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APPENDIX



## Appendix A

## Isoflavonoid contents calculation (mg/100g powder)

The 50 g dry leave powder have an isoflavonoid content	x	mg/ml
Isoflavonoid amount from HPLC using Empower program	x	mg/ml
Crude extract amount	y	g
The amount of isoflavonoid content will be	1,000xy	mg/50 g powder
	2,000xy	mg/100 g powder



## Appendix B

HPLC analysis of standard major isoflavonoids content.



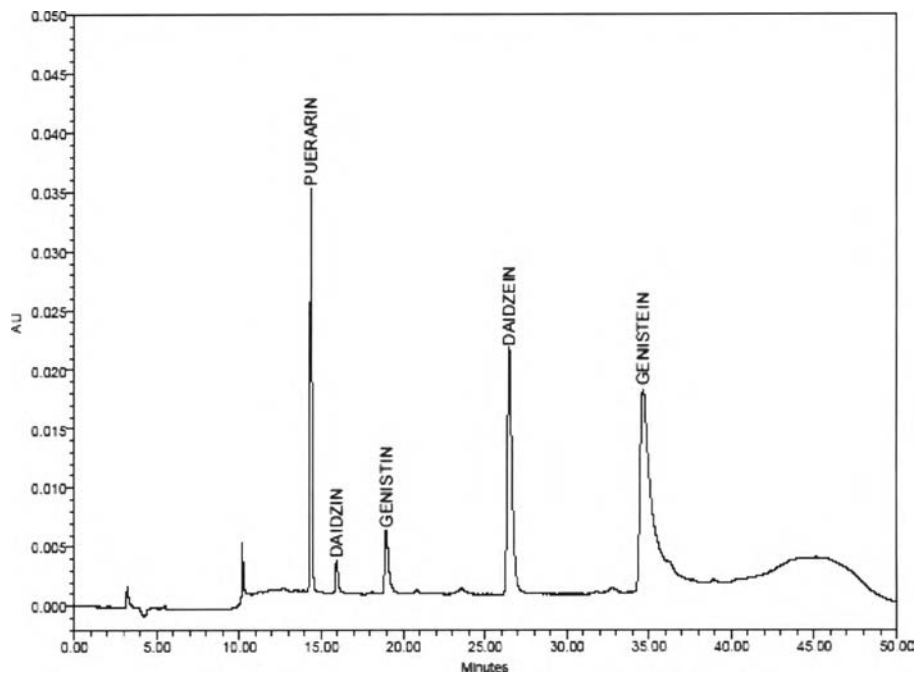
## Default Individual Report

Reported by User: System

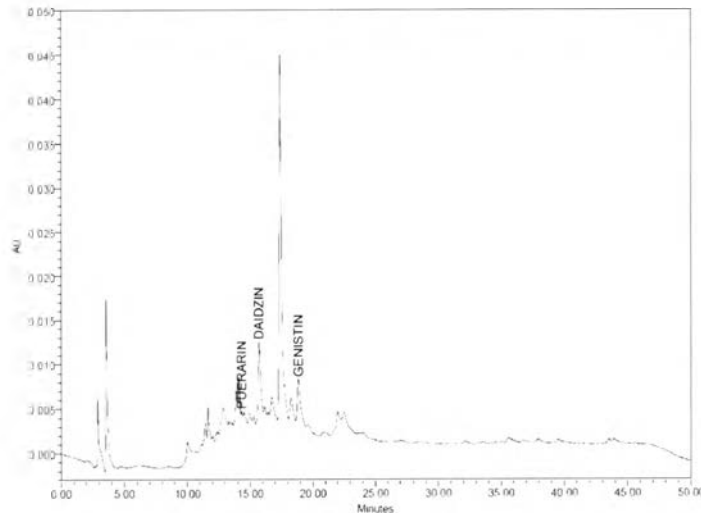
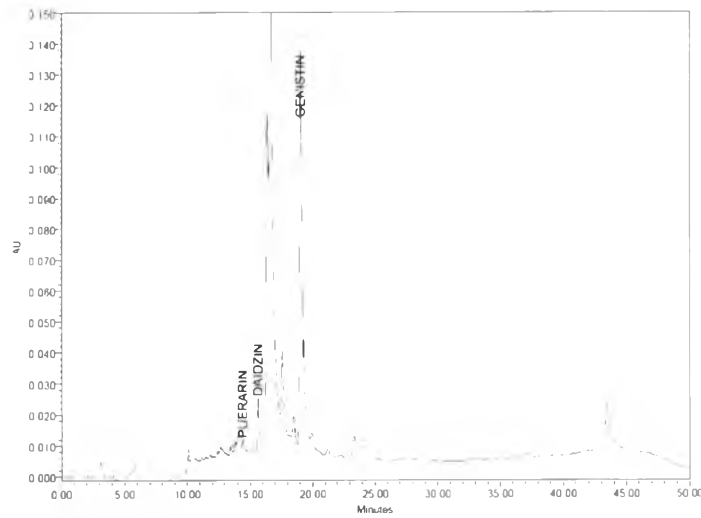
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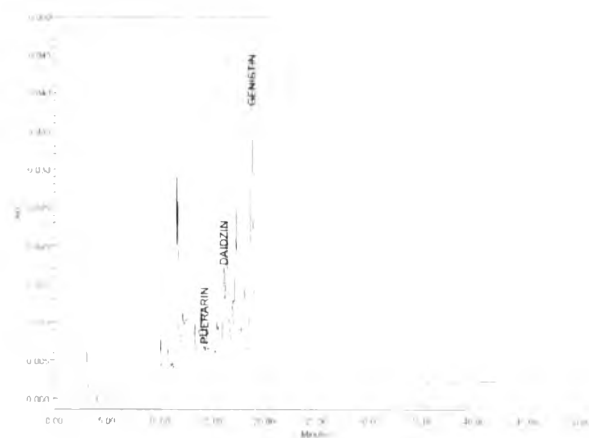
## SAMPLE INFORMATION

Sample Name:	STD Isoflavonoids 2 271207	Acquired By:	System
Sample Type:	Standard	Date Acquired:	12/28/2007 07:39:43
Vial:	85	Acq. Method Set:	Isoflavone_water@_2
Injection #:	1	Date Processed:	3/7/2009 01:08:25
Injection Volume:	10.00 ul	Processing Method:	Std Iso Isoing
Run Time:	80.0 Minutes	Channel Name:	254nm
Sample Set Name:	STD ISOFLAVONOIDS	Proc. Chnl. Descr.:	PDA 254.0 nm

