Supporting Information

Antidiarrhoeal Evaluation of Root Extract, Its Bioactive Fraction, and Lupinifolin Isolated from *Eriosema chinense*

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ISOLATION OF LUPINIFOLIN

Chloroform fraction (15 g) of *E. chinense* (CEC) was subjected to column chromatography on silica gel and eluted with hexane with increasing amounts of ethyl acetate. A total of 25 fractions with volume of 500 mL each were eluted, and the fraction eluted with 5% ethyl acetate was further purified with preparative TLC using hexane: dichloromethane: ethyl acetate (60: 30: 10 v/v/v) as mobile phase. This yielded (6.0% w/w) yellow coloured needle shaped crystals, which showed single spot with TLC using hexane: ethyl acetate (85:15 v/v) as the mobile phase. Further, the compound was characterized by IR, ^{1}H NMR ^{13}C NMR and mass spectroscopy. Melting point was recorded by using Sonar melting point apparatus, ISO 9001-2000 in open capillary tubes (Associated Scientific Technologies). The IR spectral analysis was performed on Shimadzu FT/IR 8400 infrared spectrophotometer, Japan, using KBr disc method for sample preparation. ^{1}H NMR and ^{13}C NMR spectroscopical analysis was performed on DDR X – 500 m/z Bruker Deltonics NMR spectrophotometer. The ESI-MS was recorded on a Micromass Quattro II triple quadrupole MASS spectrometer (Waters).

CHARACTERIZATION

The compounds melting point was found to be 117-119 °C. UV λ_{max} (MeOH) nm (log ε): 276 (4.23), 310 (3.69), 365 (3.27); IR bands (KBr, υ cm^{-1}): 3392, 3068, 2928, 1656, 1631, 1573, 1464, 1421, 1388, 1261, 1201, 1170, 1126; ^{1}HNMR ($CDCl_3$) δ: 5.33 (1H, dd, J = 3.2,12.6, H-2), 3.04 (1H, dd, J = 9.6, 16.8, H-3α), 2.60 (1H, dd, J = 3.6, 16.5, H-3β), 7.32 (1H, d, J = 8.4, H-2'), 7.32 (1H, d, J = 8.4, H-6'), 6.87 (1H, d, J = 8.4, H-3'), 6.87 (1H, d, J = 8.4, H-5'), 5.59 (1H, d, J = 10.2, H3''), 6.61 (1H, d, J = 10.2, H-4''), 1.45 (3H, s, H 5''), 1.36 (3H, s, H-6''), 3.36 (2H, d, J = 7.2, H-1'''), 5.14 (t, J = 7.8, H-2'''), 1.68 (3H, s, H-4'''), 1.68 (3H, s, H-5'''), 11.51 (1H, s, 5-OH).

^{13}C NMR (CDCl3) δ: 78.58 (C-2), 43.24 (C-3), 196.56 (C-4), 156.55 (C-5), 102.82 (C-6),159.92 (C-7), 108.63 (C-8), 159.33 (C-9), 102.63 (C-10), 131.11(C-1'), 127.84 (C-2'), 115.53 (C-3'), 155.95 (C-4'), 115.53 (C-5'), 127.84 (C-6'), 78.16 (C-2''), 126.69 (C-3''),115.63 (C-4''), 28.52 (C-5''), 28.33 (C-6''), 17.84 (C-1'''), 122.55 (C-2'''), 131.04 (C-3'''), 25.78 (C-4'''), 25.78 (C-5'''). The structure drawn of the isolated compound from the above interpretation was found to resemble lupinifolin (**Figure 1S**), which was further confirmed by comparing its melting point, IR, NMR, and mass spectrum to those of lupinifolin reported in literature [1-3].

