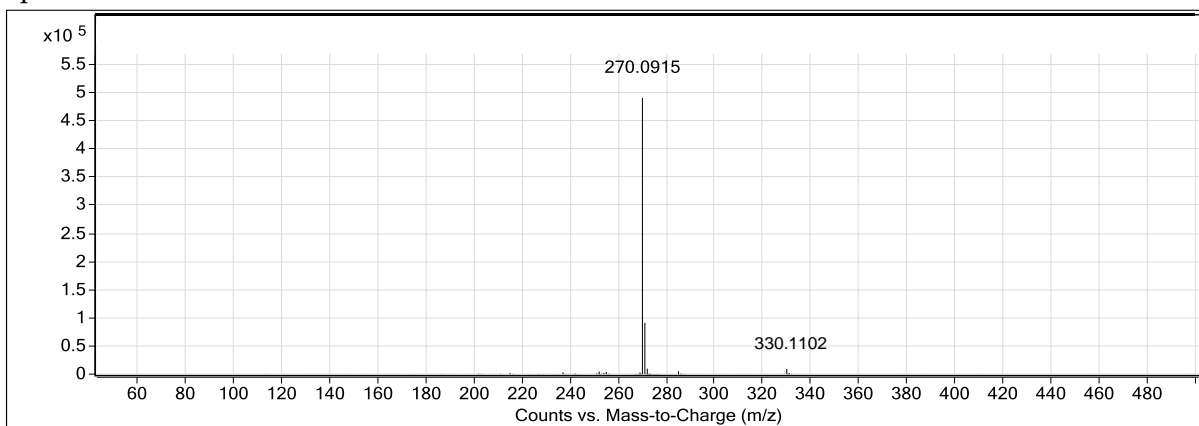
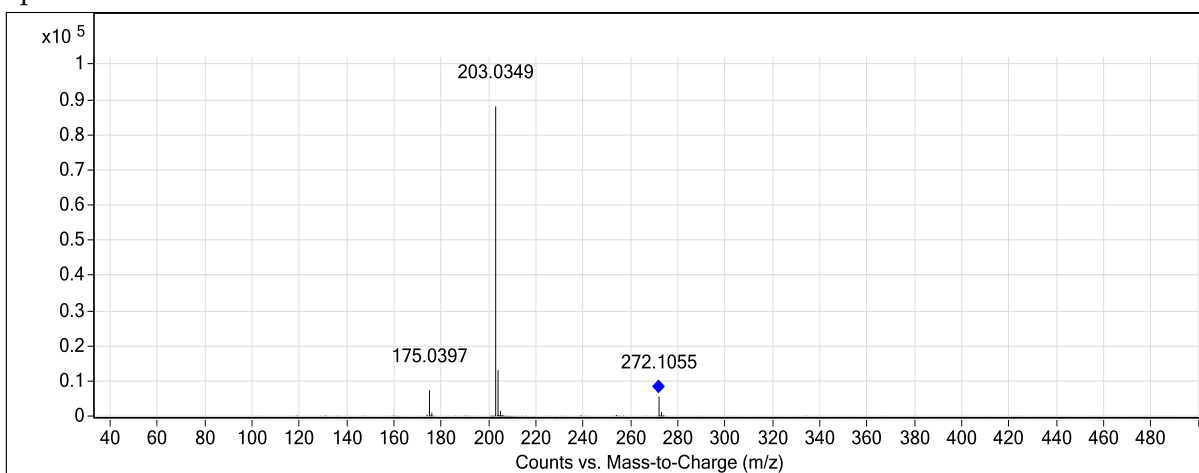