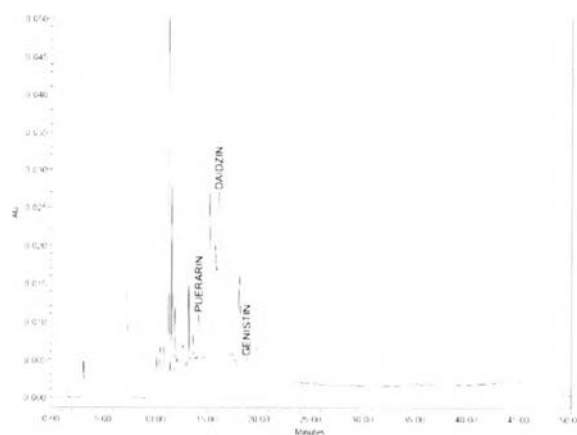


## Appendix C

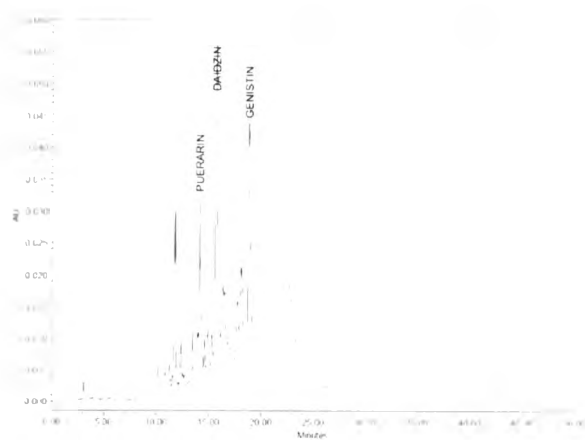
HPLC analysis of major isoflavonoids in *P. mirifica* leavesHPLC fingerprint of *P. mirifica* leaves (PM-III) in MarchHPLC fingerprint of *P. mirifica* leaves (PM-IV) in April



(A)



(B)

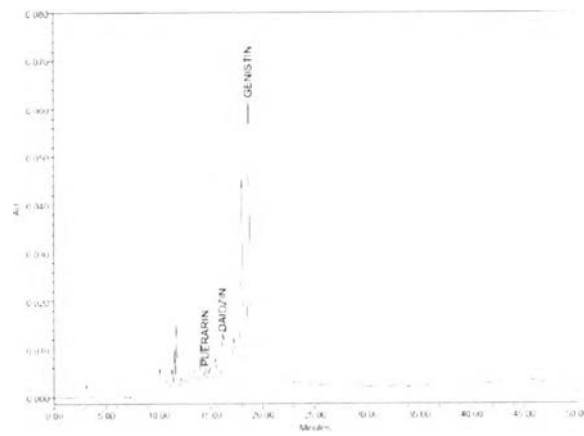


(C)

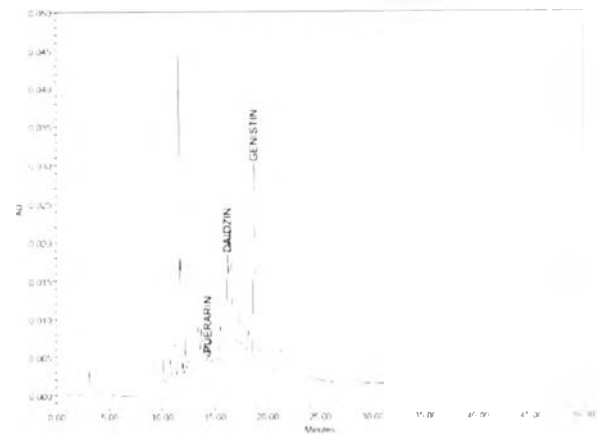
HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in May



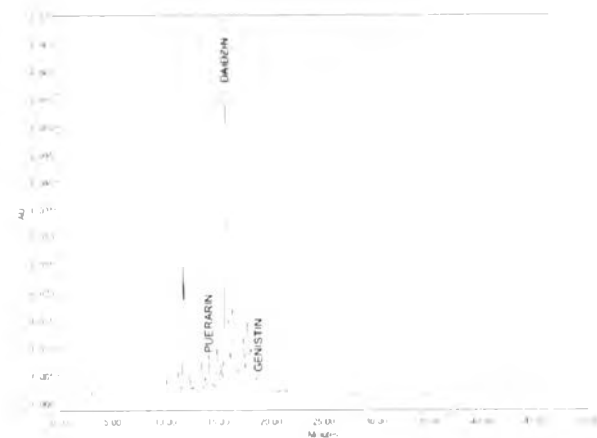




(A)



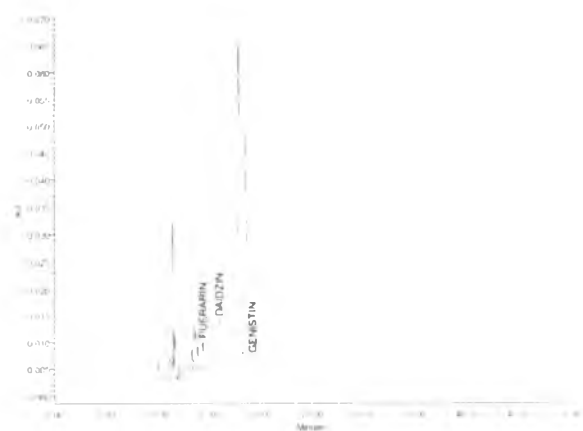
(B)



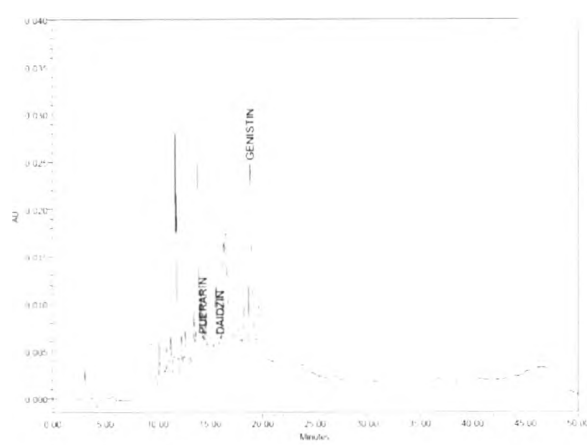
(C)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in June

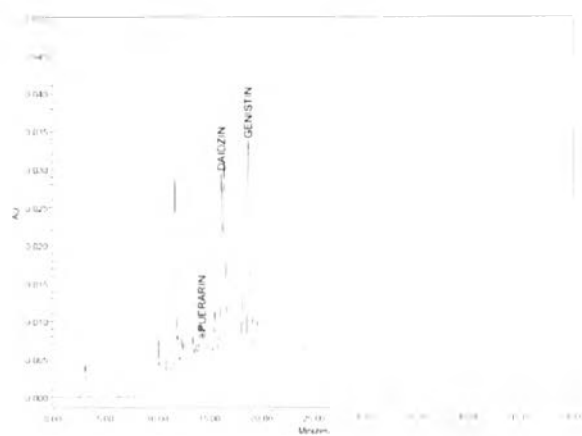




(A)

