



## APPENDIX A THE CALIBRATION CURVE AND CHROMATOGRAMS

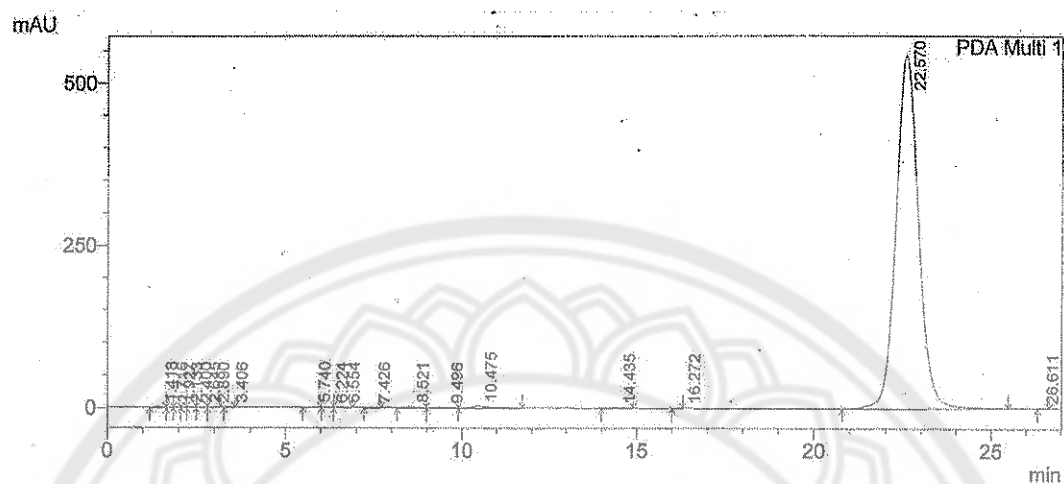


Figure 42 The HPLC chromatogram of artocarpin standard of 0.2 mg/mL.

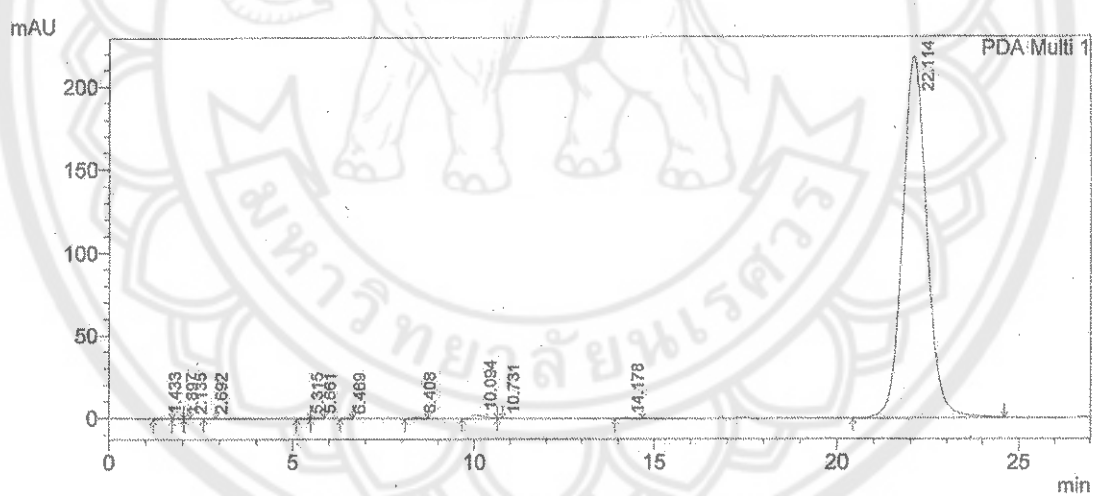


Figure 43 The HPLC chromatogram of artocarpin standard of 0.04 mg/mL.

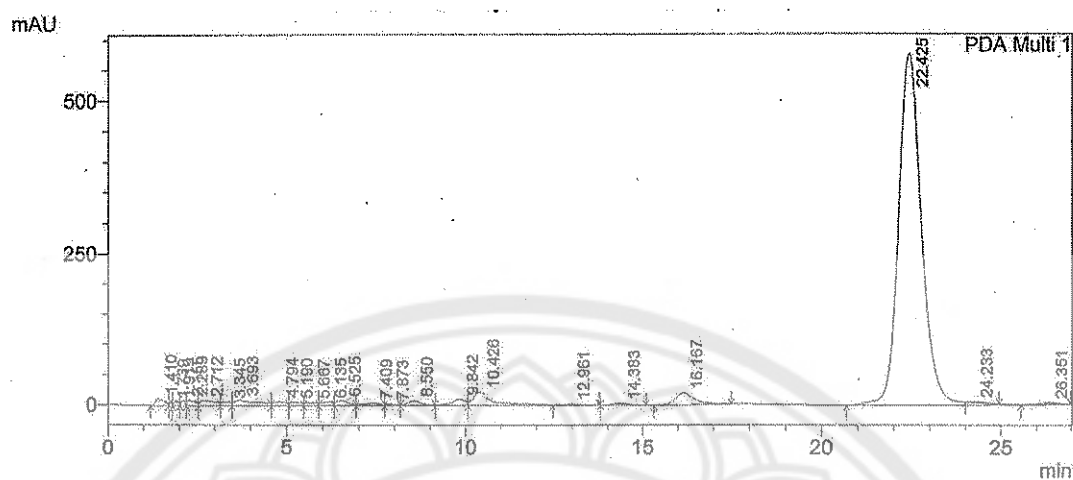


Figure 44 The HPLC chromatogram of *A. incisus* extract.

