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APPENDICES

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APPENDIX A

NMR and IR spectra

1. NMR and IR spectra of the water extract of *S.venosa* tuber.

1.1 ^1H NMR (D_2O) spectra (Figure 11) showed the peaks at δ 3-5 (glucoside) and at δ 6-8 (aromatics such as coumarin, quinone or cinnamic acid derivatives).

1.2 IR (Neat) spectra (Figure 12) showed λ_{max} at 3422(OH), 2521 and 1638 cm^{-1}

Based on the ^1H NMR and IR spectral data of the water extract, its could possibly contain a mixture of glucoside and coumarin, quinone or cinnamic acid derivatives.

2. NMR and IR of the ethanol extract of *S.venosa* tuber.

1.3 ^1H NMR (CDCl_3) spectra (Figure 13) showed the peaks at δ 0.8-2.2 (long chain hydrocarbon) and δ 3-5.5 (glucoside).

1.4 IR(Neat) spectra (Figure 14) showed λ_{max} at 3374(OH), 2924, 2853(CH), 1713(CO ester), 1633,1499,1273 and 1074 cm^{-1}

Based on the ^1H NMR and IR spectral data of the ethanol extract, its could possibly contain a mixture of long chain hydrocarbon and glucoside.

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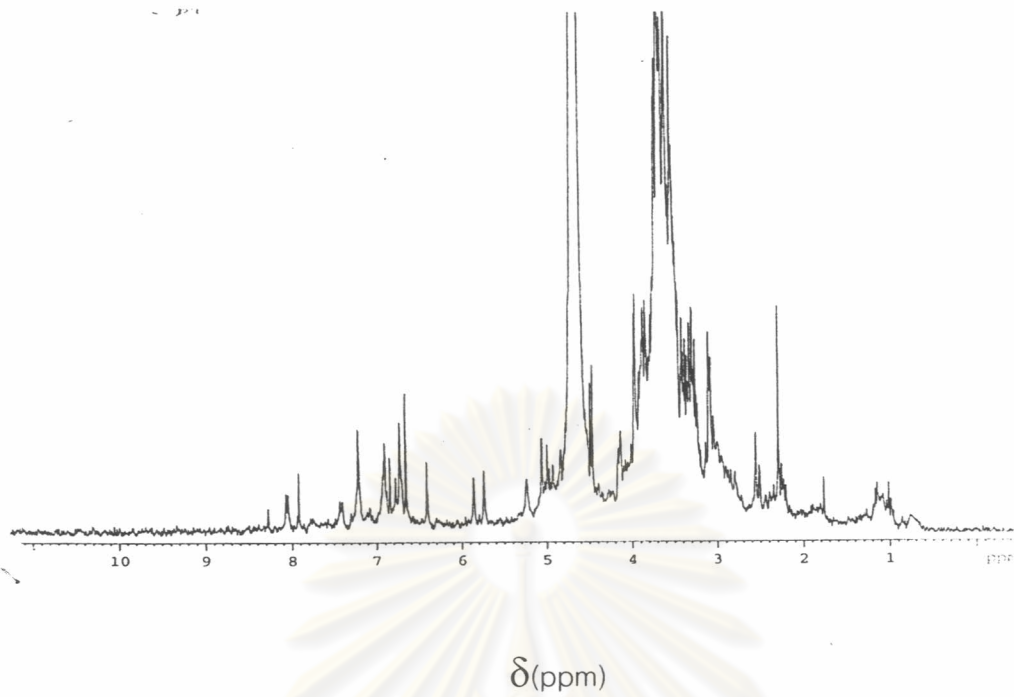


