Appendices

Appendix A: Real-time quantitative reverse transcription-PCR (RT-PCR)

A1: RNA extraction protocol
1. Disrupt samples in Lysis/Binding Solution
2. Add an equal volume of 64% Ethanol and mix
3. Draw the lysate/ethanol mixture through a Filter Cartridge
4. Wash with 700µl Wash Solution #1
5. Wash with 2 x 500µl Wash Solution #2/3
6. Elute RNA with 40-60µl preheated Elution Solution
7. Elute with a second 10-60µl aliquot of Elution Solution

A2: Reverse transcription of RNA to cDNA
Reaction Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per Reaction</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Master Mix</td>
<td>4µl</td>
<td>1X</td>
</tr>
<tr>
<td>RNA</td>
<td>_µl</td>
<td>1µg</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>_µl</td>
<td>-</td>
</tr>
<tr>
<td>Total volume</td>
<td>20µl</td>
<td>-</td>
</tr>
</tbody>
</table>

* Volume needed for 1µg RNA
** Volume needed after subtracted volume of Complete Master Mix and RNA

Thermal cycler conditions
Step 1: 5 minutes at 25°C
Step 2: 30 minutes at 42°C
Step 3: 5 minutes at 85°C
Step 4: Hold at 4°C

A3: Gene expression analysis
Reaction Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per Reaction</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taqman Gene Expression Master Mix</td>
<td>5µl</td>
<td>1X</td>
</tr>
<tr>
<td>cDNA</td>
<td>1µl</td>
<td>-</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>4µl</td>
<td>-</td>
</tr>
<tr>
<td>Total volume</td>
<td>10µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Thermal cycler conditions
Step 1: 2 minutes at 50°C
Step 2: 10 minutes at 95°C
Step 3: 40 cycles of 15 seconds at 95°C and 1 minutes at 60°C
Appendix B: Graph of gallic acid standard

The phenolic content of F.deltoidea’s extracts and its fractions were calculated based on the equation y=0.0178x.
Appendix C: Dose-response curve of samples and ascorbic acid in DPPH assays

C1: Crude sample curve

![Crude sample dose-response curve]

C2: Fraction 30 curve

![Fraction 30 dose-response curve]
C3: Fraction 60 curve

Fraction 60 dose-response curve

C4: Fraction 90 curve

Fraction 90 dose-response curve

C5: Ascorbic acid curve

Ascorbic acid curve
Appendix D: Dose-response curve of samples and ascorbic acid in lipid peroxidation assays

D1: Crude sample curve

![Crude sample curve](image1)

D2: Fraction 30 curve

![Fraction 30 dose response curve](image2)
D3: Fraction 60 curve

Fraction 60 dose response curve

D4: Fraction 90 curve

Fraction 90 dose response curve

D5: Ascorbic acid curve

Ascorbic acid curve
Appendix E: Viable cells curve upon different treatment

E1: Treatment of SF crude and fractions towards Ca Ski cells

E2: Treatment of SF crude and fractions towards Hep G2 cells

E3: Treatment of SF crude and fractions towards Chang Liver cells
E4: Treatment of doxorubicin towards different cell lines

Cell viability upon doxorubicin treatment

- **Ca Ski**
- **Hep G2**
- **Chang liver**
Appendix F: Catalase standard curve

The catalase concentration of cell lysate upon treatment were estimated and calculated based on the equation \( y = -4.4286x^2 + 5.7438x - 0.0172 \).

\[ R^2 = 0.9968 \]
Appendix G: DNA fragmentation results

G1: Ca Ski cells upon different treatments

(a) SF

Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml, Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

(b) SF30

(c) SF60

(d) SF90

(e) Doxorubicin

SF samples: left to right
Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml, Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

Doxorubicin: left to right
Lane 1: 100bp marker, Lane 2: 0.125µg/ml, Lane 3: 0.25µg/ml, Lane 4: 0.5µg/ml, Lane 5: 1µg/ml, Lane 6: 2µg/ml, Lane 7: control, Lane 8: 1kb marker.
G2: Hep G2 cells upon different treatments

(a) SF  
(b) SF30  
(c) SF60  
(d) SF90  
(e) Doxorubicin

SF samples: left to right
Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml, Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

Doxorubicin: left to right
Lane 1: 100bp marker, Lane 2: 0.125µg/ml, Lane 3: 0.25µg/ml, Lane 4: 0.5µg/ml, Lane 5: 1µg/ml, Lane 6: 2µg/ml, Lane 7: control, Lane 8: 1kb marker.
G3: Chang Liver cells upon different treatments

(a) SF  
(b) SF30  
(c) SF60  
(d) SF90  
(e) Doxorubicin

SF samples: left to right  
Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml,  
Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

Doxorubicin: left to right  
Lane 1: 100bp marker, Lane 2: 0.125µg/ml, Lane 3: 0.25µg/ml, Lane 4: 0.5µg/ml,  
Lane 5: 1µg/ml, Lane 6: 2µg/ml, Lane 7: control, Lane 8: 1kb marker.
Appendix H

H1: Hep G2 cells treated with SF30
H2: Hep G2 cells treated with SF60
H3: Hep G2 cells treated with SF90
H4: Hep G2 cells treated with doxorubicin
H5: Ca Ski cells treated with SF30
H6: Ca Ski cells treated with SF60
H7: Ca Ski cells treated with SF90
H8: Ca Ski cells treated with doxorubicin
H9: Chang Liver cells treated with SF30
H10: Chang Liver cells treated with SF60
H11: Chang Liver cells treated with SF90
H12: Chang Liver cells treated with doxorubicin