**Table 8 The peak areas of artocarpin at various concentrations of artocarpin standard regarding to the absorbance at wavelength of 282 nm.**

| Artocarpin Conc.<br>(mg/mL) | Absorbance of Peak Areas |             |             | Mean (SD)         |
|-----------------------------|--------------------------|-------------|-------------|-------------------|
|                             | Sample No.1              | Sample No.2 | Sample No.3 |                   |
| 0.04                        | 4862647                  | 4851238     | 4840112     | 4856202 (11006)   |
| 0.08                        | 9924699                  | 9824561     | 9784231     | 9875642 (81446)   |
| 0.12                        | 14219456                 | 14265722    | 14804851    | 14265906 (304974) |
| 0.16                        | 18967894                 | 18841557    | 18987967    | 18925641 (106757) |
| 0.20                        | 23698650                 | 23736219    | 23801231    | 23741231 (96252)  |

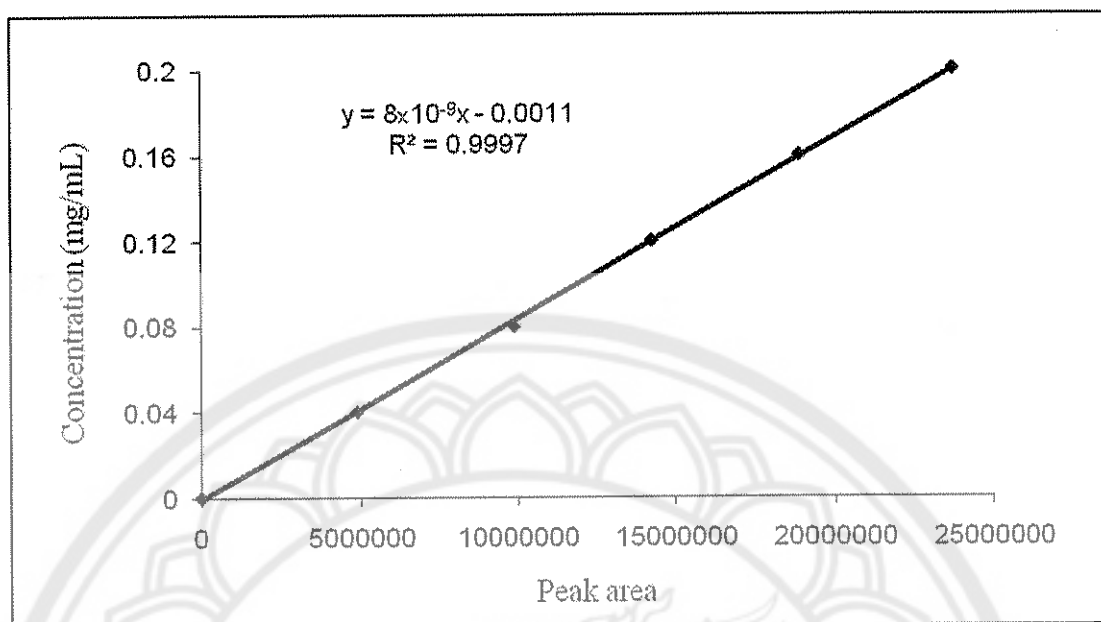


Figure 45 The calibration curve of artocarpin standard.

Where;  $y$  = Concentration (mg/ml)

$x$  = Peak area

Table 9 The content of artocarpin contained in extract of *A. incisus*'s heartwood.

| Samples                                | Sample | Peak area | Artocarpin content (mg) | Mean of artocarpin content (mg $\pm$ SD) |
|----------------------------------------|--------|-----------|-------------------------|------------------------------------------|
| 1.0 mg/mL of <i>A. incisus</i> extract | 1      | 27858463  | 4.44                    | 4.44 $\pm$ 0.01                          |
|                                        | 2      | 27965892  | 4.45                    |                                          |
|                                        | 3      | 27895648  | 4.44                    |                                          |
|                                        | 4      | 28056471  | 4.47                    |                                          |

### The calculation of artocarpin content of *A. incisus* extracts

#### Example:

The concentration (y) can be calculated by using the equation;

$$y = 8 \times 10^{-9}(x) - 0.0011$$

If peak area of 0.5 mg/mL *A. incisus* extract is 27858463, therefore, the concentration is calculated as follow:

$$y = 8 \times 10^{-9}(27858463) - 0.0011$$

$$y = 0.222$$

Therefore, Artocarpin content of 1.0 mg/mL *A. incisus* extract is calculated as follow:

$$= 0.222 \times 2$$

$$= 0.444$$

The methods of artocarpin content calculation are the following:

1. 1.0 mg/mL of the extract was prepared by dissolving 10.0 mg original weight of the extract with methanol. At this concentration, the extract provided the 0.444 mg/mL of artocarpin.
2. Then, to multiply the concentration with 10 mL, the final volume of the extract, therefore, the content of artocarpin contained in the extract of *A. incisus*'s heartwood;

$$= 0.444 \text{ mg/mL} \times 10 \text{ mL}$$

$$= 4.44 \text{ mg}$$

3. The obtained artocarpin was calculated in the term of percent artocarpin content;

$$= (4.44 \text{ mg} / \text{original weight}) \times 100$$

$$= (4.44 \text{ mg} / 10 \text{ mg}) \times 100$$

$$= 44.4\% \text{ w/w}$$

## APPENDIX B DETERMINATION OF CELL VIABILITY AND MELANIN CONTENT BY USING CELL CULTURE

**Table 10 The cell number of time course study.**

| Time<br>(day) | Cell number x 10 <sup>5</sup> |          |          | Mean ±SD |
|---------------|-------------------------------|----------|----------|----------|
|               | Sample 1                      | Sample 2 | Sample 3 |          |
| 0             | 1.4                           | 1.1      | 1.2      | 1.2 ±0.2 |
| 1             | 2.1                           | 2.6      | 2.3      | 2.3±0.3  |
| 2             | 4.9                           | 5.1      | 5.4      | 5.1±0.3  |
| 3             | 11.9                          | 12.1     | 12.5     | 12.2±0.3 |
| 4             | 23.9                          | 24.1     | 24.3     | 24.1±0.2 |
| 5             | 27.8                          | 28.4     | 28.1     | 28.1±0.3 |
| 6             | 28.0                          | 27.7     | 28.1     | 27.9±0.2 |
| 7             | 28.2                          | 27.8     | 27.9     | 28.0±0.2 |

