Table 1 Colour and moisture removal (%) of *G. pseudochina* leaves with various leaf ages and drying methods.

Drying process	Age of leaves	Moisture removal (%)	Dried leaf color		
			Lightness (L^*)	Red (a^*)	Yellow (b^*)
Freeze dry	Young	92.1±0.2[b]			
	Developing	92.2±0.1[ab]	64.43±0.01[a]	-4.54±0.01[c]	25.08±0.01[a]
	Mature	92.6±0.3[ab]			
Microwave	Young	92.7±0.5[ab]			
	Developing	92.8±0.3[ab]	44.75±0.01[b]	-0.75±0.02[b]	22.75±0.01[b]
	Mature	93.1±0.4[ab]			
Oven	Young	92.8±0.2[ab]			
	Developing	92.9±0.4[ab]	37.17±0.02[c]	2.79±0.02[a]	18.17±0.01[c]
	Mature	93.2±0.4[a]			

Different letter(s) (a-c) in same column are significant differences according to Scheffe's test ($p < 0.05$). Data are given as means ± *SD* ($n = 3$).

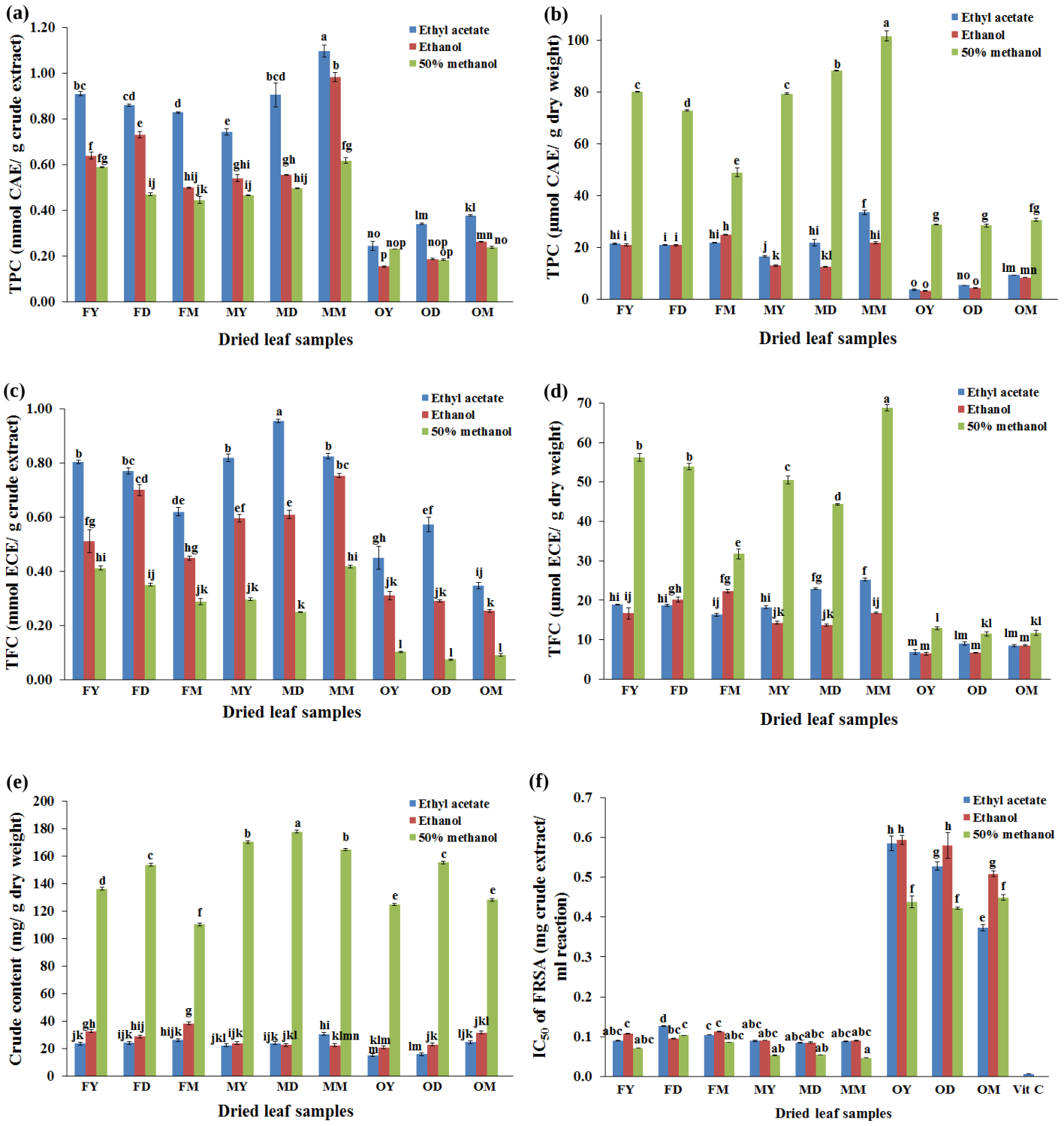


Fig. 1 TPC of *G. pseudochina* leaf extracts per g dry weight (a) and per g crude extract (b), TFC of *G. pseudochina* leaf extracts per g dry weight (c) and per g crude extract (d), crude content (e) and 50% of FRSA activity (IC_{50}) (f) prepared with different drying processes, leaf ages and polarity of solvents. Abbreviations of sample names: first letter, F is freeze dry, M is microwave and O is oven; second letter, Y is young leaf, D is developing leaf and M is mature leaf. Different letter (s) (a-p) are significant differences according to Scheffe's test ($p < 0.05$). Data are presented as the means ± *SD* ($n = 3$).