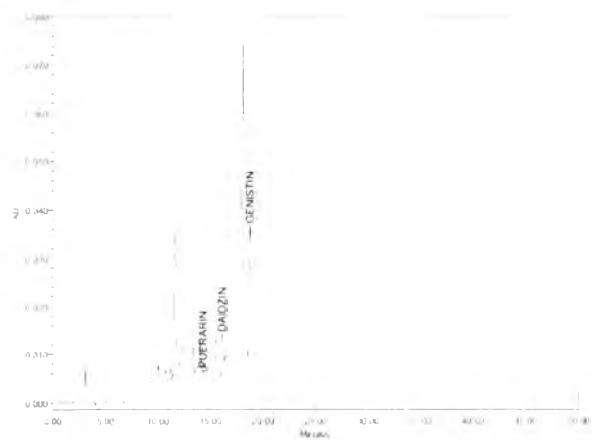


(B)

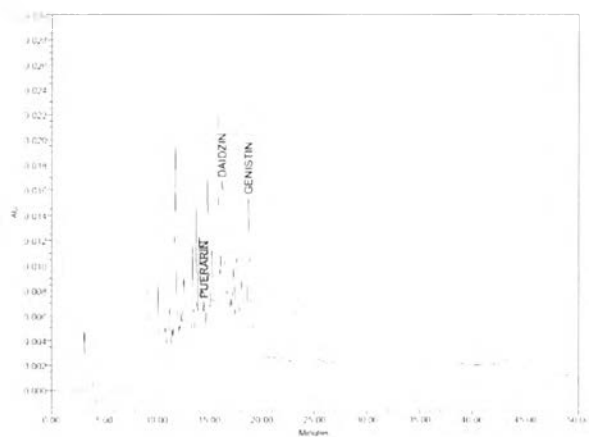


(C)

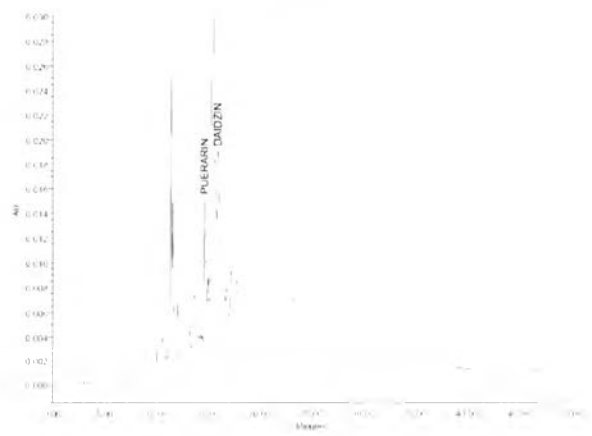
HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in July



(A)



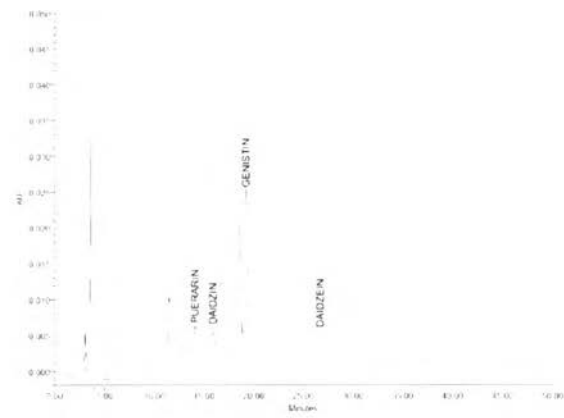
(B)



(C)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in August

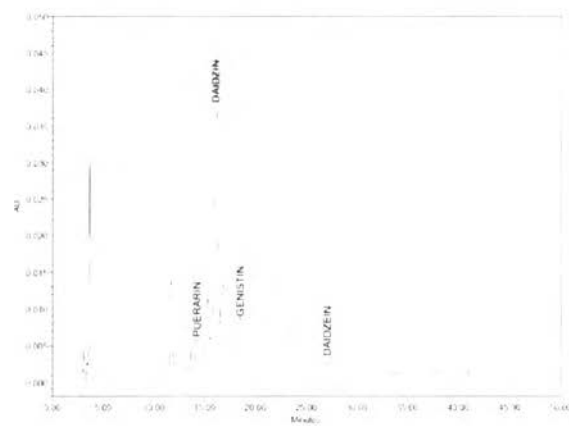




(A)



(B)



(C)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in September





(A)



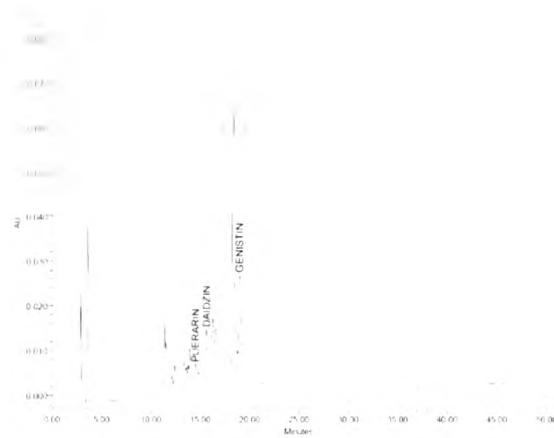
(B)



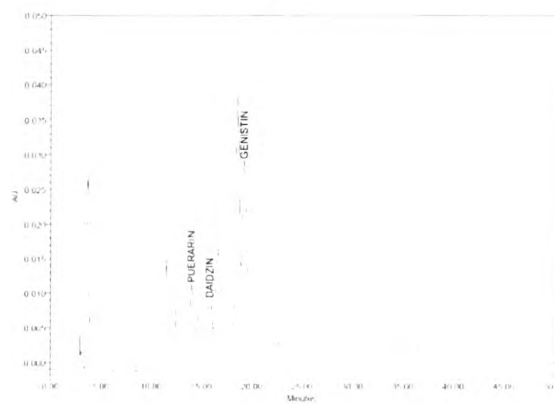
(C)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in October





(A)



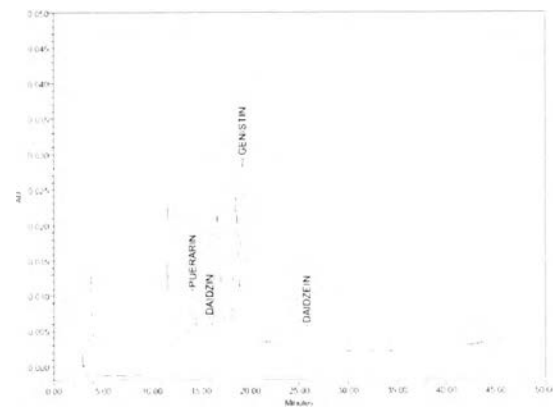
(B)