Table 11 The effect of *A. incisus* extract on cells number.

| Sample                                    | Day 1 |      |      | Day 2          |      |      | Day 3 |                |       | Day 4 |       |                 |                 |
|-------------------------------------------|-------|------|------|----------------|------|------|-------|----------------|-------|-------|-------|-----------------|-----------------|
|                                           | 1     | 2    | 3    | Mean<br>(SD)   | 1    | 2    | 3     | Mean<br>(SD)   | 1     | 2     | 3     | Mean<br>(SD)    |                 |
| Control                                   | 2.39  | 2.42 | 2.35 | 2.39<br>(0.04) | 5.24 | 5.08 | 5.38  | 5.23<br>(0.15) | 14.93 | 14.84 | 14.67 | 14.81<br>(0.13) | 24.77<br>(0.07) |
| 10 µg/mL<br><i>A. incisus</i><br>extract  | 2.29  | 2.46 | 2.32 | 2.36<br>(0.09) | 4.79 | 4.89 | 5.12  | 4.93<br>(0.17) | 14.35 | 14.46 | 14.39 | 14.4<br>(0.06)  | 21.59<br>(0.05) |
| 40 µg/mL<br><i>A. incisus</i><br>extract  | 1.76  | 1.81 | 1.92 | 1.83<br>(0.08) | 4.23 | 4.31 | 4.39  | 4.31<br>(0.08) | 12.84 | 12.92 | 12.76 | 12.84<br>(0.08) | 20.85<br>(0.11) |
| 100 µg/mL<br><i>A. incisus</i><br>extract | 1.42  | 1.49 | 1.54 | 1.48<br>(0.06) | 3.56 | 3.61 | 3.64  | 3.60<br>(0.04) | 8.97  | 8.87  | 8.74  | 8.86<br>(0.12)  | 12.78<br>(0.09) |



## APPENDIX C DETERMINATION OF ANTIOXIDANT ACTIVITY BY USING DPPH ASSAY

**Table 13** The antioxidant activity of *A. incisus* extract.

| Concentrations<br>( $\mu\text{g/mL}$ ) | Sample<br>No. | Absorbance (515 nm) |                 |                                |
|----------------------------------------|---------------|---------------------|-----------------|--------------------------------|
|                                        |               | Sample              | Blank of sample | Blank (DPPH<br>without sample) |
| 0.5                                    | 1             | 1.178               | 0.001           | 1.225                          |
|                                        | 2             | 1.158               | 0.001           | 1.185                          |
|                                        | 3             | 1.162               | 0.001           | 1.207                          |
| 1.0                                    | 1             | 1.121               | 0.001           | 1.225                          |
|                                        | 2             | 1.114               | 0.001           | 1.185                          |
|                                        | 3             | 1.112               | 0.001           | 1.207                          |
| 5.0                                    | 1             | 1.089               | 0.002           | 1.225                          |
|                                        | 2             | 1.091               | 0.002           | 1.185                          |
|                                        | 3             | 1.061               | 0.002           | 1.207                          |
| 10                                     | 1             | 1.069               | 0.002           | 1.225                          |
|                                        | 2             | 1.051               | 0.002           | 1.185                          |
|                                        | 3             | 1.005               | 0.003           | 1.207                          |
| 25                                     | 1             | 0.982               | 0.003           | 1.225                          |
|                                        | 2             | 0.953               | 0.004           | 1.185                          |
|                                        | 3             | 0.979               | 0.005           | 1.207                          |
| 50                                     | 1             | 0.769               | 0.005           | 1.225                          |
|                                        | 2             | 0.782               | 0.006           | 1.185                          |
|                                        | 3             | 0.759               | 0.005           | 1.207                          |

Table 13 (Cont.)

| Concentrations<br>( $\mu\text{g/mL}$ ) | Sample<br>No. | Absorbance (515 nm) |                 |                                |
|----------------------------------------|---------------|---------------------|-----------------|--------------------------------|
|                                        |               | Sample              | Blank of sample | Blank (DPPH<br>without sample) |
| 100                                    | 1             | 0.506               | 0.009           | 1.225                          |
|                                        | 2             | 0.546               | 0.100           | 1.185                          |
|                                        | 3             | 0.531               | 0.110           | 1.207                          |
| 200                                    | 1             | 0.553               | 0.201           | 1.225                          |
|                                        | 2             | 0.502               | 0.203           | 1.185                          |
|                                        | 3             | 0.54                | 0.203           | 1.207                          |
| 500                                    | 1             | 0.412               | 0.215           | 1.225                          |
|                                        | 2             | 0.396               | 0.212           | 1.185                          |
|                                        | 3             | 0.403               | 0.211           | 1.207                          |
| 2000                                   | 1             | 0.337               | 0.270           | 1.225                          |
|                                        | 2             | 0.336               | 0.250           | 1.185                          |
|                                        | 3             | 0.327               | 0.250           | 1.207                          |

Table 14 The antioxidant activity of L-ascorbic acid.

| Concentrations<br>( $\mu\text{g/mL}$ ) | Sample<br>No. | Absorbance (515 nm) |                 |                                |
|----------------------------------------|---------------|---------------------|-----------------|--------------------------------|
|                                        |               | Sample              | Blank of sample | Blank (DPPH<br>without sample) |
| 0.25                                   | 1             | 1.215               | 0.001           | 1.326                          |
|                                        | 2             | 1.199               | 0.001           | 1.231                          |
|                                        | 3             | 1.231               | 0.001           | 1.351                          |
| 0.50                                   | 1             | 1.115               | 0.001           | 1.326                          |
|                                        | 2             | 1.108               | 0.001           | 1.231                          |
|                                        | 3             | 1.106               | 0.001           | 1.351                          |

Table 14 (Cont.)