Table 2 Summary of TPC and TFC in *G. pseudochina* leaf extracts obtained through different drying processes and leaf ages.

Drying process	Leaf age	Content/ g dry weight			Content/ g crude extract	
		TPC (μmol CAE)	TFC (μmol ECE)	Crude content (mg)	TPC (mmol CAE)	TFC (mmol ECE)
Freeze dry	Young	122.41±0.83^{b}	91.78±0.61^{b}	192.50±1.08^{cd}	2.14±0.02^{b}	1.73±0.04^{c}
	Developing	114.78±0.22^{c}	92.74±0.64^{b}	206.67±0.36^{b}	2.06±0.00^{bc}	1.82±0.01^{c}
	Mature	95.60±1.81^{d}	70.39±1.26^{d}	186.25±2.86^{cd}	1.77±0.02^{d}	1.36±0.02^{d}
Microwave	Young	108.99±0.25^{c}	83.05±0.97^{c}	216.46±0.72^{a}	1.75±0.00^{d}	1.71±0.01^{c}
	Developing	122.49±1.24^{b}	81.01±0.52^{c}	224.17±4.61^{a}	1.96±0.05^{c}	1.81±0.02^{b}
	Mature	157.18±3.25^{a}	110.85±0.83^{a}	217.71±0.95^{a}	2.70±0.06^{a}	2.00±0.02^{a}
Oven	Young	35.77±0.27^{f}	26.17±0.73^{e}	160.83±3.15^{e}	0.63±0.02^{f}	0.86±0.04^{e}
	Developing	38.14±0.68^{f}	27.19±0.34^{e}	194.17±2.37^{c}	0.71±0.01^{f}	0.94±0.03^{e}
	Mature	48.27±0.69^{e}	28.32±0.57^{e}	184.58±1.57^{d}	0.88±0.01^{e}	0.69±0.01^{f}

Different letter(s) (a-f) in same column are significant differences according to Scheffe's test ($p < 0.05$). Data are given as means ± *SD* ($n = 3$)