(C)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in November





(A)



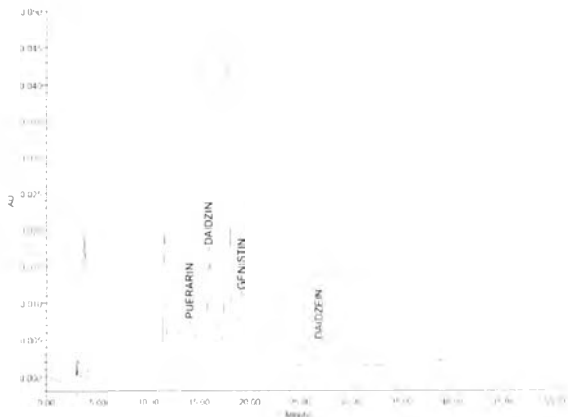
(B)



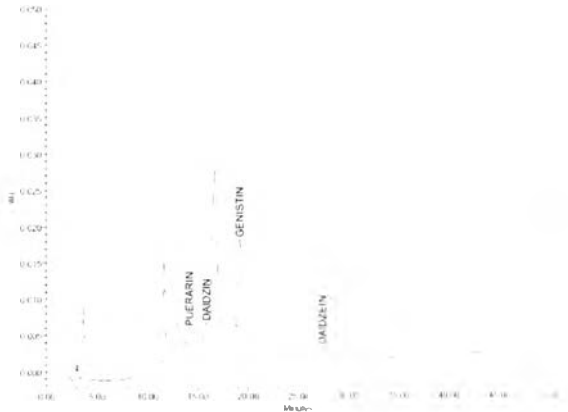
(C)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in December

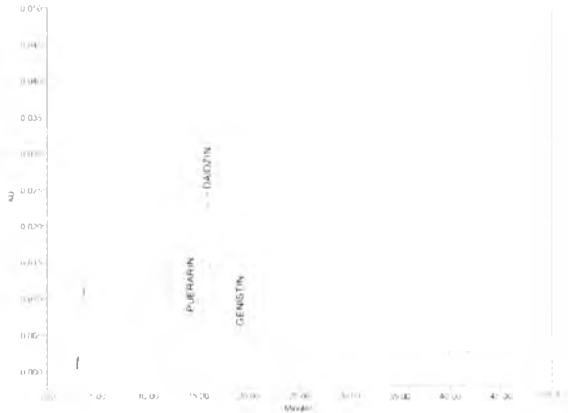




(A)



(B)

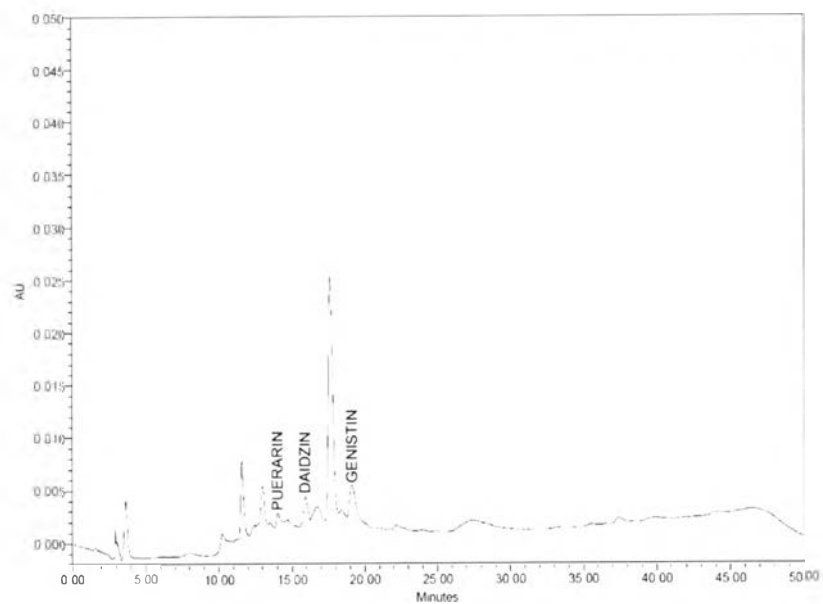


(C)

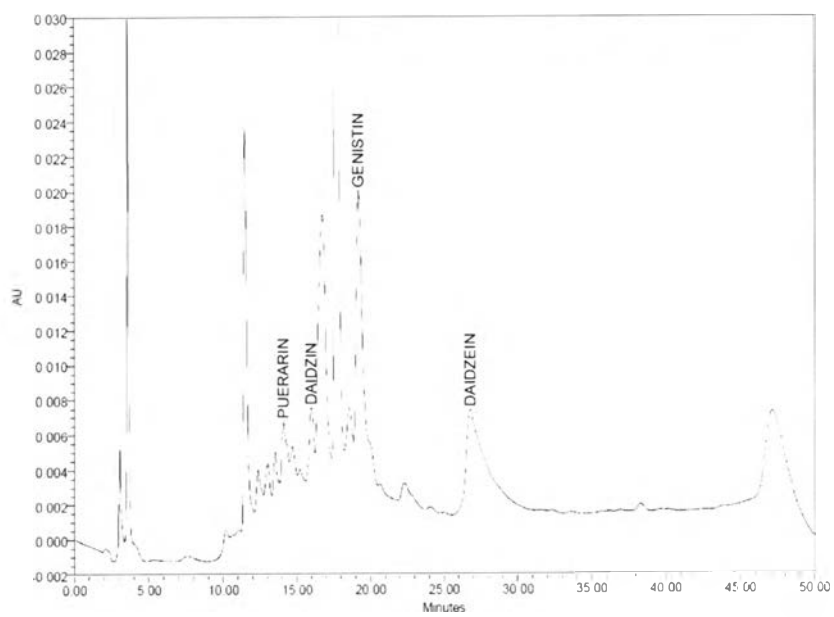
HPLC fingerprint of *P. mirifica* leaves (A) PM-III, (B) PM-IV and (C) PM-V in January







(A)



(B)

HPLC fingerprint of *P. mirifica* leaves (A) PM-III and (B) PM-IV in February



## Appendix D

HPLC analysis of major isoflavonoids in *P. mirifica* tubers.