Supplementary Materials

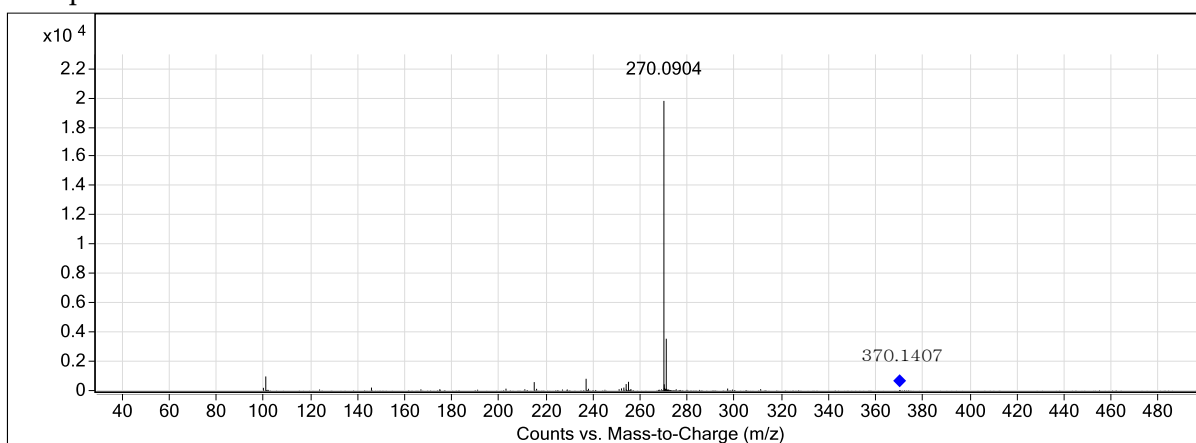
a) Compound 1



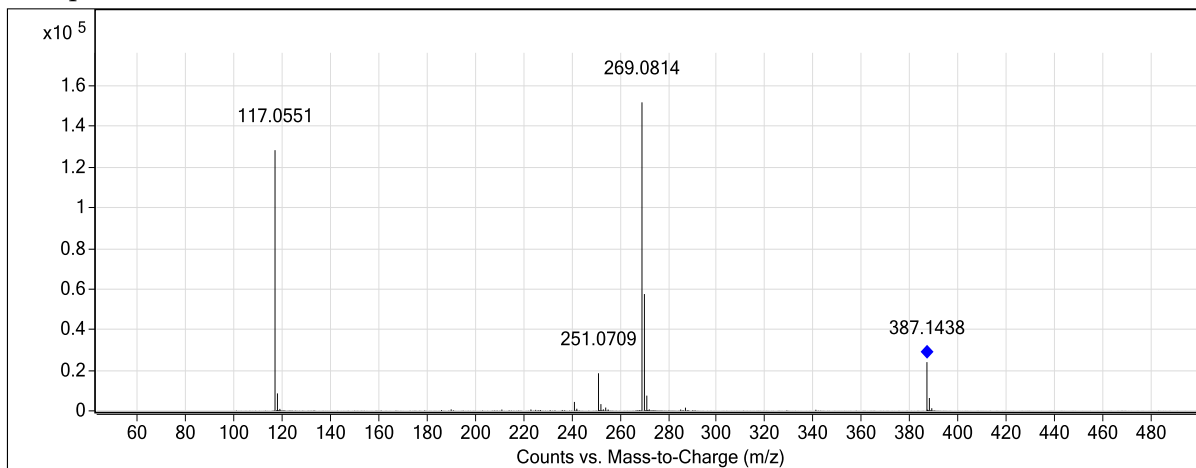
b) Compound 2



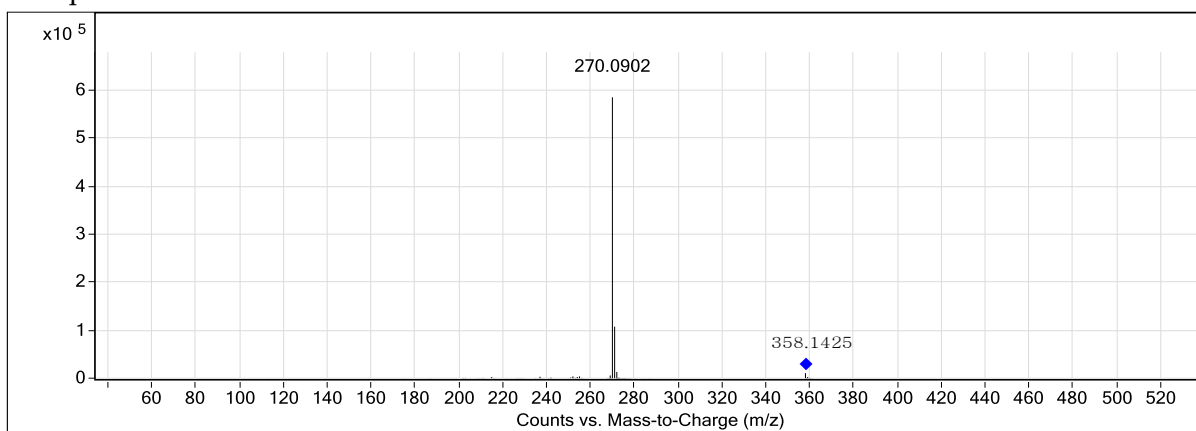
c) Compound 3