| Concentrations<br>( $\mu\text{g/mL}$ ) | Sample<br>No. | Absorbance (515 nm) |                 |                                |
|----------------------------------------|---------------|---------------------|-----------------|--------------------------------|
|                                        |               | Sample              | Blank of sample | Blank (DPPH<br>without sample) |
| 2.5                                    | 1             | 0.781               | 0.001           | 1.326                          |
|                                        | 2             | 0.759               | 0.001           | 1.231                          |
|                                        | 3             | 0.776               | 0.001           | 1.351                          |
| 5.0                                    | 1             | 0.398               | 0.001           | 1.326                          |
|                                        | 2             | 0.387               | 0.001           | 1.231                          |
|                                        | 3             | 0.401               | 0.001           | 1.351                          |
| 25                                     | 1             | 0.152               | 0.001           | 1.326                          |
|                                        | 2             | 0.16                | 0.001           | 1.231                          |
|                                        | 3             | 0.154               | 0.001           | 1.351                          |
| 50                                     | 1             | 0.075               | 0.001           | 1.326                          |
|                                        | 2             | 0.071               | 0.001           | 1.231                          |
|                                        | 3             | 0.069               | 0.001           | 1.351                          |
| 125                                    | 1             | 0.084               | 0.002           | 1.326                          |
|                                        | 2             | 0.08                | 0.002           | 1.231                          |
|                                        | 3             | 0.077               | 0.002           | 1.351                          |
| 250                                    | 1             | 0.084               | 0.002           | 1.326                          |
|                                        | 2             | 0.075               | 0.002           | 1.231                          |
|                                        | 3             | 0.078               | 0.002           | 1.351                          |
| 1000                                   | 1             | 0.079               | 0.002           | 1.326                          |
|                                        | 2             | 0.077               | 0.002           | 1.231                          |
|                                        | 3             | 0.081               | 0.002           | 1.351                          |
| 2500                                   | 1             | 0.08                | 0.006           | 1.326                          |
|                                        | 2             | 0.078               | 0.006           | 1.231                          |
|                                        | 3             | 0.076               | 0.007           | 1.351                          |

**Table 15 The antioxidant activity of butylated hydroxytoluene (BHT).**

| Concentrations<br>( $\mu\text{g/mL}$ ) | Sample<br>No. | Absorbance (515 nm) |                 |                             |
|----------------------------------------|---------------|---------------------|-----------------|-----------------------------|
|                                        |               | Sample              | Blank of sample | Blank (DPPH without sample) |
| 0.25                                   | 1             | 1.238               | 0.007           | 1.375                       |
|                                        | 2             | 1.165               | 0.007           | 1.381                       |
|                                        | 3             | 1.172               | 0.007           | 1.362                       |
| 0.50                                   | 1             | 1.166               | 0.009           | 1.375                       |
|                                        | 2             | 1.169               | 0.008           | 1.381                       |
|                                        | 3             | 1.158               | 0.009           | 1.362                       |
| 2.5                                    | 1             | 0.689               | 0.061           | 1.375                       |
|                                        | 2             | 0.671               | 0.065           | 1.381                       |
|                                        | 3             | 0.663               | 0.068           | 1.362                       |
| 5.0                                    | 1             | 0.385               | 0.103           | 1.375                       |
|                                        | 2             | 0.409               | 0.098           | 1.381                       |
|                                        | 3             | 0.396               | 0.101           | 1.362                       |
| 25                                     | 1             | 0.108               | 0.047           | 1.375                       |
|                                        | 2             | 0.128               | 0.045           | 1.381                       |
|                                        | 3             | 0.184               | 0.055           | 1.362                       |
| 50                                     | 1             | 0.098               | 0.029           | 1.375                       |
|                                        | 2             | 0.101               | 0.028           | 1.381                       |
|                                        | 3             | 0.105               | 0.033           | 1.362                       |
| 125                                    | 1             | 1.238               | 0.007           | 1.375                       |
|                                        | 2             | 1.165               | 0.007           | 1.381                       |
|                                        | 3             | 1.172               | 0.007           | 1.362                       |

Table 15 (Cont.)

| Concentrations<br>( $\mu\text{g/mL}$ ) | Sample<br>No. | Absorbance (515 nm) |                 |                             |
|----------------------------------------|---------------|---------------------|-----------------|-----------------------------|
|                                        |               | Sample              | Blank of sample | Blank (DPPH without sample) |
| 250                                    | 1             | 0.074               | 0.013           | 1.375                       |
|                                        | 2             | 0.079               | 0.008           | 1.381                       |
|                                        | 3             | 0.064               | 0.010           | 1.362                       |
| 1000                                   | 1             | 0.079               | 0.043           | 1.375                       |
|                                        | 2             | 0.092               | 0.048           | 1.381                       |
|                                        | 3             | 0.086               | 0.041           | 1.362                       |
| 2500                                   | 1             | 0.076               | 0.035           | 1.375                       |
|                                        | 2             | 0.064               | 0.031           | 1.381                       |
|                                        | 3             | 0.079               | 0.032           | 1.362                       |

#### The calculation of % free radical scavenging

##### Example

At the concentration of 2000  $\mu\text{g/mL}$  *A. incisus* extract dissolved in DMSO, the % free radical scavenging is calculated by using the equation:

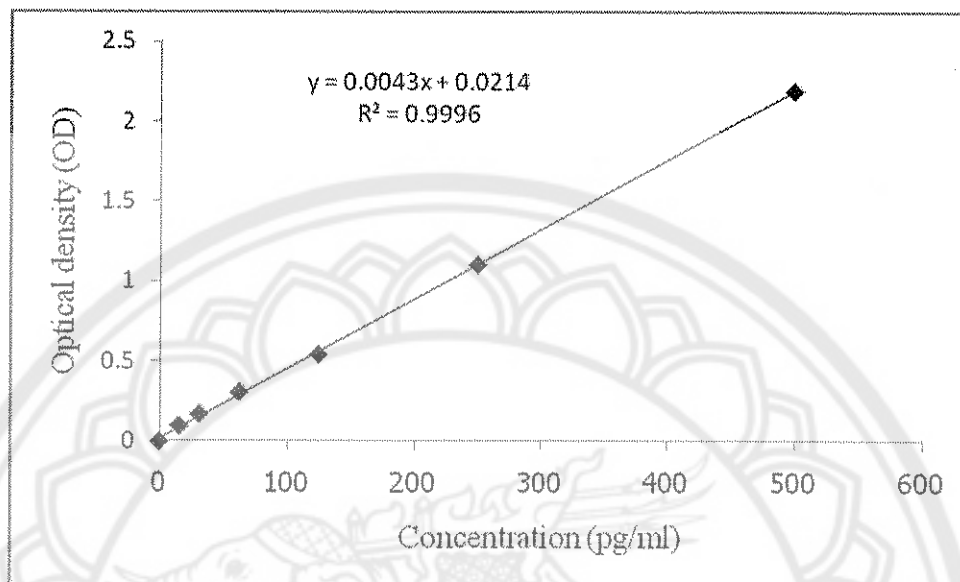
$$\% \text{ Free radical scavenging} = [ 1 - ( A_S / A_B ) ] \times 100$$