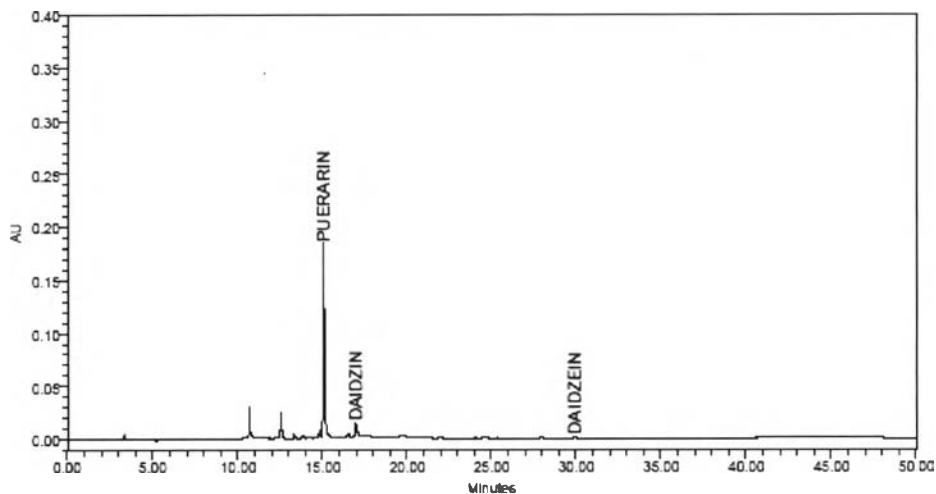
## Default Individual Report

Reported by User: System

Project Name: Isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 4:29:35
Vial:	5	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	26/5/2554 16:47:54
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.085	1180970	78.09	0.032500	mg/ml
2	DAIDZIN	16.988	154069	10.10	0.032500	mg/ml
3	GENISTIN	20.925				
4	DAIDZEIN	29.931	12018	0.79	0.032500	mg/ml
5	GENISTEN	40.400				

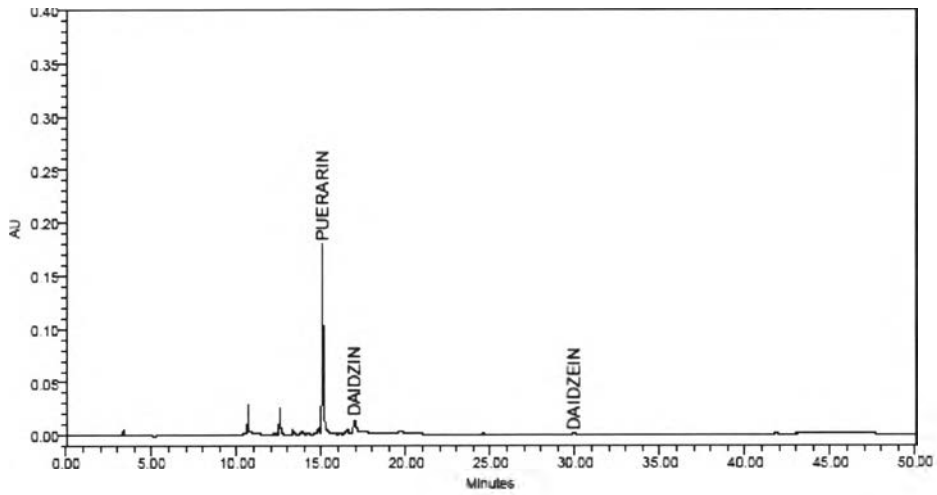


Default Individual Report

Reported by User: System

Project Name: isoflavone2

SAMPLE INFORMATION			
Sample Name:	Tuber PM-IV Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 3:28:44
Vial:	5	Acq. Method Set:	Isoflavone_water9_2
Injection #:	2	Date Processed:	20/5/2554 16:47:54
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.078	1183528	78.21	0.032500	mg/ml
2	DAIDZIN	16.066	152331	10.07	0.032500	mg/ml
3	GENISTIN	20.025				
4	DAIDZEIN	29.925	11225	0.74	0.032500	mg/ml
5	GENISTEN	40.400				

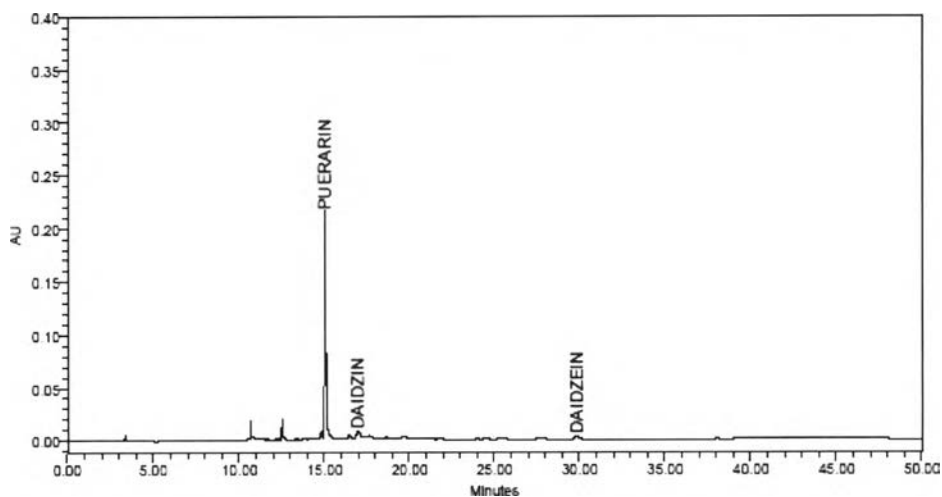


Default Individual Report

Reported by User: System

Project Name: isoflavone2

SAMPLE INFORMATION			
Sample Name:	Tuber PM-IV Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 11:35:51
Vial:	8	Acq. Method Set:	Isoflavone_water9_2
Injection #:	1	Date Processed:	28/5/2554 18:47:48
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.085	1334828	80.15	0.025000	mg/ml
2	DAIDZIN	18.974	117482	7.05	0.025000	mg/ml
3	GENISTIN	20.925				
4	DAIDZEIN	29.861	37582	2.26	0.025000	mg/ml
5	GENISTEN	40.400				