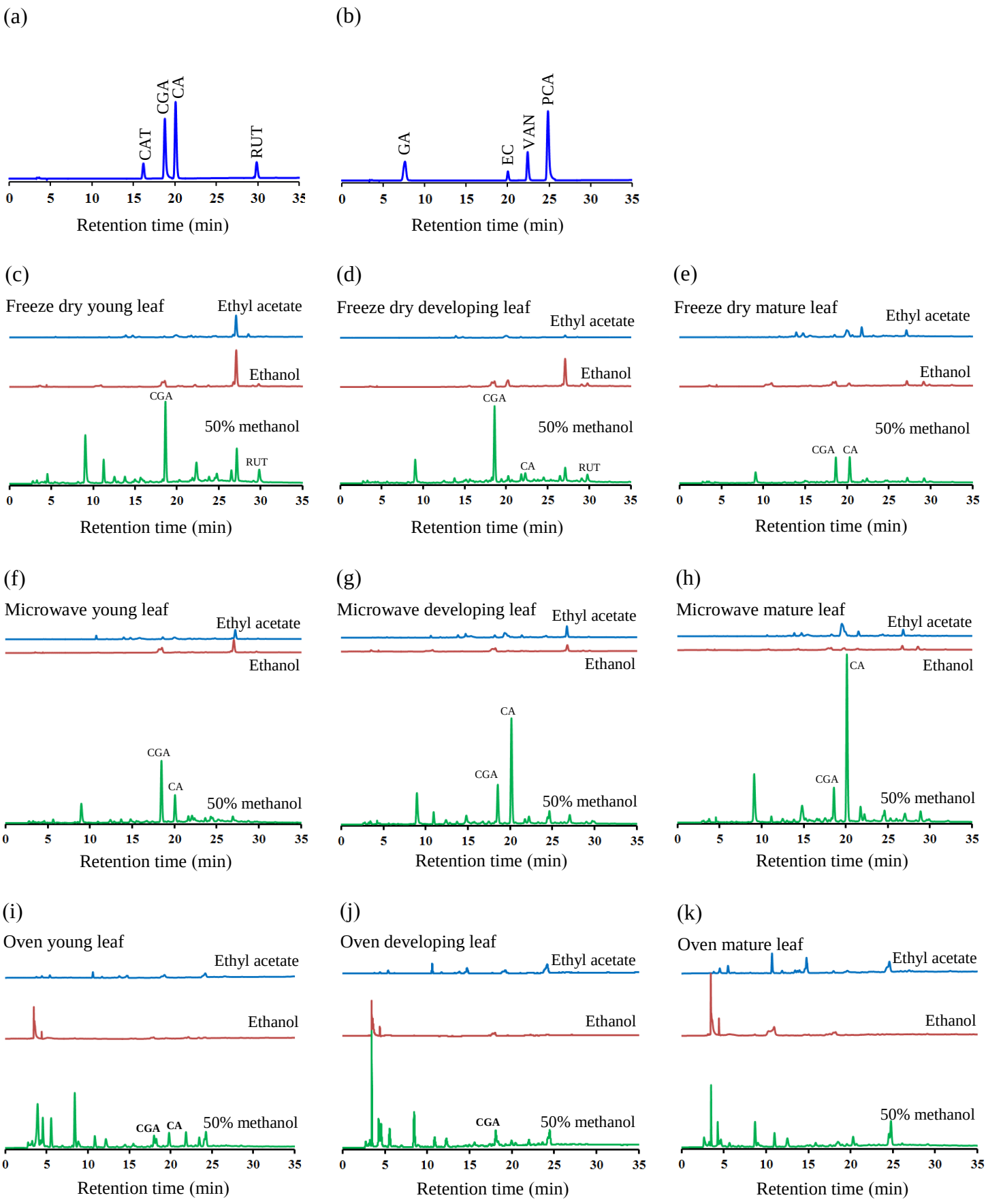


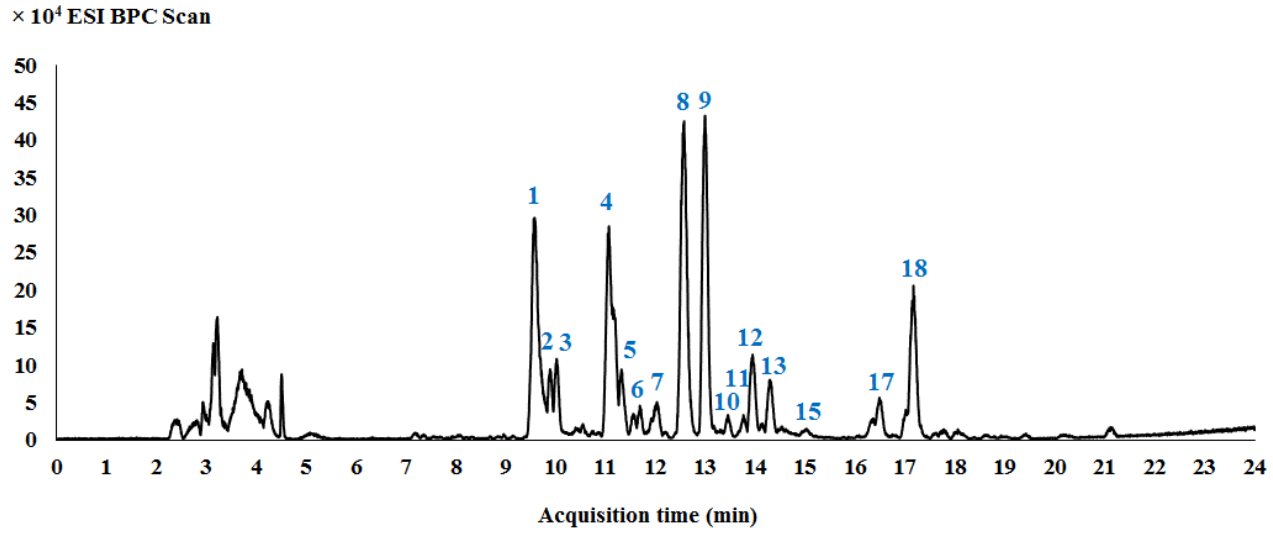
Fig. 2 Normalized HPLC chromatograms with retention times of (a, b) standards of phenolic compounds, and *G. pseudochina* extracts from continuous extracts with ethyl acetate, ethanol and 50% methanol of (c-e) the freeze-dried leaves, (f-h) microwave-dried leaves and (i-k) oven-dried leaves at various leaf ages.

Table 3 TPC, TFC, 50% free radical scavenging activity (IC_{50}), CGA, CA and RUT content of the MLM extracts from separate extractions with various ethanol concentrations of 25, 50, 75 and 100%.

Solvent	TPC (µmol CAE/ g dry weight)	TFC (µmol ECE/ g dry weight)	Crude (g/ g dry weight)	IC_{50} of FRSA (µg crude extract/ ml)	CGA (mg/ g crude extract)	CA (mg/ g crude extract)	RUT (mg/ g crude extract)