Where;  $A_S$  is an absorbance of DPPH with tested sample

$A_B$  is an absorbance of DPPH without tested sample

$$\begin{aligned}
 \text{Therefore, \% Free radical scavenging} &= [ 1 - ( (0.327-0.250) / 1.207 ) ] \times 100 \\
 &= [ 1 - ( 0.077 / 1.207 ) ] \times 100 \\
 &= 93.6
 \end{aligned}$$

## APPENDIX D DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY



Where;  $y$  = Optical density  
 $x$  = Concentration (pg/mL)

Figure 46 The standard curve of mouse TNF- $\alpha$  ELISA Ready-SET-Go.



Table 16 (cont.)

| Concentration<br>( $\mu\text{g/mL}$ ) | Type            | Absorbance (490 nm) |        |        | % TNF- $\alpha$ release |      |            |
|---------------------------------------|-----------------|---------------------|--------|--------|-------------------------|------|------------|
|                                       |                 | 1                   | 2      | 3      | 1                       | 2    | 3          |
| 120                                   | Sample          | 0.5365              | 0.528  | 0.5157 |                         |      |            |
|                                       | Blank of sample | 0.123               | 0.142  | 0.140  | 54.3                    | 50.5 | 49.1       |
|                                       | $\Delta$        | 0.4135              | 0.386  | 0.3757 |                         |      | 51.3 (2.7) |
| 140                                   | Sample          | 0.4999              | 0.4498 | 0.4516 |                         |      |            |
|                                       | Blank of sample | 0.170               | 0.154  | 0.162  | 42.8                    | 38.0 | 37.2       |
|                                       | $\Delta$        | 0.3299              | 0.2958 | 0.2896 |                         |      | 39.3 (3.0) |
| 160                                   | Sample          | 0.4585              | 0.4443 | 0.4277 |                         |      |            |
|                                       | Blank of sample | 0.192               | 0.187  | 0.181  | 34.1                    | 32.7 | 31.2       |
|                                       | $\Delta$        | 0.2665              | 0.2573 | 0.2467 |                         |      | 32.6 (1.4) |

**Note:**  $\Delta$  represents to the different absorbance between sample and blank of sample.



Table 17 (cont.)

| Concentration<br>n<br>(µg/mL) | Type            | Absorbance (490 nm) |        |        | % TNF-α release |      |            |
|-------------------------------|-----------------|---------------------|--------|--------|-----------------|------|------------|
|                               |                 | 1                   | 2      | 3      | 1               | 2    | 3          |
| 25                            | Sample          | 0.3835              | 0.3459 | 0.3783 |                 |      |            |
|                               | Blank of sample | 0.002               | 0.001  | 0.002  | 49.9            | 44.8 | 49.2       |
|                               | Δ               | 0.3815              | 0.3449 | 0.3763 |                 |      | 48.0 (2.7) |
| 30                            | Sample          | 0.2766              | 0.3063 | 0.3282 |                 |      |            |
|                               | Blank of sample | 0.002               | 0.002  | 0.002  | 35.1            | 39.2 | 42.2       |
|                               | Δ               | 0.2746              | 0.3043 | 0.3262 |                 |      | 38.8 (3.6) |
| 35                            | Sample          | 0.2343              | 0.2124 | 0.2226 |                 |      |            |
|                               | Blank of sample | 0.002               | 0.002  | 0.001  | 29.2            | 26.2 | 27.7       |
|                               | Δ               | 0.2323              | 0.2104 | 0.2216 |                 |      | 27.7 (1.5) |

Note: Δ represents to the different absorbance between sample and blank of sample.



Table 18 (cont.)

| Concentration<br>( $\mu\text{g/mL}$ ) | Type            | Absorbance (490 nm) |        |        | %Cell viability |      |            |
|---------------------------------------|-----------------|---------------------|--------|--------|-----------------|------|------------|
|                                       |                 | 1                   | 2      | 3      | 1               | 2    | 3          |
| 120                                   | Sample          | 0.7772              | 0.7781 | 0.7625 |                 |      |            |
|                                       | Blank of sample | 0.106               | 0.109  | 0.108  | 90.1            | 89.8 | 87.7       |
|                                       | $\Delta$        | 0.6712              | 0.6691 | 0.6545 |                 |      | 89.2 (1.3) |
| 140                                   | Sample          | 0.7817              | 0.7894 | 0.7759 |                 |      |            |
|                                       | Blank of sample | 0.123               | 0.126  | 0.122  | 88.3            | 89.0 | 87.7       |
|                                       | $\Delta$        | 0.6587              | 0.6634 | 0.6539 |                 |      | 88.3 (0.6) |
| 160                                   | Sample          | 0.7793              | 0.7828 | 0.7737 |                 |      |            |
|                                       | Blank of sample | 0.147               | 0.140  | 0.145  | 84.7            | 86.1 | 84.2       |
|                                       | $\Delta$        | 0.6323              | 0.6428 | 0.6287 |                 |      | 85.0 (1.0) |

**Note:**  $\Delta$  represents to the different absorbance between sample and blank of sample.



Table 19 (cont.)