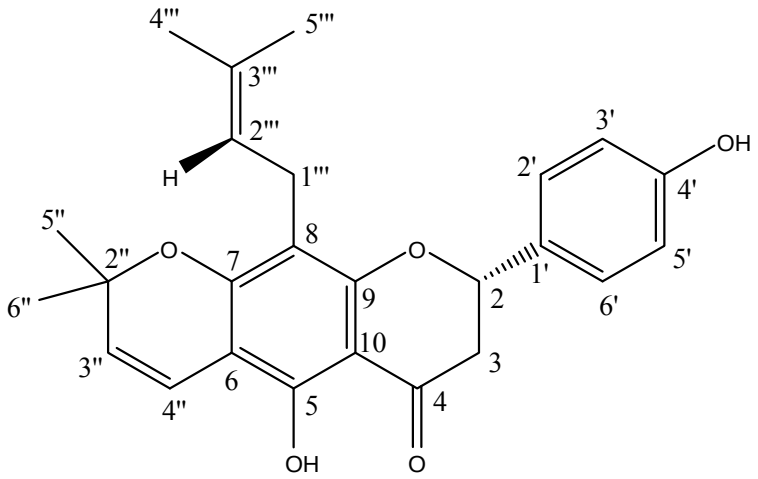

Figure1S. Structure of lupinifolin.

References

1. *Itoigawa M, Ito C, Ju-ichi M, Nobukuni T, Ichiishi E, Tokuda H, Nishino H, Furukawa, H.* Cancer chemopreventive activity of flavanones on Epstein–Barr virus activation and two-stage mouse skin carcinogenesis. Cancer Lett 2002; 176: 25-29.
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3. *Joycharat N, Thammavong S, Limsuwan S, Homlaead S, Voravuthikunchai SP, Yingyongnarongkul B, Dej-adisai S, Subhadhirasakul S.* Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*. Arch Pharm Res 2013; DOI: 10.1007/s12272-013-0085-7.

Preliminary antidiarrhoeal screening of *E. chinense* extract, its different fractions, and isolated lupinifolin on normal faecal excretion rate and castor oil-induced diarrhoea models.

Table 1S. Effect of *E. chinense* extract, its fractions, and isolated lupinifolin on normal faecal excretion.

Treatment	Faecal wet weight (g) at various times (h) after treatment				Wet/dry weight of faeces
	1	3	5	7	
Extract and its fraction					
Control	0.154 ±0.010	0.355 ±0.016	0.544 ±0.020	0.922 ±0.028	1.632 ±0.049
EEC 100	0.153 ±0.013	0.328 ±0.029	0.538 ±0.058	0.872 ±0.076	1.515 ±0.057
EEC 200	0.139 ±0.012	0.277 ±0.034	0.488 ±0.034	0.693 ±0.085^{abefgklm}	1.457 ±0.030
EEC 400	0.121 ±0.013	0.201 ±0.027^{abehklm}	0.354 ±0.036^{abcefghklm}	0.611 ±0.074^{abcefghklm}	1.225 ±0.054^{a}
EAEC 50	0.151 ±0.019	0.324 ±0.038	0.559 ±0.043	0.952 ±0.069	1.496 ±0.081
EAEC 100	0.147 ±0.014	0.320 ±0.035	0.517 ±0.043	0.932 ±0.035	1.428 ±0.042
EAEC 200	0.136 ±0.011	0.312 ±0.023	0.499 ±0.025	0.822 ±0.030^{ek}	1.366 ±0.046
CEC 50	0.145 ±0.013	0.377 ±0.029	0.514 ±0.033	0.789 ±0.044^{aefkl}	1.481 ±0.062
CEC 100	0.141 ±0.016	0.225 ±0.022^{ahkl}	0.308 ±0.029^{abcefghklm}	0.527 ±0.043^{abcefghklm}	1.241 ±0.034^{a}
CEC 200	0.126 ±0.011	0.193 ±0.029^{abefhklm}	0.289 ±0.037^{abcefghklm}	0.508 ±0.032^{abcefghklm}	1.198 ±0.070^{a}
HEC 50	0.161 ±0.013	0.382 ±0.021	0.579 ±0.028	0.963 ±0.041	1.449 ±0.068
HEC 100	0.150 ±0.010	0.366 ±0.023	0.572 ±0.034	0.914 ±0.025	1.422 ±0.048
HEC 200	0.146 ±0.013	0.338 ±0.019	0.508 ±0.037	0.825 ±0.034^{ek}	1.384 ±0.035
Loperamide	0.052 ±0.017	0.077 ±0.018^{abcdefghiklm}	0.095 ±0.024^{abcdefghijklm}	0.091 ±0.024^{abcdefghijklm}	1.144 ±0.234^{a}
Values are mean ±SEM (n = 6). Where a: $p < 0.05$ vs control, b: $p < 0.05$ vs EEC 100, c: $p < 0.05$ vs EEC 200, d: $p < 0.05$ vs EEC 400, e: $p < 0.05$ vs EAEC 50, f: $p < 0.05$ vs EAEC 100, g: $p < 0.05$ vs EAEC 200, h: $p < 0.05$ vs CEC 50, i: $p < 0.05$ vs CEC 100, j: $p < 0.05$ vs CEC 200, k: $p < 0.05$ vs HEC 50, l: $p < 0.05$ vs HEC 100 and m: $p < 0.05$ vs HEC 200.					
Lupinifolin					
Control	0.162 ±0.012	0.337 ±0.015	0.525 ±0.019	0.831 ±0.035	1.613 ±0.034
LUP 2.5	0.164 ±0.011	0.319 ±0.024	0.489 ±0.024	0.791 ±0.037	1.456 ±0.048
LUP 5	0.156 ±0.010	0.271 ±0.018	0.368 ±0.020^{ab}	0.561 ±0.030^{ab}	1.318 ±0.033
LUP 10	0.141 ±0.011	0.228 ±0.016^{a}	0.326 ±0.021^{ab}	0.517 ±0.028^{ab}	1.178 ±0.027^{a}
Loperamide	0.054 ±0.017^{abcd}	0.085 ±0.020^{abcd}	0.104 ±0.027^{abcd}	0.097 ±0.025^{abcd}	1.089 ±0.222^{a}
Values are mean ±SEM (n = 6). Where a: $p < 0.05$ vs control, b: $p < 0.05$ vs LUP 2.5, c: $p < 0.05$ vs LUP 5 and d: $p < 0.05$ vs LUP 10.					