Figure 12. ^1H NMR of the water extract of *S.venosa*

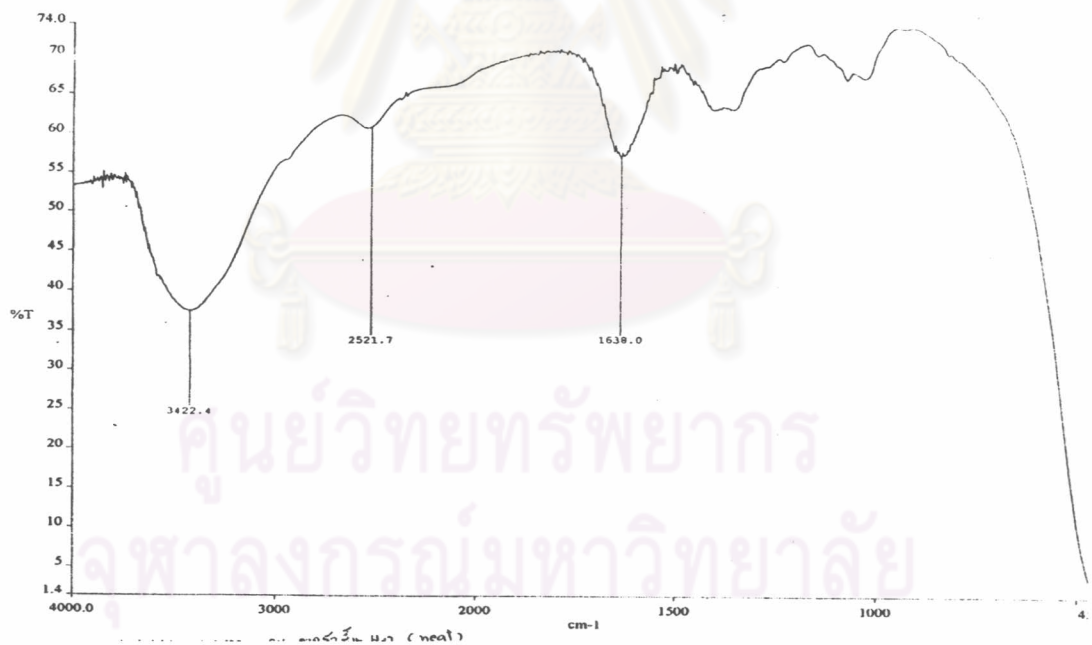


Figure 13. IR of the water extract of *S.venosa*

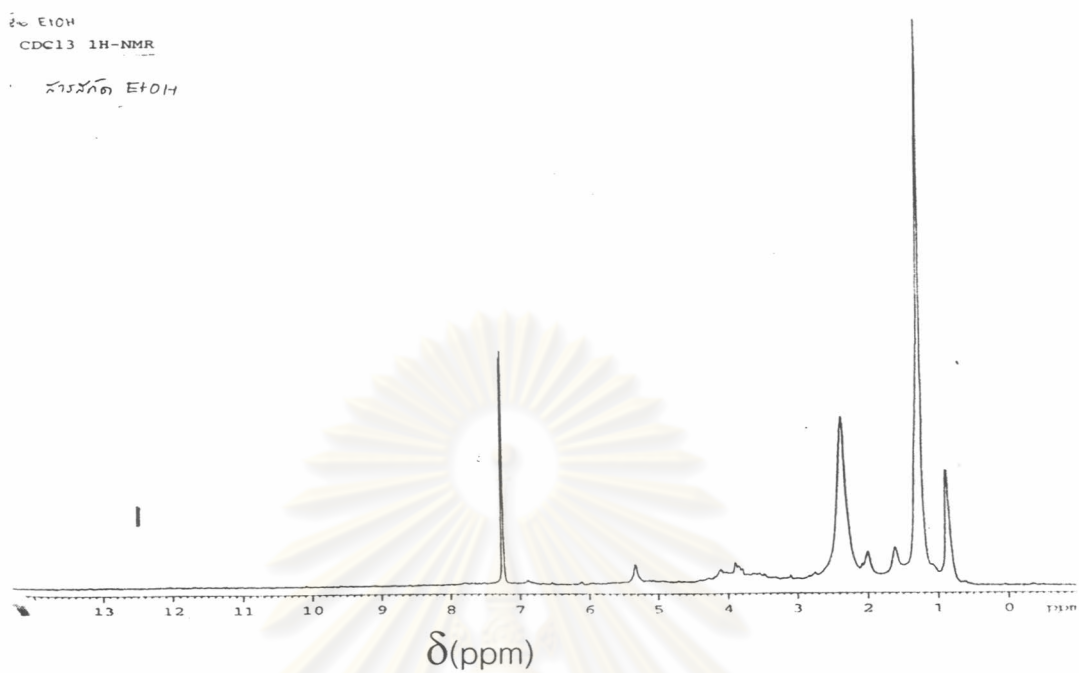


Figure 14. ^1H NMR of the ethanol extract of *S.venosa*.