| Concentration<br>( $\mu\text{g/mL}$ ) | Type            | Absorbance (490 nm) |        |        | %Cell viability |      |            |
|---------------------------------------|-----------------|---------------------|--------|--------|-----------------|------|------------|
|                                       |                 | 1                   | 2      | 3      | 1               | 2    | 3          |
| 25                                    | Sample          | 0.6587              | 0.6698 | 0.6443 |                 |      |            |
|                                       | Blank of sample | 0.002               | 0.002  | 0.002  | 88.1            | 89.6 | 86.1       |
|                                       | $\Delta$        | 0.6567              | 0.6678 | 0.6423 |                 |      | 87.9 (1.8) |
| 30                                    | Sample          | 0.6377              | 0.6208 | 0.6265 |                 |      |            |
|                                       | Blank of sample | 0.002               | 0.001  | 0.002  | 85.1            | 82.9 | 83.6       |
|                                       | $\Delta$        | 0.6357              | 0.6198 | 0.6245 |                 |      | 83.9 (1.1) |
| 35                                    | Sample          | 0.5962              | 0.6027 | 0.6068 |                 |      |            |
|                                       | Blank of sample | 0.002               | 0.002  | 0.002  | 79.4            | 80.3 | 80.9       |
|                                       | $\Delta$        | 0.5942              | 0.6007 | 0.6048 |                 |      | 80.2 (0.7) |

**Note:**  $\Delta$  represents to the different absorbance between sample and blank of sample.

## APPENDIX E DEVELOPMENT OF NANOEMULSION CONTAINING A. *INCISUS* EXTRACT

**Table 20 Mean droplet size and polydispersity index value of nanoemulsions  
formulated with cetareth-10.**

| Formulation<br>cetareth-10 | Mean droplet size (nm) |     |     |         | Polydispersity index (PI) |      |      |           |
|----------------------------|------------------------|-----|-----|---------|---------------------------|------|------|-----------|
|                            | 1                      | 2   | 3   | Mean±SD | 1                         | 2    | 3    | Mean±SD   |
| C 4% + G 5%                | 512                    | 533 | 492 | 512±20  | 0.51                      | 0.46 | 0.48 | 0.48±0.03 |
| C 8% + G 5%                | 309                    | 326 | 339 | 325±15  | 0.32                      | 0.29 | 0.31 | 0.31±0.02 |
| C 10% + G 5%               | 414                    | 448 | 421 | 428±18  | 0.43                      | 0.38 | 0.40 | 0.40±0.02 |
| C 12% + G 5%               | 659                    | 634 | 676 | 656±21  | 0.65                      | 0.59 | 0.62 | 0.62±0.03 |
| C 16% + G 5%               | 875                    | 857 | 892 | 875±18  | 0.87                      | 0.78 | 0.83 | 0.83±0.04 |
| C 8% + G 1%                | 395                    | 410 | 435 | 413±20  | 0.41                      | 0.37 | 0.38 | 0.39±0.02 |
| C 8% + G 2%                | 372                    | 388 | 408 | 389±18  | 0.39                      | 0.35 | 0.36 | 0.37±0.02 |
| C 8% + G 3%                | 364                    | 358 | 373 | 365±8   | 0.36                      | 0.33 | 0.34 | 0.34±0.02 |
| C 8% + G 4%                | 335                    | 358 | 321 | 338±19  | 0.34                      | 0.30 | 0.32 | 0.32±0.02 |
| C 8% + G 5%                | 309                    | 326 | 339 | 325±15  | 0.32                      | 0.29 | 0.31 | 0.31±0.02 |
| C 8% + G 6%                | 612                    | 638 | 607 | 619±17  | 0.62                      | 0.55 | 0.58 | 0.58±0.03 |
| C 8% + G 7%                | 742                    | 763 | 725 | 743±19  | 0.74                      | 0.66 | 0.71 | 0.70±0.03 |
| C 8% + G 8%                | 956                    | 978 | 932 | 955±23  | 0.95                      | 0.85 | 0.90 | 0.90±0.04 |

**Note:** C = Cetareth-10, G = GMS.

**Table 21 Mean droplet size and polydispersity index value of nanoemulsions formulated with cetareth-20.**

| Formulation<br>cetareth-10 | Mean droplet size (nm) |     |     |         | Polydispersity index (PI) |      |      |           |
|----------------------------|------------------------|-----|-----|---------|---------------------------|------|------|-----------|
|                            | 1                      | 2   | 3   | Mean±SD | 1                         | 2    | 3    | Mean±SD   |
| C 4% + G 5%                | 493                    | 513 | 526 | 511±17  | 0.51                      | 0.46 | 0.48 | 0.48±0.03 |
| C 8% + G 5%                | 527                    | 542 | 518 | 529±12  | 0.53                      | 0.47 | 0.49 | 0.50±0.03 |
| C 10% + G 5%               | 642                    | 671 | 656 | 656±15  | 0.65                      | 0.59 | 0.62 | 0.62±0.03 |
| C 12% + G 5%               | 754                    | 767 | 781 | 767±14  | 0.76                      | 0.69 | 0.74 | 0.73±0.04 |
| C 16% + G 5%               | 804                    | 837 | 813 | 818±17  | 0.81                      | 0.73 | 0.76 | 0.77±0.04 |
| C 8% + G 1%                | 702                    | 729 | 682 | 704±24  | 0.70                      | 0.63 | 0.66 | 0.66±0.03 |
| C 8% + G 2%                | 651                    | 641 | 667 | 653±13  | 0.65                      | 0.58 | 0.63 | 0.62±0.03 |
| C 8% + G 3%                | 612                    | 633 | 601 | 615±16  | 0.61                      | 0.55 | 0.58 | 0.58±0.03 |
| C 8% + G 4%                | 536                    | 511 | 521 | 523±13  | 0.52                      | 0.47 | 0.48 | 0.49±0.03 |
| C 8% + G 5%                | 584                    | 553 | 568 | 568±16  | 0.56                      | 0.51 | 0.53 | 0.53±0.03 |
| C 8% + G 6%                | 745                    | 721 | 732 | 733±12  | 0.73                      | 0.66 | 0.69 | 0.69±0.04 |
| C 8% + G 7%                | 691                    | 683 | 712 | 695±15  | 0.69                      | 0.62 | 0.66 | 0.66±0.03 |
| C 8% + G 8%                | 868                    | 864 | 843 | 858±13  | 0.85                      | 0.77 | 0.81 | 0.81±0.04 |

Note: C = Cetareth-20, G = GMS.