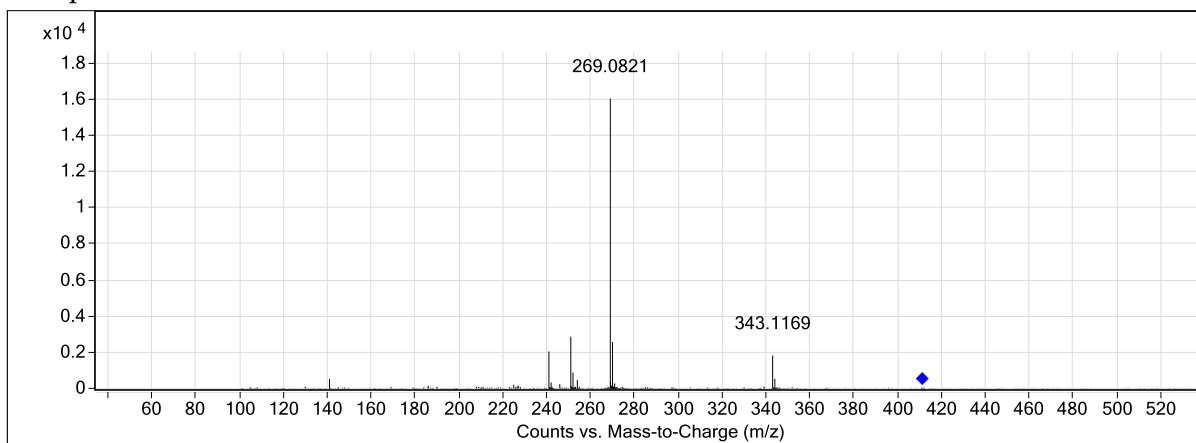
d) Compound 4



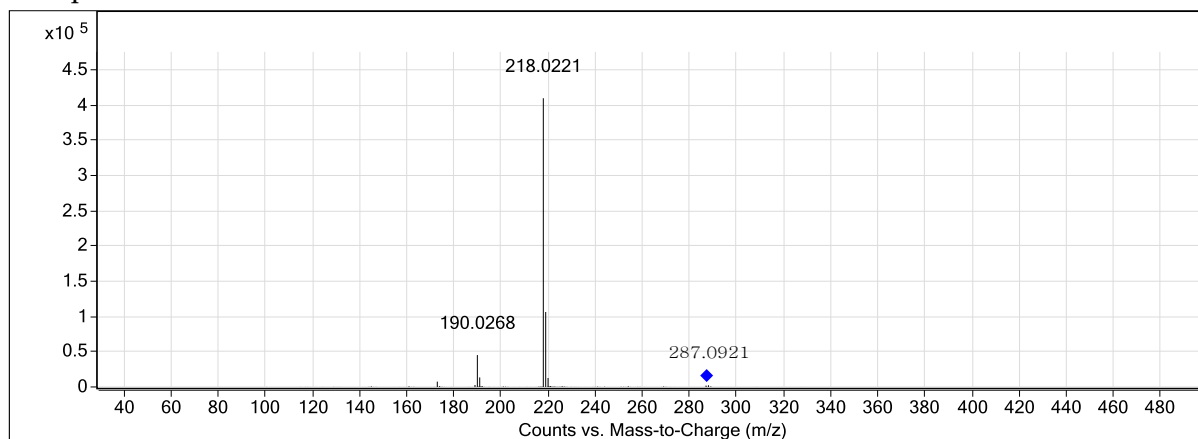
e) Compound 5



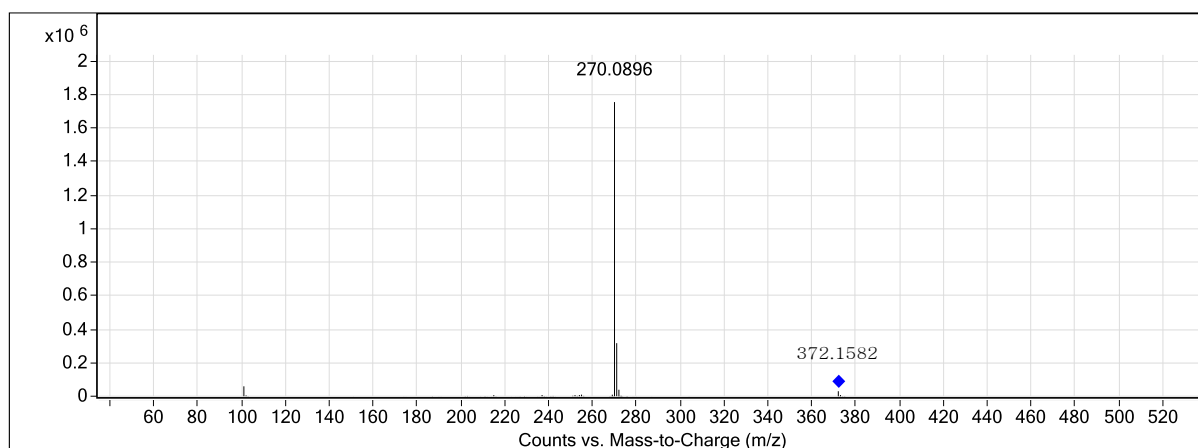
f) Compound 6



g) Compound 7



h) Compound 8