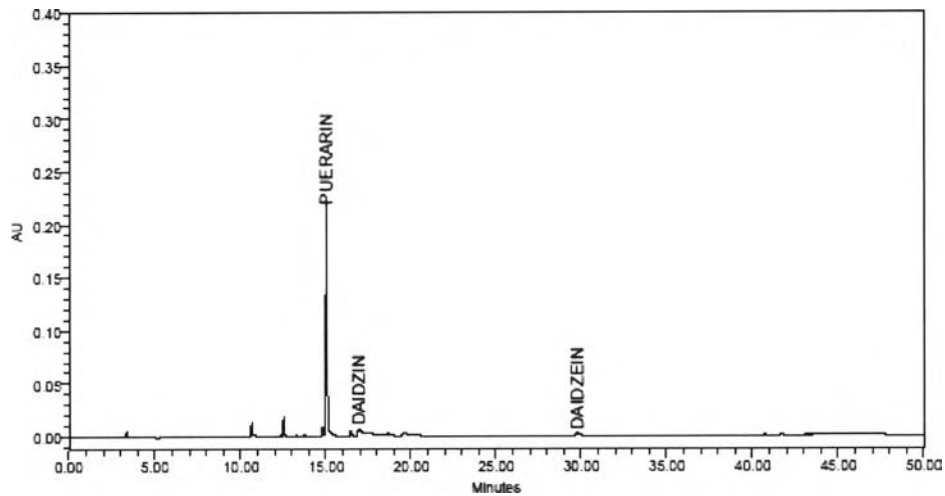
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 12:38:41
Vial:	8	Acq. Method Set:	Isoflavone_water9_2
Injection #:	2	Date Processed:	28/5/2554 18:47:47
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WVln Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.038	1334279	80.48	0.025000	mg/ml
2	DAIDZIN	18.964	117830	7.11	0.025000	mg/ml
3	GENISTIN	20.925				
4	DAIDZEIN	29.799	37834	2.27	0.025000	mg/ml
5	GENISTEIN	40.400				



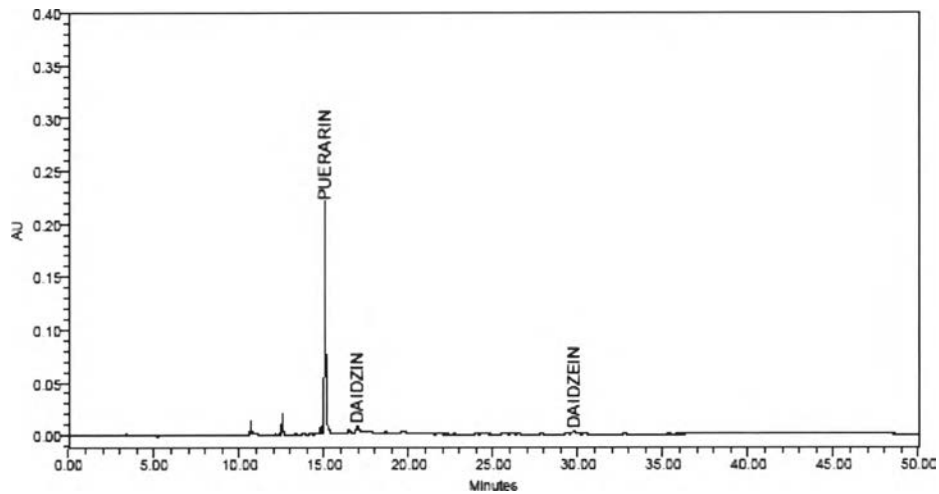
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 13:37:32
Via:	8	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	26/5/2554 16:47:46
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254 0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.063	1337256	80.54	0.025000	mg/ml
2	DAIDZIN	16.964	117335	7.07	0.025000	mg/ml
3	GENISTIN	20.925				
4	DAIDZEIN	29.782	35255	2.12	0.025000	mg/ml
5	GENISTEIN	40.400				



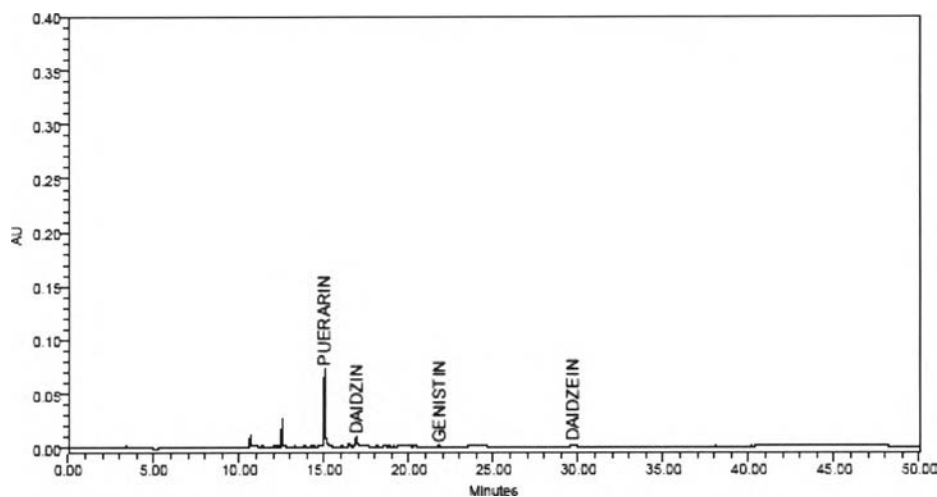
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 21:44:35
Vial:	11	Acq. Method Set:	Isoflavone_water9_2
Injection #:	2	Date Processed:	26/5/2554 16:47:40
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	Wwin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.021	443161	65.98	0.025000	mg/ml
2	DAIDZIN	16.899	74892	11.15	0.025000	mg/ml
3	GENISTIN	21.803	8192	1.22	0.025000	mg/ml
4	DAIDZEIN	29.682	25837	3.85	0.025000	mg/ml
5	GENISTIN	40.400				



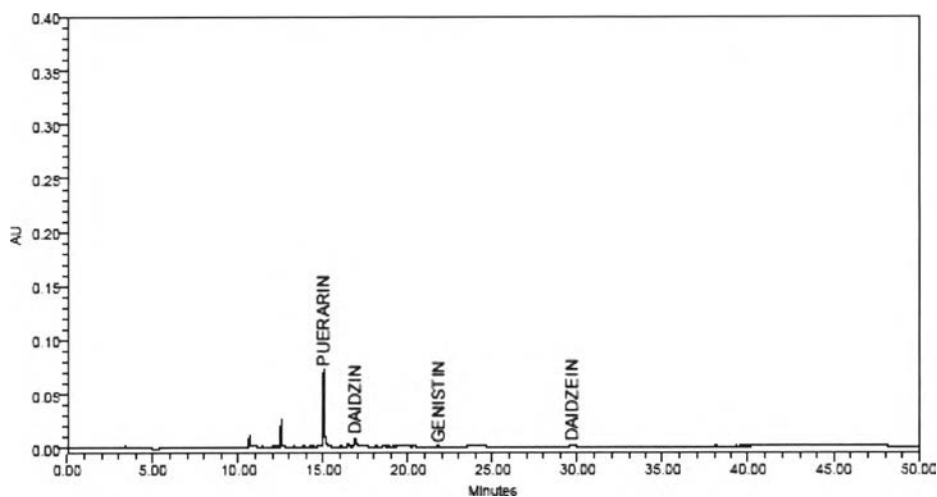
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 20:43:45
Vial:	11	Acq. Method Set:	Isoflavone_water9_2
Injection #:	1	Date Processed:	28/5/2554 16:47:40
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.014	44397.1	66.05	0.025000	mg/ml
2	DAIDZIN	16.880	7573.4	11.27	0.025000	mg/ml
3	GENISTIN	21.793	853.4	1.27	0.025000	mg/ml
4	DAIDZEIN	29.688	2666.4	3.97	0.025000	mg/ml
5	GENISTIN	40.400				