Table 2S. Effect of *E. chinense* extract, its fractions, and isolated lupinifolin on castor oil-induced diarrhoea model.

Group	Onset time (min)	Total no of faeces	Total no of wet faeces	Loss in body weight	Total wt of faeces	Mean defecation in 4h	Diarrhoea score	% protection
Extract and its fraction								
Normal		3.833 ±0.307		0.142±0.024	0.385 ±0.042	0.958 ±0.076	-	100
Castor oil	53.500 ±3.972	11.667 ±0.802^{a}	8.667 ±0.802	1.035 ±0.081^{a}	2.093 ±0.146^{a}	2.916 ±0.200^{a}	17.00 ±1.914	-
EEC 100	74.834 ±4.587^{b}	9.334 ±0.614^{a}	6.00 ±0.683	0.896 ±0.068^{a}	1.615 ±0.089^{ab}	2.334 ±0.153^{a}	12.667 ±1.173	25.490
EEC 200	105.833 ±4.300^{b}	7.667 ±0.494	5.334 ±0.667	0.809 ±0.057^{ab}	1.413 ±0.067^{ab}	1.916 ±0.178	9.500 ±0.885^{b}	44.117
EEC 400	128.00 ±3.881^{bcdfghilmn}	6.833 ±0.477^{b}	4.833 ±0.401^{b}	0.608 ±0.053^{abfgl}	1.100 ±0.063^{abcdfghilmn}	1.708 ±0.253^{b}	7.166 ±1.851^{b}	59.729
EAEC 50	73.334 ±4.120^{b}	10.166 ±0.980^{a}	7.334 ±0.714	0.956 ±0.059^{a}	1.804 ±0.092^{ab}	2.541 ±0.277^{a}	13.500 ±1.384	20.588
EAEC 100	82.166 ±4.269^{b}	9.334 ±0.667^{a}	6.833 ±0.542	0.859 ±0.045^{a}	1.740 ±0.071^{ab}	2.334 ±0.263^{a}	11.667 ±1.308	31.372
EAEC 200	99.667 ±3.422^{bcfl}	8.166 ±0.872^{a}	6.334 ±0.614	0.771 ±0.041^{ab}	1.577 ±0.083^{ab}	2.041 ±0.198^{a}	11.00 ±1.825	35.294
CEC 50	88.00 ±4.442^{b}	9.500 ±0.846^{a}	6.00 ±0.730	0.809 ±0.061^{ab}	1.745 ±0.099^{ab}	2.375 ±0.211^{a}	10.833 ±1.579	46.891
CEC 100	130.334 ±3.946^{bcdfghilmn}	7.00 ±0.632^{b}	4.500 ±0.763^{b}	0.610 ±0.040^{abcfgl}	1.083 ±0.080^{abcdfghlm}	1.750 ±0.241^{b}	7.00 ±1.238^{b}	64.156
CEC 200	137.833 ±3.166^{bcdfhgilmn}	6.833 ±0.600^{b}	4.334 ±0.421^{b}	0.598 ±0.047^{abcfgl}	0.967 ±0.062^{abcdfghilmn}	1.708 ±0.253^{b}	6.667 ±1.706^{b}	66.438
HEC 50	76.667 ±4.255^{b}	9.334 ±0.714^{a}	7.833 ±0.909	0.889 ±0.073^{a}	1.783 ±0.090^{ab}	2.334 ±0.286^{a}	13.334 ±1.706	21.568