i) Compound 9

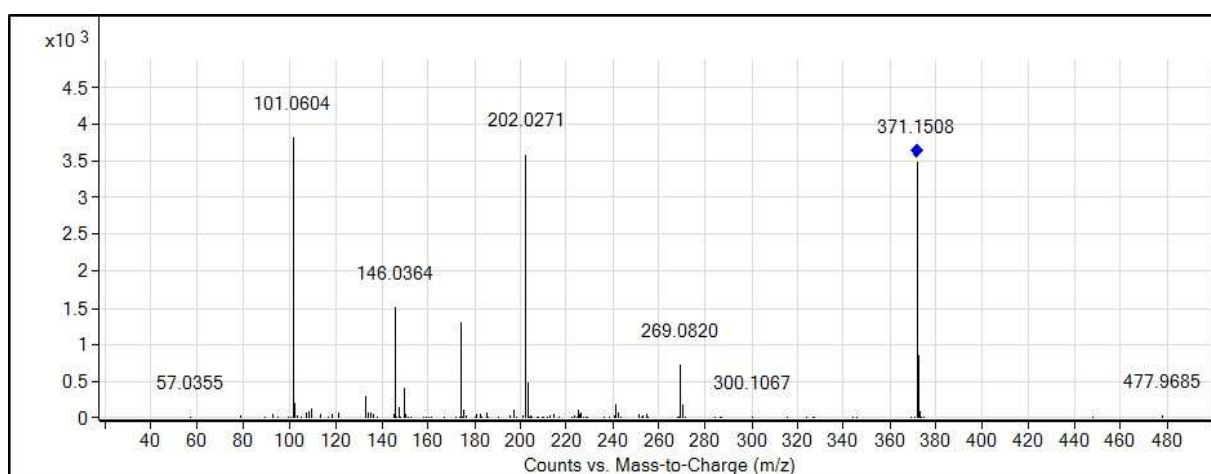


Figure 1S (a-i) – Spectral library of nine shikonins. Mass spectrum was obtained in MSMS mode using optimized collision energy (Table 1).