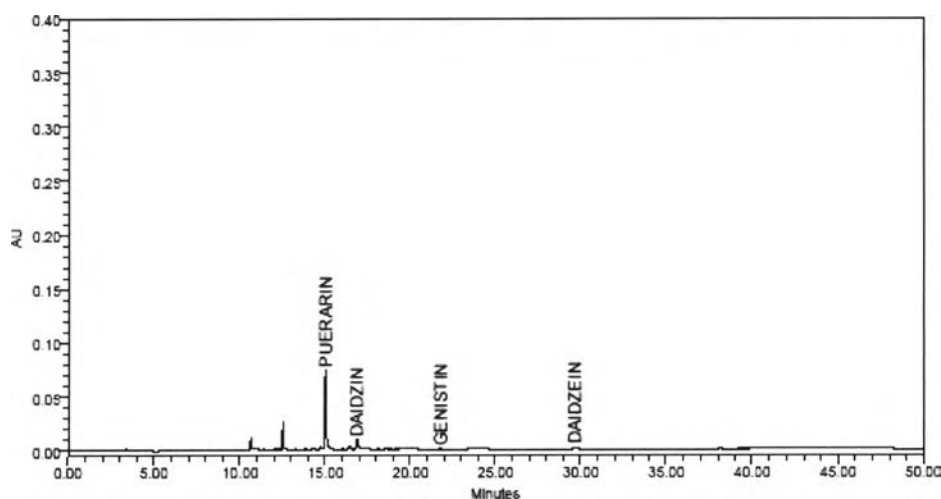
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	10/3/2554 22:45:25
Vial:	11	Acq. Method Set:	Isoflavone_water@_2
Injection #:	3	Date Processed:	20/5/2554 10:47:39
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WIn Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.017	445771	65.89	0.025000	mg/ml
2 DAIDZIN	16.883	76042	11.24	0.025000	mg/ml
3 GENISTIN	21.794	8640	1.28	0.025000	mg/ml
4 DAIDZEIN	29.869	25395	3.75	0.025000	mg/ml
5 GENISTIN	40.400				

I 27435076



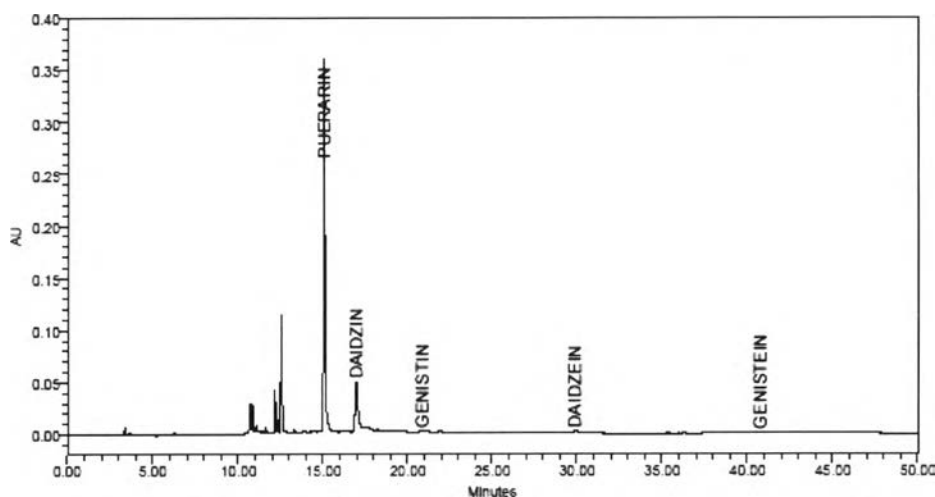
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 5:30:33
Vial:	8	Acq. Method Set:	Isoflavone_water@_2
Injection #:	1	Date Processed:	26/5/2554 16:47:53
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.074	2322493	75.72	0.025000	mg/ml
2	DAIDZIN	16.973	558686	18.22	0.025000	mg/ml
3	GENISTIN	20.879	31415	1.02	0.025000	mg/ml
4	DAIDZEIN	29.912	33837	1.11	0.025000	mg/ml
5	GENISTEIN	40.759	8279	0.27	0.050000	mg/ml



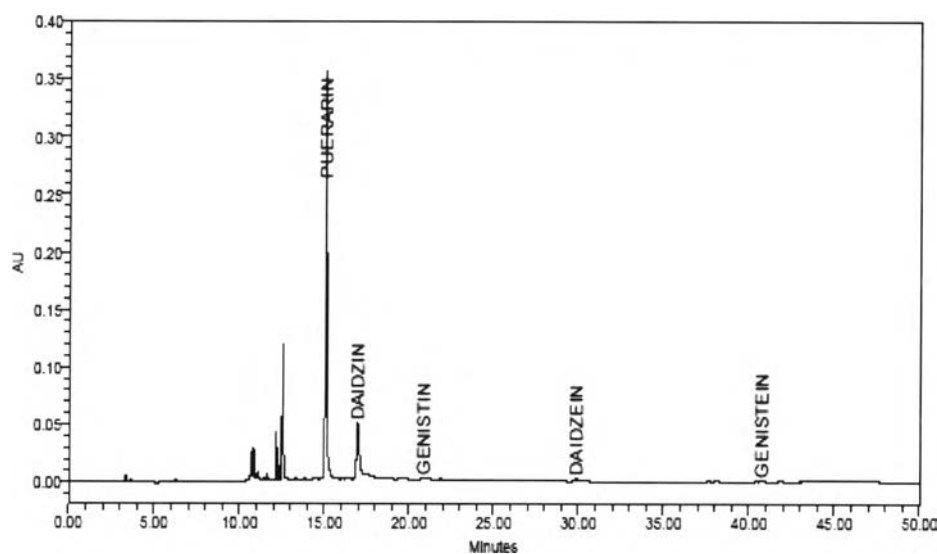
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 6:31:23
Vial:	6	Acq. Method Set:	Isoflavone_water0_2
Injection #:	2	Date Processed:	28/5/2554 16:47:52
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	Wwin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 FUERARIN	15.077	2325549	75.01	0.025000	mg/ml
2 DAIDZIN	18.974	559947	18.06	0.025000	mg/ml
3 GENISTIN	20.864	46577	1.50	0.025000	mg/ml
4 DAIDZEIN	29.902	33652	1.09	0.025000	mg/ml
5 GENISTEIN	40.763	12038	0.39	0.050000	mg/ml



