 s |
| 5 | - | - | 126.8 s | 126.7 s |
| 6 | 5.00 s | 5.04 s | 81.4 d | 81.5 d |
| 7 | - | - | 76.0 s | 75.7 s |
| 8α | 1.68 m | 1.63 m | 31.5 t | 31.3 t |
| 8β | 1.99 td (13.2, 3.6) | 1.85 td(13.5, 3.5) | | |
| 9α | 1.49 td (13.2, 3.1) | 1.49 td(13.5, 3.3) | 34.9 t | 34.7 t |
| 9β | 1.68 m | 1.63 m | | |
| 10 | - | - | 32.7 s | 32.5 s |
| 11 | - | - | 144.7 s | 144.6 s |
| 12 | - | - | 169.1 s | 169.7 s |
| 13a | 5.81 s | 5.80 s | 121.0 t | 121.2 t |
| 13b | 6.27 s | 6.15 s | | |
| 14 | 1.09 s | 1.01 s | 26.1 q | 26.0 q |
| 15 | 1.78 s | 1.71 s | 19.4 q | 19.2 q |
| OH | 2.3 br s | 5.27 s | - | - |

### 4.2.4 *In vitro* cytotoxicity activity

Table 4.3 shows the effect of crude EtOH, crude $CH_2Cl_2$, compound GM1, compound GM2, and CPT on the different cell line i.e. vero cell line (primate cell line), KB (oral cavity cancer), MCF-7 (breast cancer), and NC-H187 (small cell lung cancer). Compound GM2 showed higher cytotoxicity against KB and MCF-7 cell lines than GM1. However, lower cytotoxicity activity against NCI-H187 small lung cancer cell lines was found in GM1. Both GM1 and GM2 showed cytotoxicity activities against vero cells lines.

**Table 4.3** *In vitro* cytotoxicity of isolated compounds from *G. maderaspatana* and CPT against vero cell line and cancer cell lines.

| | $IC_{50}$ | | | | |
|---|---|---|---|---|---|
| Sample / Cell line | Crude EtOH | Crude $CH_2Cl_2$ | GM1 | GM2 | CPT |
| Vero cell line (Primate cell line) | - | - | 10.30 µM | 3.41 µM | 0.49 µM |
| KB (Oral cavity cancer) | Inactive | Inactive | 6.38 µM | 5.48 µM | 0.0086 µM |
| MCF-7 (Breast cancer) | Inactive | Inactive | 29.70 µM | 3.35 µM | 0.32 µM |
| NC-H187 (Small cell lung cancer) | 0.42 µg/ml | 1.53 µg/ml | 3.23 µM | 5.77 µM | 0.0029 µM |

## 4.3 Discussions

Chemical constituents of *G. maderaspatana* have been reported previously (Iyer and Iyer, 1978; Pandey *et al.*,1984; Singh et al,1988; Ruangrungsi *et al.*, 1989; Krishna and Singh, 1999; Rojatkar *et al.*, 1994). Some reports demonstrated cytotoxicity of crude extract or compounds from this plant against cancer cell lines (Rojatkar *et al.*, 1994). However, no previous studies have been gone into any detail of the mechanism.

In this study, the ethanolic extract of *G. maderaspatana* exhibited topoisomerae I inhibitory activity against yeast cell-based assay. Bioassay-guided fractionation and isolation led to the identification of two bioactive compounds, GM1 and GM2. Comparison of theirs $^{1}H$ and $^{13}C$ NMR, IR and MS data with previously publications (Ruangrungsi *et al.*, 1989), compound GM1 was identified as (-)-frullanolide and compound GM2 was identified as (-)-7$\alpha$-hydroxyfrullanolide.

*In vitro* cytotoxicity activities of GM1 and GM2 showed potential anti-cancer agents from *G. maderaspatana*. The two compounds exhibited cytotoxicity activities against KB oral cavity cancer cell lines ($IC_{50}$ = 6.38 and 5.48 µM respectively), MCF-7 breast cancer cell lines ($IC_{50}$ = 29.7 and 3.35 µM respectively), and NCI-H187 small lung cancer cell lines ($IC_{50}$ = 3.23 and 5.77 µM respectively). Compound GM2 showed higher cytotoxicity against KB and MCF-7 cell lines than GM1. However, lower cytotoxicity activity against NCI-H187 small lung cancer cell lines was found in GM1. Both GM1 and GM2 showed cytotoxicity activities against vero cells with $IC_{50}$ = 10.30 and 3.41 µM respectively. Comparison with CPT ($IC_{50}$ = 0.49 µM), topoisomerase I-targeted drug, however, these compounds exhibited lower cytotoxicity.

This study demonstrated the use of column chromatography for isolation of bioactive compounds in a bioassay-guided study. Yeast cell-base assay was used to test topoisomerase I inhibitory activity of fractions. As we know, this is the first report of cytotoxicity due to topoisomerase I inhibitory mechanism of two compounds from *G. maderaspatana*.