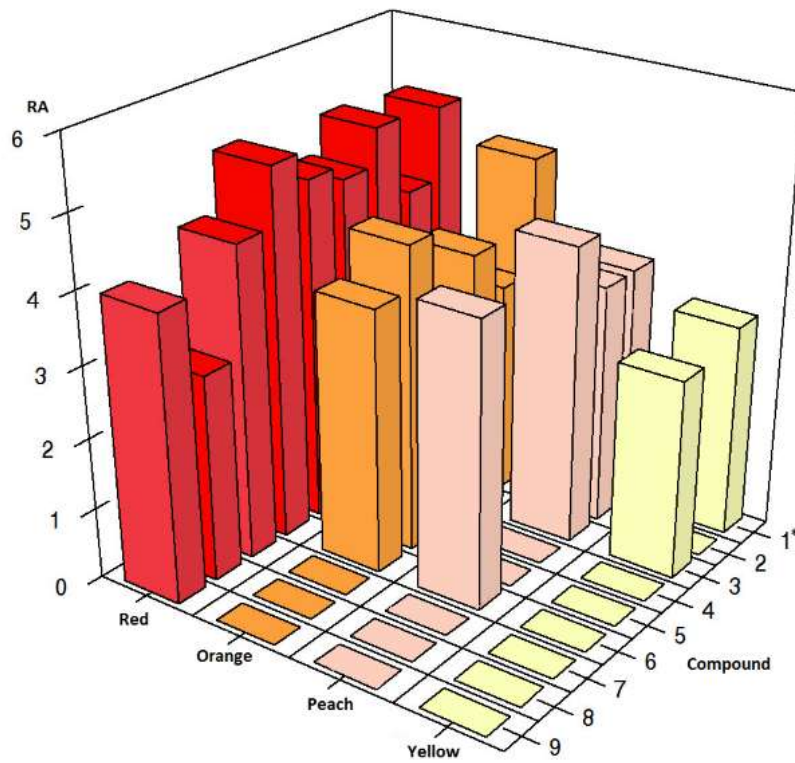
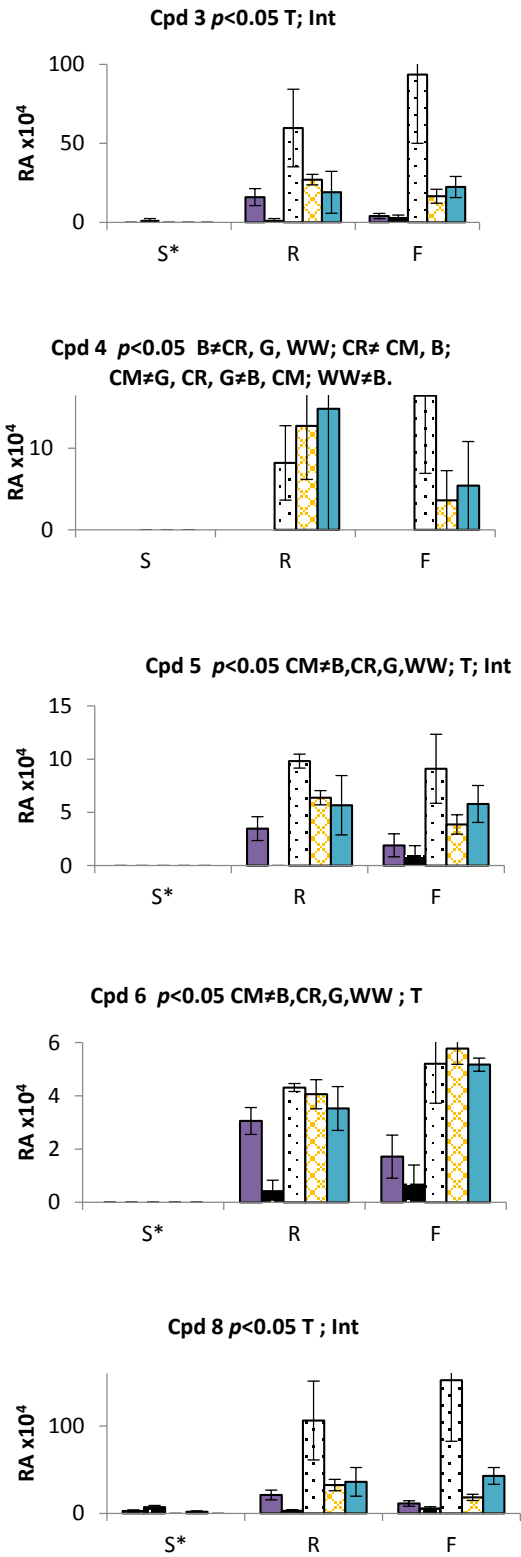
