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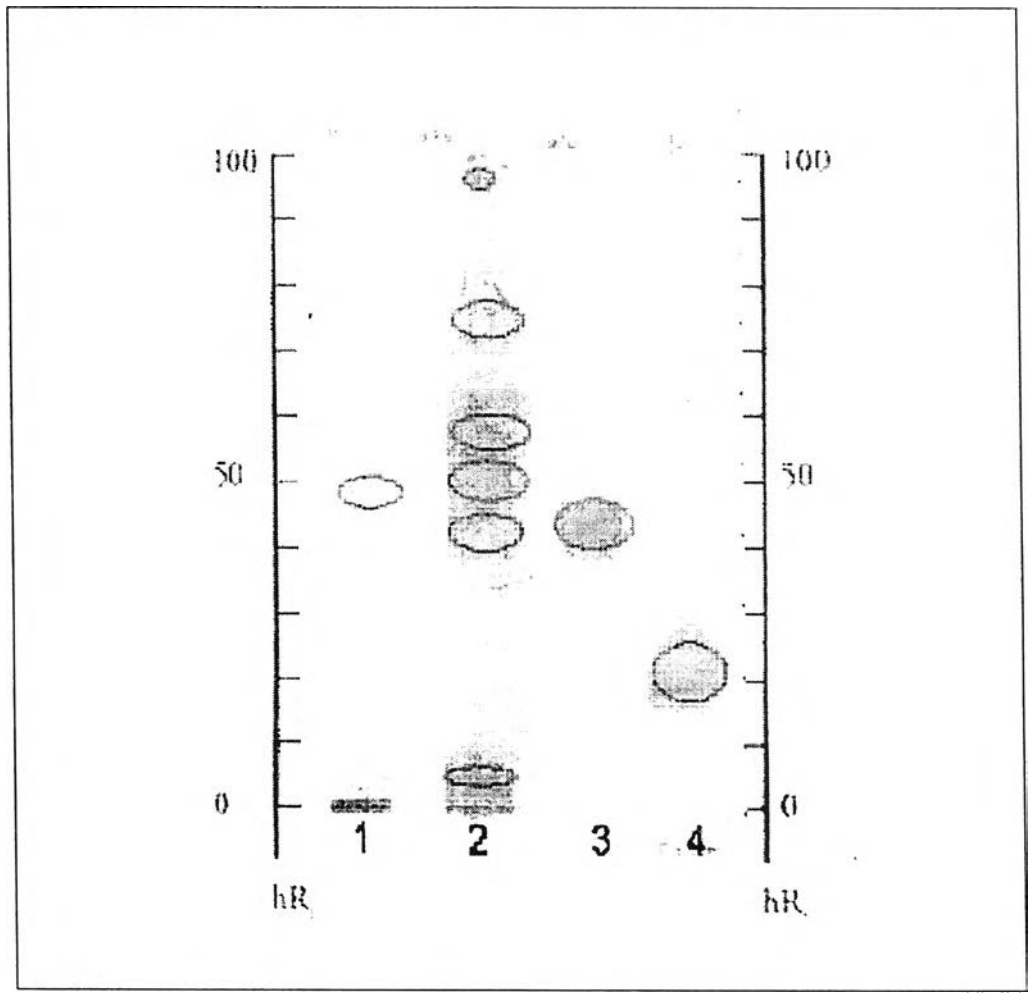
APPENDICES

APPENDIX A

Figure A Thin–layer chromatography of carbohydrates (TLC) fingerprint from *Scaphium scarphigerum* fruit extract



Adsorbent : Silica gel 60 F_{254} , precoated TLC plates (Merck, Darmstadt)

Solvent System : Dichloromethane-Methanol-Water 15 : 7 : 1

Detection : 0.2% Naphthoresorcinol in butanol and 10% phosphoric acid

Track 1 = *S. scaphigerum* fruit extract

Track 2 = Hydrolysed *S. scaphigerum* fruit extract by 2 M Trifluoroacetic acid

Track 3 = D (+) - Glucose

Tractk4 = Lactose

APPENDIX B

EXPERIMENTS RESULTS

Table A Mitogenic activity of PHA on human PBMCs by MTT assay. The results were expressed as the percentage of stimulation over untreated control (mean± S.E.M.), (n=3).

Sample	concentration (μg/ml)	% stimulation
PHA	5	21.84 ± 3.81 *
	10	53.08 ± 8.56 *
	50	102.32 ± 10.61*
	100	85.37 ± 4.30 *

Table B Mitogenic activity of *S. scaphogerum* fruit extract on PBMCs by MTT assay. The results were expressed as the percentage of stimulation over untreated control (mean± S.E.M.), (n=5). * $p < 0.05$ compared with the untreated control.