**Table 22 Artocarpin remaining in nanoemulsion under 7 heat-cool cycles and after 1-3 months.**

| Condition | Sample No. | Peak area | Artocarpin content (mg) | % Artocarpin remaining | Mean±SD    |
|-----------|------------|-----------|-------------------------|------------------------|------------|
| Intitial  | 1          | 1907023   | 0.01416                 | 100                    | 100        |
|           | 2          | 1925168   | 0.01430                 |                        |            |
|           | 3          | 1912547   | 0.01420                 |                        |            |
| Heat cool | 1          | 1636735   | 0.01199                 | 84.46                  | 84.54±0.32 |
|           | 2          | 1638019   | 0.01200                 | 84.54                  |            |
|           | 3          | 1639723   | 0.01202                 | 84.63                  |            |
| 1 mo.     | 1          | 1885143   | 0.01398                 | 98.46                  | 98.61±0.08 |
|           | 2          | 1894376   | 0.01406                 | 98.98                  |            |
|           | 3          | 1883895   | 0.01397                 | 98.39                  |            |
| 2 mo.     | 1          | 1840242   | 0.01362                 | 95.93                  | 95.99±0.20 |
|           | 2          | 1840842   | 0.01363                 | 95.96                  |            |
|           | 3          | 1842880   | 0.01364                 | 96.08                  |            |
| 3 mo.     | 1          | 1792434   | 0.01324                 | 93.23                  | 93.01±0.08 |
|           | 2          | 1787348   | 0.01320                 | 92.95                  |            |
|           | 3          | 1785764   | 0.01319                 | 92.86                  |            |

**Table 23 Droplet size of nanoemulsion under 7 heat-cool cycles and after 1-3 months.**

| Condition | Sample No. | Droplet size (nm) | Mean±SD |
|-----------|------------|-------------------|---------|
| Intitial  | 1          | 309               | 325±15  |
|           | 2          | 326               |         |
|           | 3          | 339               |         |
| Heat-cool | 1          | 342               | 347±19  |
|           | 2          | 368               |         |
|           | 3          | 331               |         |
| 1 mo.     | 1          | 372               | 354±17  |
|           | 2          | 351               |         |
|           | 3          | 338               |         |
| 2 mo.     | 1          | 351               | 355±24  |
|           | 2          | 334               |         |
|           | 3          | 381               |         |
| 3 mo.     | 1          | 428               | 453±27  |
|           | 2          | 451               |         |
|           | 3          | 481               |         |

**Table 24 Viscosity of nanoemulsion containing *A. incisus* extract under 7 heat-cool cycles and after 1-3 months.**

| Condition | Sample No. | Viscosity (cps) | Mean±SD |
|-----------|------------|-----------------|---------|
| Intitial  | 1          | 766             | 749±17  |
|           | 2          | 748             |         |
|           | 3          | 732             |         |
| Heat cool | 1          | 732             | 726±9   |
|           | 2          | 716             |         |
|           | 3          | 729             |         |
| 1 mo.     | 1          | 723             | 708±14  |
|           | 2          | 695             |         |
|           | 3          | 706             |         |
| 2 mo.     | 1          | 711             | 688±21  |
|           | 2          | 682             |         |
|           | 3          | 671             |         |
| 3 mo.     | 1          | 695             | 673±20  |
|           | 2          | 666             |         |
|           | 3          | 657             |         |

**Table 25** pH values of nanoemulsion containing *A. incisus* extract under 7 heat-cool cycles and after 1-3 months.

| Condition | Sample No. | pH values | Mean±SD   |
|-----------|------------|-----------|-----------|
| Intital   | 1          | 5.52      | 5.52±0.01 |
|           | 2          | 5.53      |           |
|           | 3          | 5.51      |           |
| Heat cool | 1          | 5.53      | 5.53±0.01 |
|           | 2          | 5.52      |           |
|           | 3          | 5.53      |           |
| 1 mo.     | 1          | 5.52      | 5.52±0.01 |
|           | 2          | 5.53      |           |
|           | 3          | 5.52      |           |
| 2 mo.     | 1          | 5.53      | 5.53±0.01 |
|           | 2          | 5.54      |           |
|           | 3          | 5.52      |           |
| 3 mo.     | 1          | 5.51      | 5.52±0.01 |
|           | 2          | 5.52      |           |
|           | 3          | 5.51      |           |