25% Ethanol	59.17 ± 6.36^{a}	70.44 ± 6.92^{b}	0.18 ± 0.02^{a}	83.66 ± 2.51^{a}	7.49 ± 0.13^{c}	4.23 ± 0.14^{a}	< LOQ*
50% Ethanol	67.01 ± 5.32^{a}	94.76 ± 4.39^{a}	0.09 ± 0.01^{b}	76.07 ± 8.21^{a}	15.04 ± 0.74^{b}	2.22 ± 0.17^{b}	7.16 ± 0.15^{c}
75% Ethanol	60.28 ± 4.80^{a}	89.26 ± 6.50^{a}	0.09 ± 0.01^{b}	82.58 ± 4.65^{a}	16.85 ± 0.35^{a}	1.33 ± 0.03^{c}	8.69 ± 0.75^{b}
100% Ethanol	27.02 ± 3.97^{b}	20.18 ± 1.20^{c}	0.04 ± 0.00^{c}	102.49 ± 4.51^{b}	5.67 ± 0.14^{d}	1.01 ± 0.04^{c}	15.71 ± 0.25^{a}

Different letter (s) (a-d) in the same column are significant differences according to Scheffe's test ($p < 0.05$). Data are presented as the means ± *SD* ($n = 3$). * The LOQ of RUT is 0.46 µg/ml.