HEC 100	82.334 ±3.611^{b}	9.166 ±0.600^{a}	7.334 ±0.667	0.809 ±0.062^{ab}	1.600 ±0.083^{ab}	2.291 ±0.253^{a}	11.166 ±1.400	34.313
HEC 200	97.00 ±3.183^{bcfl}	8.167 ±0.600^{a}	6.833 ±0.600	0.780 ±0.048^{ab}	1.521 ±0.070^{ab}	2.041 ±0.284^{a}	9.334 ±1.256^{b}	45.098
Loperamide	165.00 ±4.106^{bcdefghijklmn}	5.833 ±0.542^{b}	3.166 ±0.600^{b}	0.463 ±0.033^{abcdfghilmn}	0.828 ±0.052^{abcdfghilmn}	1.458 ±0.187^{b}	5.667 ±1.837^{b}	75.553
Values are mean ±SEM (n = 6). Where a: $p < 0.05$ vs Normal, b: $p < 0.05$ vs castor oil, c: $p < 0.05$ vs EEC 100, d: $p < 0.05$ vs EEC 200, e: $p < 0.05$ vs EEC 400, f: $p < 0.05$ vs EAEC 50, g: $p < 0.05$ vs EAEC 100, h: $p < 0.05$ vs EAEC 200, i: $p < 0.05$ vs CEC 50, j: $p < 0.05$ vs CEC 100, k: $p < 0.05$ vs CEC 200, l: $p < 0.05$ vs HEC 50, m: $p < 0.05$ vs HEC 100 and n: $p < 0.05$ vs HEC 200.								
Lupinifolin								
Normal		4.166 ±0.401		0.152 ±0.027	0.402 ±0.031	1.041 ±0.100		100
Castor oil	55.500 ±3.490	12.334 ±0.881^{a}	8.833 ±0.792	1.087 ±0.053^{a}	2.069 ±0.121^{a}	3.083 ±0.220^{a}	18.833 ±1.600	
LUP 2.5	79.500 ±3.063^{b}	10.833 ±0.833^{a}	6.500 ±0.718^{b}	0.858 ±0.051^{ab}	1.830 ±0.087^{a}	2.708 ±0.208^{a}	12.00 ±1.264^{b}	36.271
LUP 5	98.334 ±2.905^{bc}	8.667 ±0.614^{abc}	5.667 ±0.614^{b}	0.727 ±0.057^{ab}	1.478 ±0.085^{abc}	2.166 ±0.210^{ab}	10.334 ±1.229^{b}	45.123
LUP 10	126.00 ±3.033^{bcd}	7.500 ±0.562^{abc}	4.723 ±0.654^{b}	0.626 ±0.050^{abc}	1.130 ±0.084^{abcd}	1.916 ±0.229^{abc}	8.00 ±1.570^{b}	57.514
Loperamide	168.334 ±2.940^{bcde}	6.166 ±0.600^{abcd}	3.833 ±0.833^{b}	0.496 ±0.044^{abcd}	0.858 ±0.071^{abcde}	1.541 ±0.208^{bc}	5.500 ±1.648^{bc}	70.791
Values are mean ±SEM (n = 6). Where a: $p < 0.05$ vs Normal, b: $p < 0.05$ vs castor oil, c: $p < 0.05$ vs LUP 2.5, d: $p < 0.05$ vs LUP 5 and e: $p < 0.05$ vs LUP 10.								