## APPENDIX F *IN VITRO* PERMEATION STUDIES THROUGH MOUSE SKIN

**Table 26 The percentage of cumulative of artocarpin release of nanoemulsion were determined within 24 hr.**

| Time<br>(hr) | Peak area |        |        | Artocarpin concentration<br>(mg/mL) |                       |                       | % drug release |       |       |       |     |
|--------------|-----------|--------|--------|-------------------------------------|-----------------------|-----------------------|----------------|-------|-------|-------|-----|
|              | 1         | 2      | 3      | 1                                   | 2                     | 3                     | 1              | 2     | 3     | mean  | SD  |
| 0.25         | 138946    | 138797 | 139032 | $1.16 \times 10^{-5}$               | $1.04 \times 10^{-5}$ | $1.23 \times 10^{-5}$ | 12.68          | 11.37 | 13.43 | 12.49 | 1.0 |
| 0.5          | 139867    | 139727 | 140152 | $1.89 \times 10^{-5}$               | $1.78 \times 10^{-5}$ | $2.08 \times 10^{-5}$ | 21.54          | 20.23 | 23.62 | 21.80 | 1.7 |
| 1            | 140828    | 141050 | 141135 | $2.66 \times 10^{-5}$               | $2.36 \times 10^{-5}$ | $2.88 \times 10^{-5}$ | 31.27          | 27.79 | 33.85 | 30.97 | 3.0 |
| 3            | 141945    | 141882 | 142029 | $3.08 \times 10^{-5}$               | $3.19 \times 10^{-5}$ | $3.62 \times 10^{-5}$ | 37.62          | 38.46 | 43.94 | 40.01 | 3.4 |
| 6            | 142635    | 142434 | 142649 | $3.71 \times 10^{-5}$               | $3.55 \times 10^{-5}$ | $3.88 \times 10^{-5}$ | 46.65          | 44.60 | 49.23 | 46.83 | 2.3 |
| 9            | 144159    | 143931 | 144253 | $4.69 \times 10^{-5}$               | $4.34 \times 10^{-5}$ | $4.84 \times 10^{-5}$ | 59.93          | 55.77 | 62.44 | 59.38 | 3.4 |
| 12           | 145173    | 144831 | 145295 | $5.42 \times 10^{-5}$               | $5.18 \times 10^{-5}$ | $5.44 \times 10^{-5}$ | 71.15          | 67.95 | 72.26 | 70.46 | 2.2 |
| 16           | 146047    | 145948 | 146430 | $5.96 \times 10^{-5}$               | $5.48 \times 10^{-5}$ | $6.02 \times 10^{-5}$ | 80.77          | 74.72 | 82.43 | 79.31 | 4.1 |
| 24           | 147050    | 146906 | 146610 | $6.04 \times 10^{-5}$               | $6.24 \times 10^{-5}$ | $6.25 \times 10^{-5}$ | 85.75          | 86.87 | 89.01 | 87.21 | 1.6 |

**Table 27 The percentage of cumulative of artocarpin release of solution were determined within 24 hr.**

| Time<br>(hr) | Peak area |        |        | Artocarpin concentration<br>(mg/mL) |                       |                       | % drug release |       |       |       |     |
|--------------|-----------|--------|--------|-------------------------------------|-----------------------|-----------------------|----------------|-------|-------|-------|-----|
|              | 1         | 2      | 3      | 1                                   | 2                     | 3                     | 1              | 2     | 3     | mean  | SD  |
| 0.25         | 138393    | 138466 | 138427 | $7.14 \times 10^{-6}$               | $7.73 \times 10^{-6}$ | $7.42 \times 10^{-6}$ | 7.83           | 8.47  | 8.13  | 8.14  | 0.3 |
| 0.5          | 139093    | 139066 | 139112 | $1.27 \times 10^{-5}$               | $1.25 \times 10^{-5}$ | $1.29 \times 10^{-5}$ | 14.46          | 14.26 | 14.64 | 14.45 | 0.2 |
| 1            | 139666    | 139522 | 139652 | $1.73 \times 10^{-5}$               | $1.61 \times 10^{-5}$ | $1.72 \times 10^{-5}$ | 20.35          | 19.11 | 20.26 | 19.91 | 0.7 |
| 3            | 140554    | 140418 | 140450 | $2.44 \times 10^{-5}$               | $2.33 \times 10^{-5}$ | $2.36 \times 10^{-5}$ | 29.32          | 28.08 | 28.43 | 28.61 | 0.6 |
| 6            | 141022    | 141159 | 140983 | $2.82 \times 10^{-5}$               | $2.93 \times 10^{-5}$ | $2.79 \times 10^{-5}$ | 35.10          | 36.17 | 34.72 | 35.33 | 0.8 |
| 12           | 141538    | 141976 | 141273 | $3.24 \times 10^{-5}$               | $3.58 \times 10^{-5}$ | $3.02 \times 10^{-5}$ | 41.55          | 45.34 | 39.17 | 42.02 | 3.1 |
| 9            | 142060    | 142210 | 142490 | $3.65 \times 10^{-5}$               | $3.77 \times 10^{-5}$ | $3.99 \times 10^{-5}$ | 48.34          | 49.84 | 51.91 | 50.03 | 1.8 |
| 18           | 142467    | 143170 | 142961 | $3.97 \times 10^{-5}$               | $4.54 \times 10^{-5}$ | $4.37 \times 10^{-5}$ | 54.41          | 60.84 | 58.77 | 58.01 | 3.3 |
| 24           | 143015    | 143459 | 143135 | $4.41 \times 10^{-5}$               | $4.77 \times 10^{-5}$ | $4.51 \times 10^{-5}$ | 61.94          | 66.48 | 63.29 | 63.90 | 2.3 |

## APPENDIX G *IN VIVO* STUDIES ON C57BL/6 MICE WITH UVB-INDUCED HYPERPIGMENTATION

**Table 28** The Effect of nanoemulsion containing *A. incisus* extract and *A. incisus* extract solution on UVB-induced hyperpigmentation in C57BL/6 mice skin.

| Formulation  | Time (week) | Melanin values |     |     |         |
|--------------|-------------|----------------|-----|-----|---------|
|              |             | 1              | 2   | 3   | mean±SD |
| nanoemulsion | 0           | 376            | 382 | 369 | 376±7   |
|              | 1           | 365            | 370 | 373 | 369±4   |
|              | 2           | 352            | 358 | 361 | 357±5   |
|              | 3           | 340            | 348 | 332 | 340±8   |
|              | 4           | 312            | 318 | 322 | 317±5   |
|              | 5           | 309            | 302 | 310 | 307±4   |
|              | 6           | 297            | 292 | 286 | 292±6   |
| solution     | 0           | 378            | 381 | 367 | 375±7   |
|              | 1           | 374            | 371 | 369 | 371±3   |
|              | 2           | 365            | 368 | 370 | 368±3   |
|              | 3           | 358            | 350 | 355 | 354±4   |
|              | 4           | 348            | 346 | 340 | 345±4   |
|              | 5           | 335            | 332 | 336 | 334±2   |
|              | 6           | 328            | 325 | 319 | 324±5   |