(a)



(b)

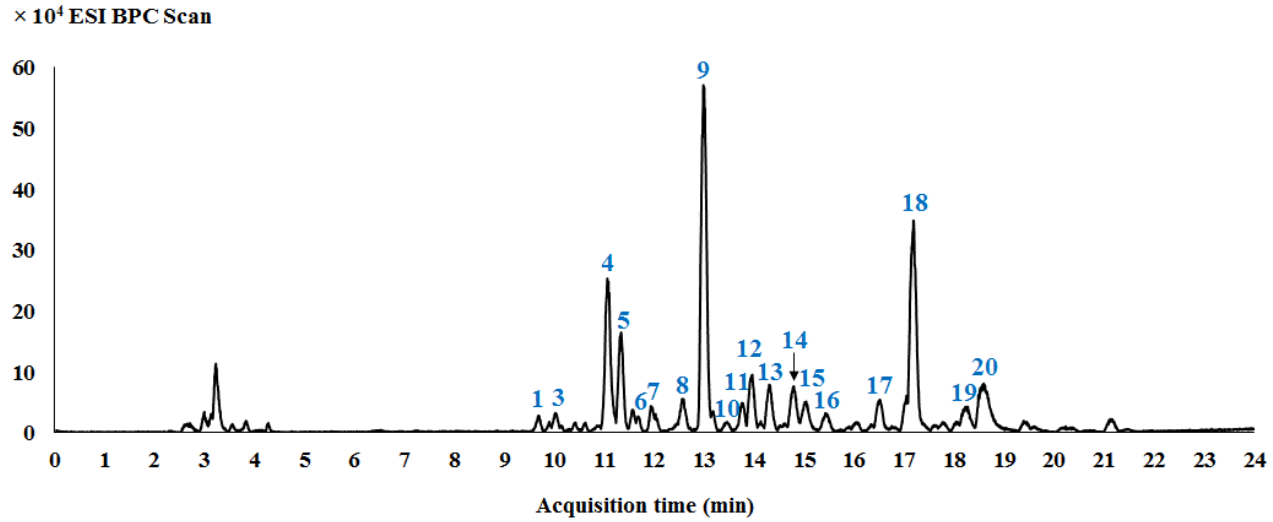


Fig. 3 LC-ESI base peak chromatograms (BPC) of the MLM extracts from separate extractions with (a) 25% ethanol and (b) 100% ethanol. For major peak assignments, see Table 4.

Table 4 LC-ESI-QTOF-MS/MS analysis of phenolic compounds from the MLM extracts from separate extractions with 25 and 100% ethanol.