Sample	concentration (μg/ml)	% stimulation
S.scaphigerum	10	-20.12 ± 4.96
	30	-6.92 ± 4.15
	100	19.89 ± 2.78 *
	300	105.53 ± 7.07 *
	500	169.10 ± 9.25 *
PHA	10	71.42 ± 4.30 *

Table C The percentage of stimulation of *S. scaphogerum* fruit extract on human PBMCs by tritiated thymidine incorporation assay. The results were expressed as the percentage of stimulation over untreated control (mean± S.E.M.), (n=5). * $p < 0.05$ compared with the untreated control.

Sample	concentration (μg/ml)	% stimulation
S.scaphigerum	75	32.34 ± 3.92 *
	150	69.01 ± 5.64 *
	300	145.86 ± 10.78 *
PHA	10	23,687.74 ± 4,690.54 *

Table D The percentage of stimulation of *S. scaphogerum* fruit extract on T cells by CD69 detection assay. The results were expressed as the percentage of stimulation over untreated control (mean± S.E.M.), (n=5). * $p < 0.05$ compared with the untreated control.

Sample	concentration (μg/ml)	% CD3/CD69 expression
RPMI 1640 medium		0.44 ± 0.14
S.scaphigerum	75	0.66 ± 0.16
	150	0.80 ± 0.17
	300	1.00 ± 0.13
	600	1.45 ± 0.07
PHA	10	25.33 ± 3.91 *

Table E Effect of *S scaphogerum* fruit extract on phagocytosis of J774A.1 cells by phagocytosis zymosan assay. The results were expressed as the percentage of stimulation over untreated control (mean± S.E.M.), (n=5). * $p < 0.05$ compared with the untreated control.

Sample	concentration (μg/ml)	% stimulation
S.scaphigerum	75	8.62 ± 0.16
	150	21.50 ± 3.75 *
	300	33.68 ± 3.13 *
	600	43.87 ± 2.56 *
LPS	5	33.44 ± 1.79 *

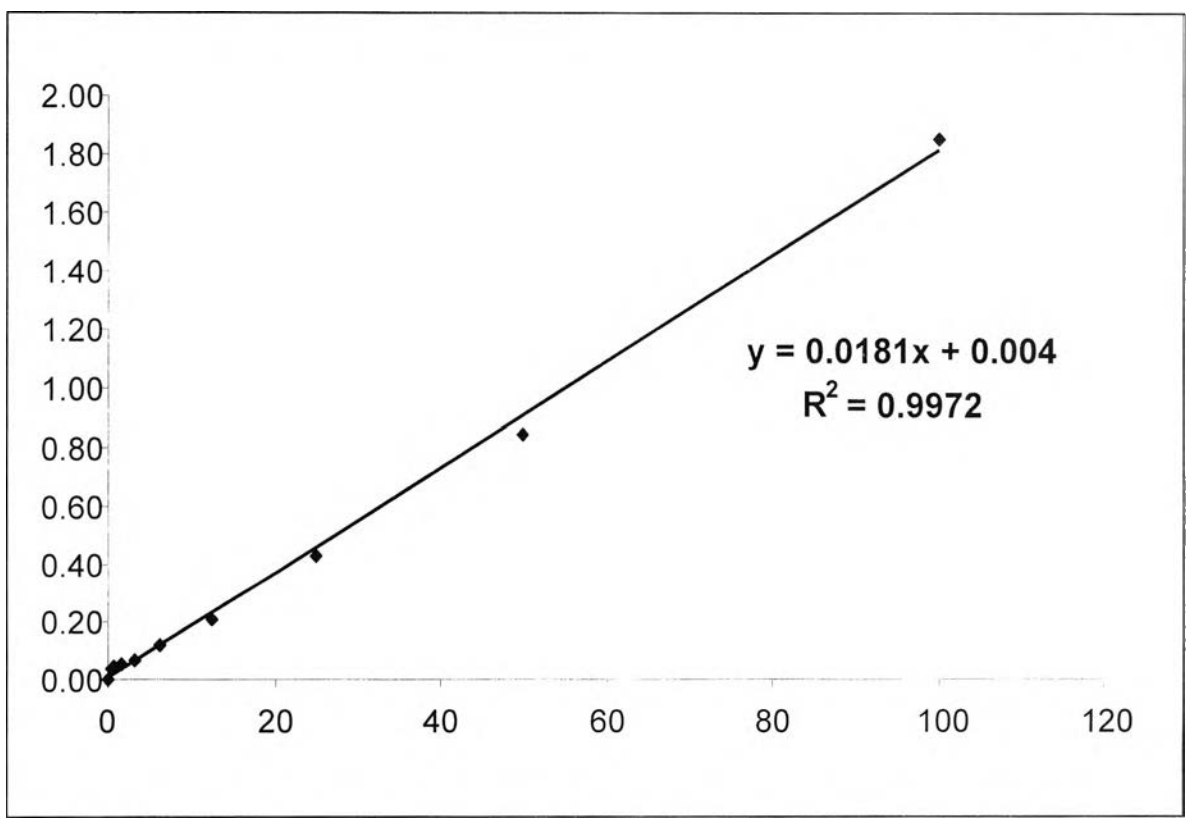
Table F Effect of LPS on nitrict oxide production from J774A.1 cells by Griess reaction (n=2).