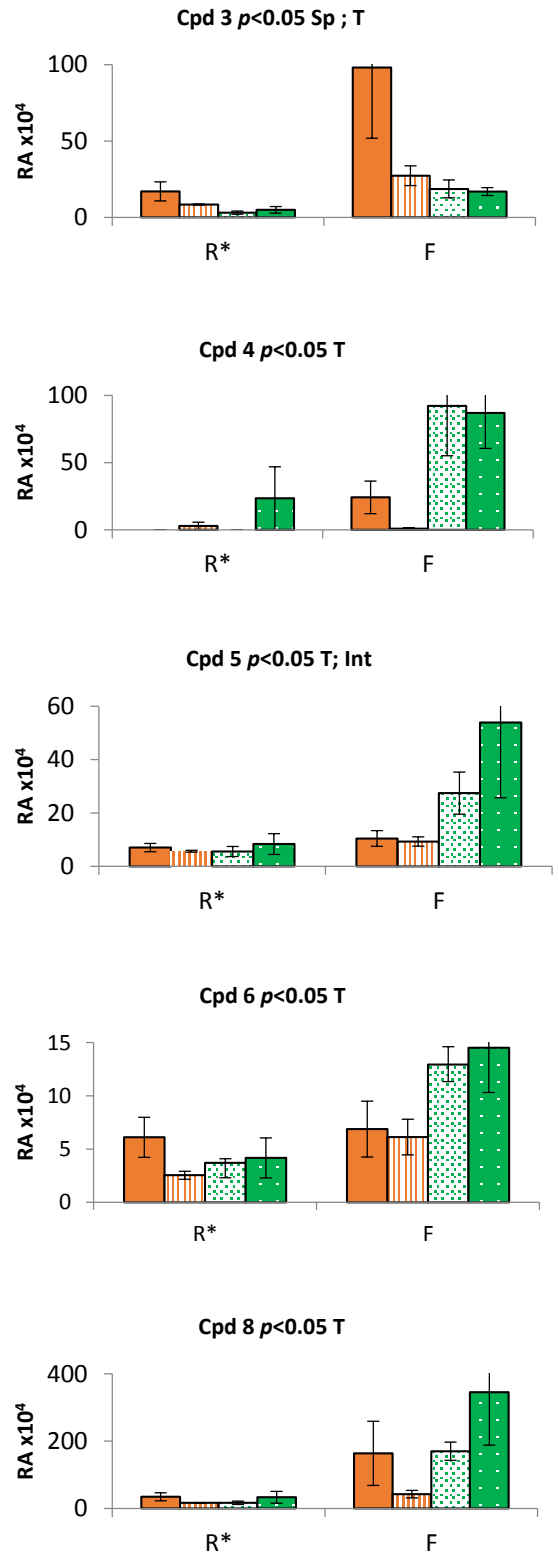