Peak no.	RT (min)	ESI-MS m/z		Tentative identification	Formula	Error (ppm)
		[M-H]	MS/MS fragment			
1	9.58	385.04	277.03,204.99,73.02	Unidentified	-	-
2	9.89	353.08	191.05,135.04	Caffeoyl quinic acid isomer1	$C_{16}H_{18}O_9$	1.15
3	10.02	423.15	363.12,113.02	(+)-Tephropurpurin	$C_{24}H_{24}O_7$	-13.37
4	11.08	353.08	191.05,135.04	Caffeoyl quinic acid isomer2 (CGA)*	$C_{16}H_{18}O_9$	1.72
5	11.32	439.18	393.17,163.05,205.06	1,3,8-Trihydroxy-4-methyl-2,7 diprenylxanthone	$C_{24}H_{26}O_5$	-12.09
6	11.68	421.16	341.11,213.04	2-(2,4-Dihydroxyphenyl)-5-hydroxy-8-methyl-8-(4-methyl-3-penten-1-yl)-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one	$C_{25}H_{26}O_6$	9.17
7	11.91	353.08	191.05	Caffeoyl quinic acid isomer3	$C_{16}H_{18}O_9$	1.15
8	12.57	179.03	135.04	CA*	$C_9H_8O_4$	8.28
9	12.99	609.14	463.08,300.02,178.99,151.00	Quercetin rutinoside (RUT)*	$C_{27}H_{30}O_{16}$	-1.14
10	13.45	367.10	179.03,135.04,99.01	3-O-Caffeoyl-1-O-methylquinic acid	$C_{17}H_{20}O_9$	6.14
11	13.76	593.15	285.03,327.04,535.21,417.24	Kaempferol rutinoside	$C_{27}H_{30}O_{15}$	-0.35
12	13.94	713.47	677.49,313.06,147.04	Unknown-C-glycoside	$C_{35}H_{70}O_{14}$	-3.51
13	14.29	826.55	790.57,656.96	Unidentified	-	-
14	14.76	515.11	353.08,173.04,179.03	Dicaffeoyl quinic acid isomer1	$C_{25}H_{24}O_{12}$	0.97
15	15.02	515.11	353.08,173.04,179.03	Dicaffeoyl quinic acid isomer2	$C_{25}H_{24}O_{12}$	0.97
16	15.55	313.07	313.07	3,4-Dihydroxycinnamoyl-(Z)-2-(3,4-dihydroxyphenyl) ethenol	$C_{17}H_{14}O_6$	4.99
17	16.49	463.25	417.24,161.04	1-(9Z-octadecenoyl)-sn-glycero-2,3-cyclic phosphate	$C_{21}H_{39}O_6P$	-13.30
18	17.16	497.21	429.20,249.14,119.0313,59.01	Unidentified	-	-
19	18.17	301.03	151.00, 121.02	Quercetin	$C_{15}H_{10}O_7$	4.23
20	18.54	623.11	311.05, 265.04, 147.04, 109.02	5-Hydroxy-2'-methoxy-6,7-methylenedioxyisoflavone	$C_{17}H_{12}O_6$	1.97

* Peaks are compared with standard compounds.

Table 5 Total pyrrolizidine alkaloid content (TPAsC) in *G. pseudochina* leaf extracts prepared from different drying processes (freeze drying and microwave and oven drying) and serial extraction with 25% and 50% ethanol.

Solvent fraction and dried leaf sample	mmol MCTE/ g crude extract
25% Ethanol MLF	0.004 ± 0.001^{e}
50% Ethanol MLF	0.142 ± 0.015^{d}
25% Ethanol MLM	0.003 ± 0.002^{e}
50% Ethanol MLM	0.065 ± 0.011^{c}
25% Ethanol MLO	0.233 ± 0.038^{b}
50% Ethanol MLO	0.684 ± 0.125^{a}

Different letter(s) (a-e) in same column are significant differences according to Scheffe's test ($p < 0.05$). Data are given as means ± *SD* ($n = 3$).

Table 6 Cytotoxicity of the EMLM extract and marker compounds on HaCaT cells, non-stimulated and stimulated by TNF-α, and quantity of each marker compound in the EMLM extract.

Samples	Cytotoxicity IC_{50} values (μg/ml)	
	Non TNF-α	TNF-α
EMLM	$680.80 \pm 23.98^{c*}$	$744.02 \pm 62.2^{c*}$
CGA	$180.61 \pm 7.09^{d*}$	$284.50 \pm 45.76^{d**}$
CA	$36.18 \pm 2.78^{e*}$	$60.19 \pm 1.76^{e**}$
RUT	$4393.41 \pm 436.11^{a*}$	$1497.99 \pm 205.50^{b**}$
PCA	$1682.78 \pm 107.96^{b*}$	$2840.76 \pm 174.12^{a**}$
PTX (positive control)	$2.69 \pm 0.32^{f*}$	$1.84 \pm 0.49^{f*}$

Different letter(s) (a-f) in same column are significant differences according to Scheffe's test ($p < 0.05$).
Different symbols (*, **) in same column are significant differences according to T-test ($p < 0.05$).
Data are given as means ± *SD* ($n = 3$).