Sample	concentration (μg/ml)	NO_2 (μM)
RPMI 1640 medium		4.76 ± 0.52
LPS	1	8.56 ± 0.78 *
	2.5	9.58 ± 0.82 *
	5	10.60 ± 0.63 *
	10	12.81 ± 1.06 *

Table G Effect of *S. scaphogerum* fruit extract on nitrict oxide production release from J774A.1 cells by Griess reaction (n=3).

Sample	concentration (μg/ml)	NO_2 (μM)
Negative		6.17 ± 0.19
S.scaphigerum	75	7.47 ± 0.21
	150	8.56 ± 0.25 *
	300	9.60 ± 0.38 *
	600	15.01 ± 0.89 *
LPS	1	12.06 ± 0.25 *

Figure H Representative of NO_2 standard curve of J774A.1 cells by Griess reaction.



APPENDIX C

Buffers and Reagents

1. RPMI 1640 stock solution 1 liter

RPMI powder	10.4	g
$NaHCO_3$	1.5	g
Glucose		
Hepe	10	ml
Penicillin/Streptomycin	10	ml
ddH_2O	900	ml

Adjust pH to 7.2 with 1M HCl

Add ddH_2O to 1 liter and Sterilized by filtering through a 0.45 membrane filter

2. Complete RPMI 1640 medium 20 ml

RPMI stock	18	ml
L-glutamine	200	µl
Fetal Bovine Serum	2	ml

3. HBSS stock solution 1 liter

HBSS powder	9.25	g
$NaHCO_3$	0.35	g
ddH_2O	900	ml

Adjust pH to 7.2 with 1M HCl

Add ddH_2O to 1 liter and Sterilized by filtering through a 0.45 membrane filter

4. 2µl/ml Heparin in HBSS

HBSS stock	22.5	ml
Heparin	45	µl

5. 10x Phosphate Buffered Saline (PBS) 1 liter

NCl	80	g
KCl	2	g
Na_2HPO_4	9.136	g
NH_2PO_4	2	g
ddH_2O	900	ml

Adjust pH to 7.4 with 1M HCl

Add ddH_2O to 1 liter and sterilized by autoclaving

6. 1x Phosphate Buffered Saline (PBS) 1 liter

10xPBS	100	ml
ddH_2O	900	ml

Sterilized by autoclaving

7. DMEM stock solution 1 liter

DMEM powder	10.4	g
$NaHCO_3$	1.5	g
Hepe	10	ml
ddH_2O	900	ml

Adjust pH to 7.2 with 1M HCl

Add ddH_2O to 1 liter and Sterilized by filtering through a 0.45 membrane filter

8. Complete DMEM 150 ml

RPMI stock	135	ml
L-glutamine	1.5	ml
Fetal Bovine Serum	15	ml

9. Scintillation fluid

PPO	1	g
POPOP	0.1	g
Toluene	1	litre

10. Wash buffer

PBS with 0.5% BSA and 0.1% NaN_3

11. 1% paraformaldehyde (PFA) in PBS 500 ml

Paraformaldehyde	5	ml
PBS	495	ml

12. NBT 2 mg/ml 10 ml

NBT	20	mg
Ultrapure water	10	ml

13. Zymosan A 4 mg/ml 10 ml

Zymosan A	40	mg
0.9% NaCl	10	ml

14. NBT – Zymosan A mixure (NBT 600 µg/ml + Zymosan A 800 µg/ml)

PBS	1440	µl
NBT 2 mg/ml	1440	µl
DMEM	960	µl
Zymosan A 4 mg/ml	960	µl

15. 2M KOH

KOH	56.11	g

Add ddH_2O to 1 liter

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