Figure 2S Distribution of nine shikonins in 36 periderm extracts separated by colouration including red, orange, peach, and yellow. Data was averaged over nine replicates for each colour treatment and log transformed. RA = relative abundance.

a)



b)



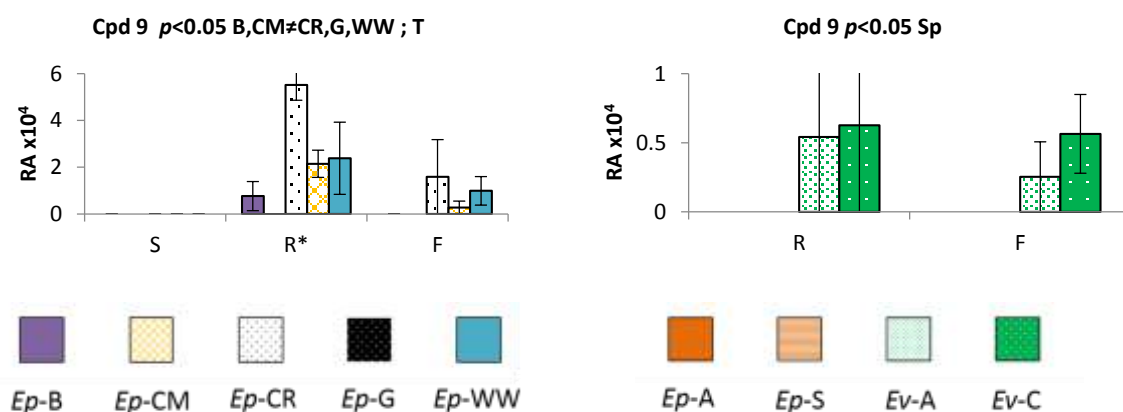


Figure 3S Relative abundance (RA) of 1, 2 and 7 based on the peak area and averaged over replication in *Echium plantagineum* (*Ep*) and *E. vulgare* (*Ev*) in glasshouse controlled conditions. Error bars represent standard error of the mean (SEM). Key: (a) *Ep*-B-Bendigo, *Ep*-CM-Coombah, *Ep*-CR-Cobar, *Ep*-G-Grenfell, *Ep*-WW-Wagga Wagga, (b) *Ep*-A-Adelong, *Ep*-S-Silverton, *Ev*-A-Adaminaby, *Ev*-C-Cooma. Significant differences ($p < 0.05$): ' \neq ' - between populations, Sp - between species and T - between time points. Int - significant interaction, time \times population. S- seedling, R- rosette, F-flowering. Data was log transformed prior to statistical analysis.

Table 1S. Results of repeated measures analysis of variance of relative abundance of compounds 1-9 and significance of time (growth stage), population and their interaction among five tested populations of *Echium plantagineum*. * denotes significant positive correlation of compound abundance with extract colour.

Compound	Source	df	Means square	F value	p value
Acetylshikonin (1)	Population	4	1.93531	5.95	0.0103
	Error Population*Replicates	10	0.32547		
	Time	2	5.03370	13.07	0.0003
	Population*Time	8	2.37977	6.18	0.0005
	Error Population*Replicates*Time	19	0.38517		
Deoxyshikonin (2)	Population	4	7.0020	3.24	0.0598
	Error Population*Replicates	10	2.1594		
	Time	2	40.3426	13.20	0.0003
	Population*Time	8	3.0016	0.98	0.4793
	Error Population*Replicates*Time	19	3.0568		
Dimethylacrylshikonin (3)	Population	4	1.0848	0.70	0.6122
	Error Population*Replicates	10	1.5601		
	Time	2	89.1036	88.10	0.0000

β-hydroxyisovalerylshikonin (4)	Population*Time	8	3.9200	3.88	0.0073
	Error Population*Replicates*Time	19	1.0113		
	Population	4	9.1710	7.27	0.0052
	Error Population*Replicates	10	1.2613		
	Time	2	12.4138	2.39	0.1185
Isobutyrylshikonin (5)	Population*Time	8	2.8478	0.55	0.8058
	Error Population*Replicates*Time	19	5.1923		
	Population	4	11.2710	12.70	0.0006
	Error Population*Replicates	10	0.8873		
	Time	2	67.0454	69.35	0.0000
Propionylshikonin (6)	Population*Time	8	3.6352	3.76	0.0084
	Error Population*Replicates*Time	19	0.9667		
	Population	4	6.2132	11.12	0.0011
	Error Population*Replicates	10	0.5585		
	Time	2	71.7415	48.85	0.0000
Shikonin (7)	Population*Time	8	2.0915	1.42	0.2495
	Error Population*Replicates*Time	19	1.4686		
	Population	4	1.328E+10	3.07	0.0686
	Error Population*Replicates	10	4.333E+09		
	Time	2	5.486E+10	10.75	0.0008
Compound 8	Population*Time	8	4.934E+09	0.97	0.4896
	Error Population*Replicates*Time	19	5.105E+09		
	Population	4	1.4135	1.37	0.3124
	Error Population*Replicates	10	1.0337		
	Time	2	47.8666	47.37	0.0000
Compound 9	Population*Time	8	6.5141	6.45	0.0004
	Error Population*Replicates*Time	19	1.0106		
	Population	4	9.1710	7.27	0.0052
	Error Population*Replicates	10	1.2613		
	Time	2	12.4138	2.39	0.1185
	Population*Time	8	2.8478	0.55	0.8058
	Error Population*Replicates*Time	19	5.1923		

Table 2S Results of repeated measures analysis of variance of relative abundance of compounds **1-9** and significance of time, species (mean of 2 populations per species) and their interaction between *Echium plantagineum* and *E. vulgare*.