Table 7 Interleukin 8 (IL-8) content in HaCaT cell lysate after treatment with the EMLM extract and marker compounds.

TNF-α stimulation	Sample for treatment	Concentration (μg/ml)	IL-8 contents (pg/ml)
Non TNF-α	Control	-	10.17±2.01^{g}
TNF-α	Control non DMSO	-	342.80±34.62^{a}
	Control 0.7% DMSO	-	322.88±36.03^{ab}
	EMLM crude extracts	375	133.79±18.18^{ef}
		750	148.82±28.15^{e}
	CGA	140	284.82±35.92^{bc}
		240	90.10±16.39^{f}
	CA	30	254.60±42.63^{c}
		60	87.19±11.76^{f}
	RUT	750	115.17±20.50^{ef}
		1500	93.68±14.33^{f}
	PCA	1400	369.71±41.43^{a}
		2800	201.21±24.38^{d}
	CUR (positive control)	50	14.35±9.78^{g}

Different letter(s) (a-f) in same column are significant differences according to Duncan's test ($p < 0.05$). Data are given as means ± *SD* ($n = 3$).

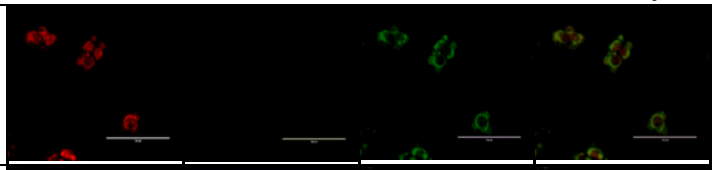
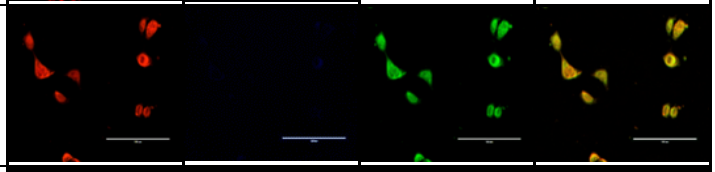
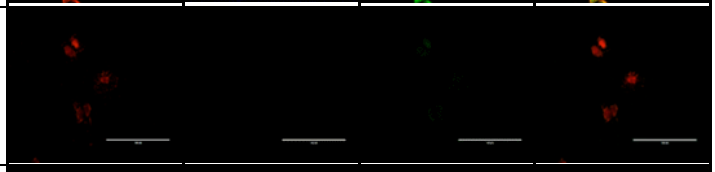
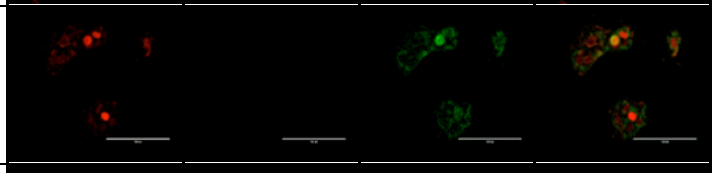