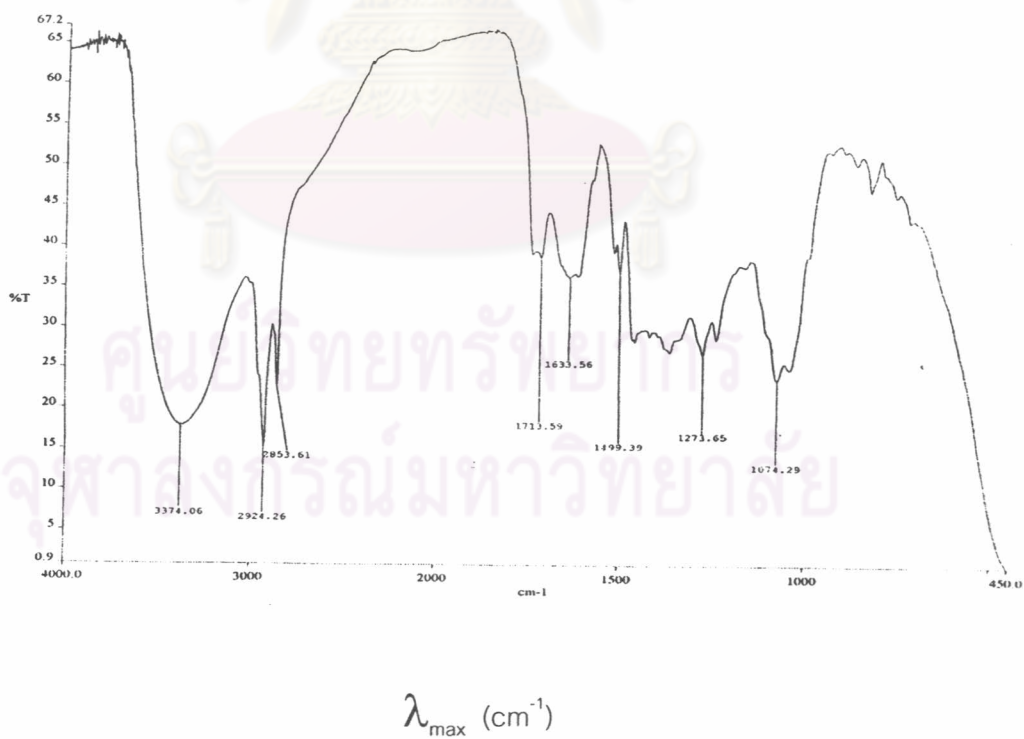


Figure 15. IR of the ethanol extract of *S.venosa*

APPENDIX B

Buffers and Reagents

1. RPMI¹⁶⁴⁰ stock solution 1 liter

RPMI powder	10.4 g
Na HCO ₃	1.5 g
Glucose	2.5 g
HEPES	10 ml
Penicilin/Streptomycin	10 ml
ddH ₂ O	900 ml

Adjust pH to 7.2 with 1 M HCl.

Add ddH₂O to 1 liter and sterilized by filtering through a 0.22 μ membrane filter.

2. HBSS stock solution 1 liter

HBSS powder	9.52 g
NaHCO ₃	0.35 g
ddH ₂ O	900 ml

Adjust pH to 7.2 with 1 M HCl.

Add ddH₂O to 1 liter and sterilized by filtering through a 0.22 μ membrane filter.

3. 10x Phosphate Buffered Saline (PBS) 1 liter

NaCl	80 g
KCl	2 g
Na ₂ HPO ₄	14.4 g
KH ₂ PO ₄	2.4 g
ddH ₂ O	900 ml

Adjust pH to 7.4 with 1 M HCl.

Sterilized by autoclaving

4. 1x Phosphate Buffer Saline (PBS) 1 liter
- | | |
|--------------------|--------|
| 10x PBS | 100 ml |
| ddH ₂ O | 900 ml |
- Sterilized by autoclaving.
5. 2 μ l / ml Heparin in HBSS
- | | |
|------------|------------|
| HBSS stock | 22.5 ml |
| Heparin | 45 μ l |
6. Complete RPMI ¹⁶⁴⁰ medium 30 ml.
- | | |
|--------------------|-------------|
| RPMI stock | 27 ml |
| L-Glutamine | 150 μ l |
| Fetal Bovine Serum | 3 ml |
7. HBSS/ 2 μ l/ml Heparin / 1% Fetal Bovine Serum
- | | |
|----------------------------|------------|
| HBSS | 33 ml |
| Heparin (5000i.u./u.i./ml) | 66 μ l |
| Fetal Bovine Serum | 0.33 ml |
8. 10x Assay buffer 10 ml
- | | |
|-------------------------|--------|
| 1 M HEPES pH 7.4 | 1 ml |
| 5 M NaCl | 2.8 ml |
| 0.1 M CaCl ₂ | 2.5 ml |
| ddH ₂ O | 4.7 ml |
9. 1x Assay buffer 10 ml
- | | |
|--------------------|------|
| 10x Assay buffer | 1 ml |
| ddH ₂ O | 9 ml |

APPENDIX C

Table 5. Cytotoxic effect of the DMSO at various concentrations by alamarBlue reduction assay on PBMCs. The data were represented as mean \pm S.E.M (n=10).

Conditions	% Cell death of water DMSO (Mean \pm SEM)
Negative control	0
0.1 % DMSO	0 \pm 1.24
0.25 % DMSO	3.64 \pm 1.02
0.5 % DMSO	4.64 \pm 1.93
1.5 μ g/ml doxorubicin (positive control)	52.35 \pm 2.83 *

*Significance when compared to the negative control group at $p < 0.05$

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BIOGRAPHY

Miss. Metta Kheiamsawang was born on September 17, 1977 in Nakhon Prathom province, Thailand. She received the Bachelor degree of Nursing Science (second class honors) in 1999 from Mahidol University, Bangkok, Thailand. She has enrolled at Chulalongkorn University in graduate program for the Degree of Master of Science in Pharmacology and graduated in 2004.



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