Compound	Source	df	Means square	F value	p value
Acetylshikonin (1)	Species	1	1.94303	35.27	0.0001
	Error Species*Replicates	10	0.05508		
	Time	1	0.21809	3.02	0.1130
	Species*Time	1	1.42654	19.74	0.0012
	Error Species*Replicates*Time	10	0.07227		
Deoxyshikonin (2)	Species	1	22.1328	7.65	0.0199
	Error Species*Replicates	10	2.8927		
	Time	1	0.4439	0.10	0.7535
	Species*Time	1	7.9111	1.86	0.2029
	Error Species*Replicates*Time	10	4.2607		
Dimethylacrylshikonin (3)	Species	1	1.30689	10.40	0.0091
	Error Species*Replicates	10	0.12564		
	Time	1	2.49425	24.65	0.0006
	Species*Time	1	0.05531	0.55	0.4767
	Error Species*Replicates*Time	10	0.10117		
β-hydroxyisovalerylshikonin (4)	Species	1	17.9384	3.46	0.0926
	Error Species*Replicates	10	5.1898		
	Time	1	66.8822	17.52	0.0019
	Species*Time	1	14.9050	3.90	0.0764
	Error Species*Replicates*Time	10	3.8180		
Isobutyrylshikonin (5)	Species	1	0.33309	3.73	0.0823
	Error Species*Replicates	10	0.08931		
	Time	1	1.44927	23.48	0.0007
	Species *Time	1	0.58393	9.46	0.0117
	Error Species*Replicates*Time	10	0.06173		
Propionylshikonin (6)	Species	1	0.08855	0.97	0.3478
	Error Species*Replicates	10	0.09125		
	Time	1	1.08504	10.27	0.0094
	Species*Time	1	0.35474	3.36	0.0968
	Error Species*Replicates*Time	10	0.10566		
Shikonin (7)	Population	1	0.00137	0.00	0.9718
	Error Species*Replicates	10	1.04371		

Compound 8	Time	1	0.74312	0.54	0.4803
	Species*Time	1	2.94288	2.13	0.1753
	Error Species*Replicates*Time	10	1.38267		
	Population	1	0.28700	2.56	0.1407
	Error Species*Replicates	10	0.11214		
Compound 9	Time	1	3.30200	21.82	0.0009
	Species*Time	1	0.56719	3.75	0.0816
	Error Species*Replicates*Time	10	0.15131		
	Species	1	17.0331	7.33	0.0220
	Error Species*Replicates	10	2.3223		
	Time	1	0.4395	0.19	0.6761
	Species*Time	1	0.4395	0.19	0.6761
Error Species*Replicates*Time	10	2.3729			

Table 3S *Echium plantagineum* samples collected from glasshouse experiment in the study of phenological cycle for each population including: Bendigo, Grenfell, Coombah, Cobar, Silverton, and Wagga Wagga.

Plant age [weeks]	Phenological stage	Block
1	S	1
1	S	2
1	S	3
7	R	1
8	R	2
9	R	3
12	F	1
13	F	2
14	F	3

Table 4S Plant samples obtained from controlled conditions experiment using two populations of *E. plantagineum* and two populations of *E. vulgare*, harvested at two time points. Each sample was a composite of four plant extracts. Key: F-flowering stage; R-rosette stage. Note: at both harvest dates, *E. vulgare* was at rosette stage.

Species	Location	Plant age [weeks]	Phenological state	Block
<i>E. plantagineum</i>	Adelong 1	6	R	1
	Adelong 1	27	F	1
	Adelong 2	7	R	2
	Adelong 2	28	F	2
	Adelong 3	8	R	3
	Adelong 3	29	F	3
	Silverton 1	6	R	1
	Silverton 1	27	F	1
	Silverton 2	7	R	2
	Silverton 2	28	F	2
	Silverton 3	8	R	3
	Silverton 3	29	F	3
<i>E. vulgare</i>	Adaminaby 1	6	R	1
	Adaminaby 1	27	R	1
	Adaminaby 2	7	R	2
	Adaminaby 2	28	R	2
	Adaminaby 3	8	R	3
	Adaminaby 3	29	R	3
	Cooma 1	6	R	1
	Cooma 1	27	R	1
	Cooma 2	7	R	2
	Cooma 2	28	R	2
	Cooma 3	8	R	3
	Cooma 3	29	R	3