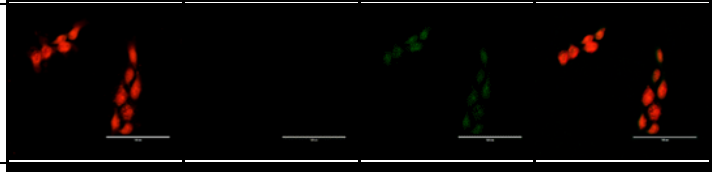
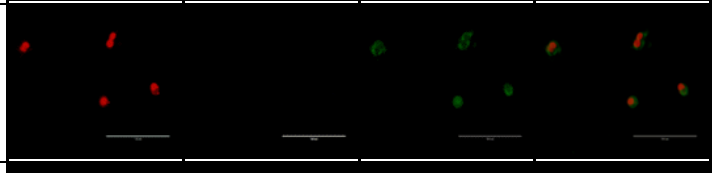
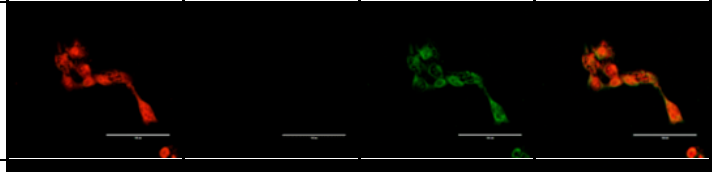
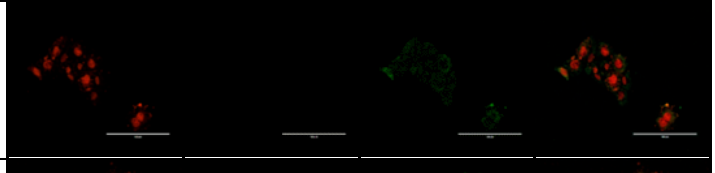
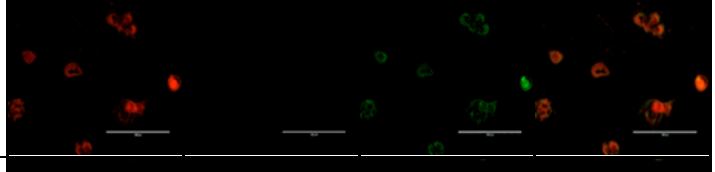
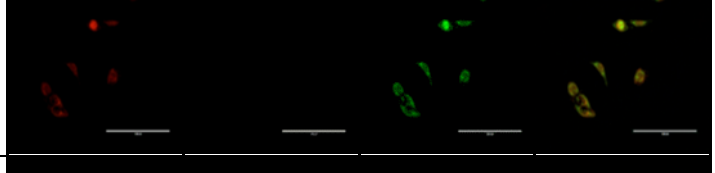
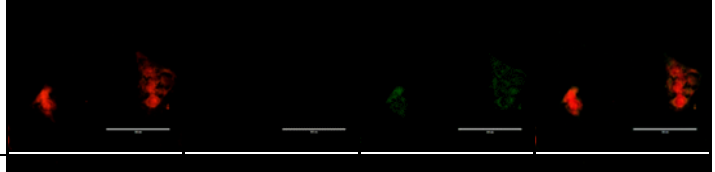
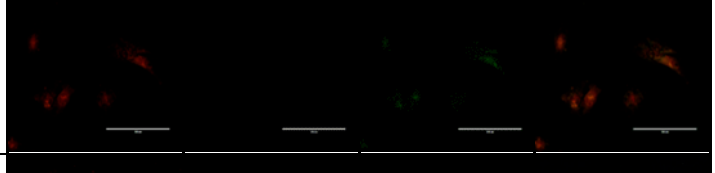
Treatment	Nucleus	RelA	RelB	Overlay
(-)TNF-α Control				
(+)TNF-α Control				
(+)TNF-α EMLM 375 µg/ml				
(+)TNF-α EMLM 750 µg/ml				
(+)TNF-α CGA 140 µg/ml				
(+)TNF-α CGA 280 µg/ml				
(+)TNF-α CA 30 µg/ml				
(+)TNF-α CA 60 µg/ml				
(+)TNF-α RUT 750 µg/ml				
(+)TNF-α RUT 1500 µg/ml				
(+)TNF-α PCA 1400 µg/ml				
(+)TNF-α PCA 2800 µg/ml				
(+)TNF-α CUR 50 µg/ml				

Fig. 4 Localization of RelA and RelB on HaCaT cells due to TNF-α stimulation. HaCaT cells were pre-treated with 50 ng/ml of TNF-α for 12 h and treated with the EMLM extracts, marker compounds (CGA, CA, PCA and RUT) and CUR (positive control) for 24 h.