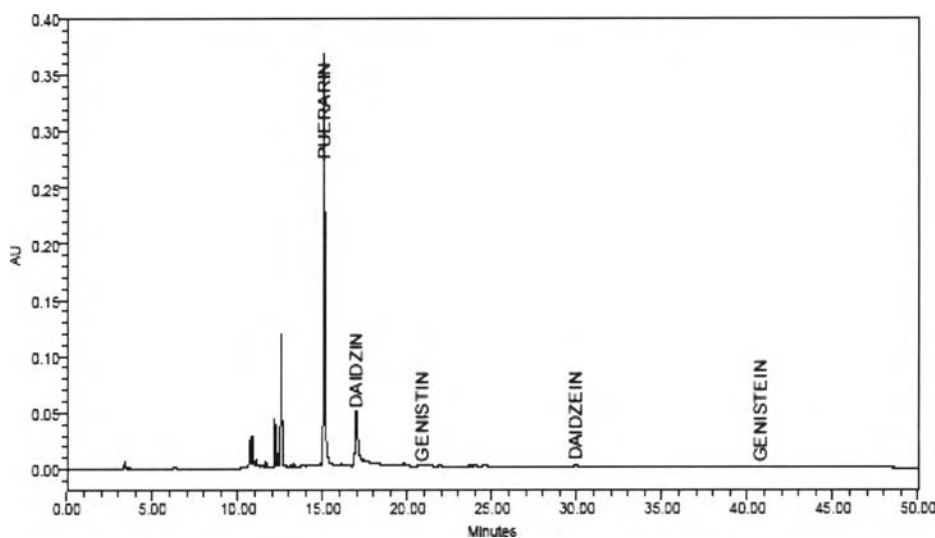
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 7:32:14
Vial:	6	Acq. Method Set:	Isoflavone_water@_2
Injection #:	3	Date Processed:	26/5/2554 16:47:52
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	Wvin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.083	2327438	75.07	0.025000	mg/ml
2 DAIDZIN	18.989	564056	18.22	0.025000	mg/ml
3 GENISTIN	20.884	45173	1.46	0.025000	mg/ml
4 DAIDZEIN	29.943	33394	1.08	0.025000	mg/ml
5 GENISTEIN	40.762	9084	0.29	0.050000	mg/ml



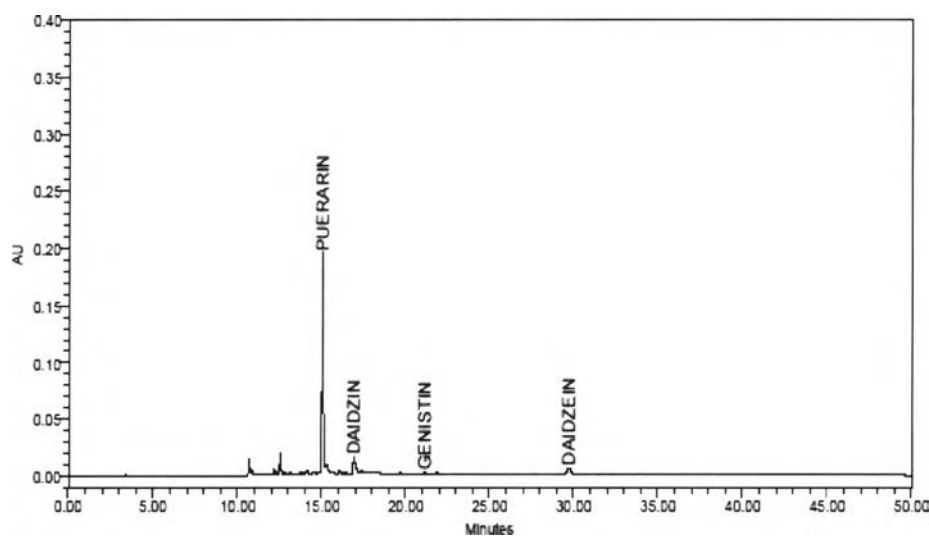
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 14:38:30
Vial:	9	Acq. Method Set:	Isoflavone_water9_2
Injection #:	1	Date Processed:	26/5/2554 16:47:45
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WVln Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUE RARIN	15.063	1148940	75.00	0.025000	mg/ml
2 DAIDZIN	18.932	152480	9.95	0.025000	mg/ml
3 GENISTIN	21.148	6067	0.40	0.025000	mg/ml
4 DAIDZEIN	29.742	74667	4.87	0.025000	mg/ml
5 GENISTIN	40.400				



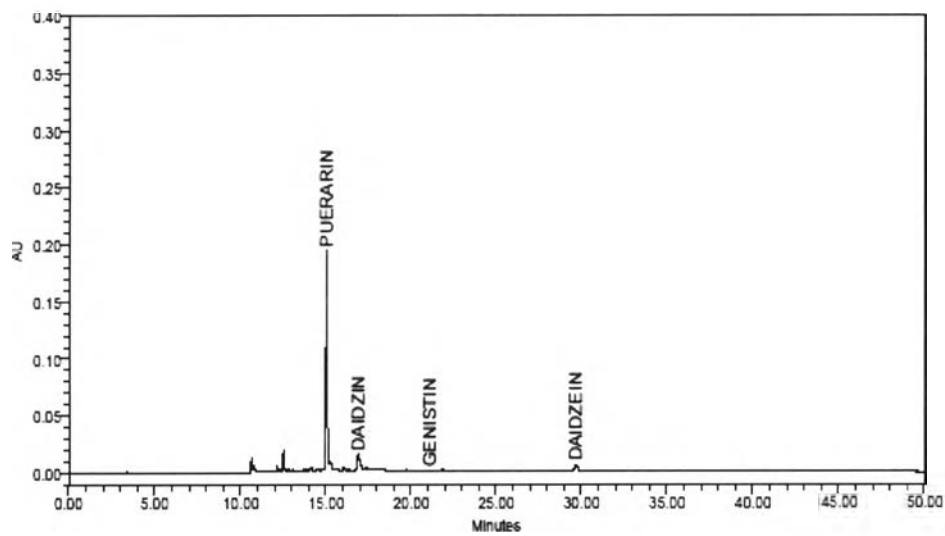
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	10/3/2554 15:39:20
Vial:	9	Acq. Method Set:	Isoflavone_water0_2
Injection #:	2	Date Processed:	26/5/2554 16:47:44
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WVln Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.041	1158563	75.31	0.025000	mg/ml
2 DAIDZIN	16.920	152677	9.92	0.025000	mg/ml
3 GENISTIN	21.135	6041	0.39	0.025000	mg/ml
4 DAIDZEIN	29.715	75187	4.88	0.025000	mg/ml
5 GENISTIN	40.400				



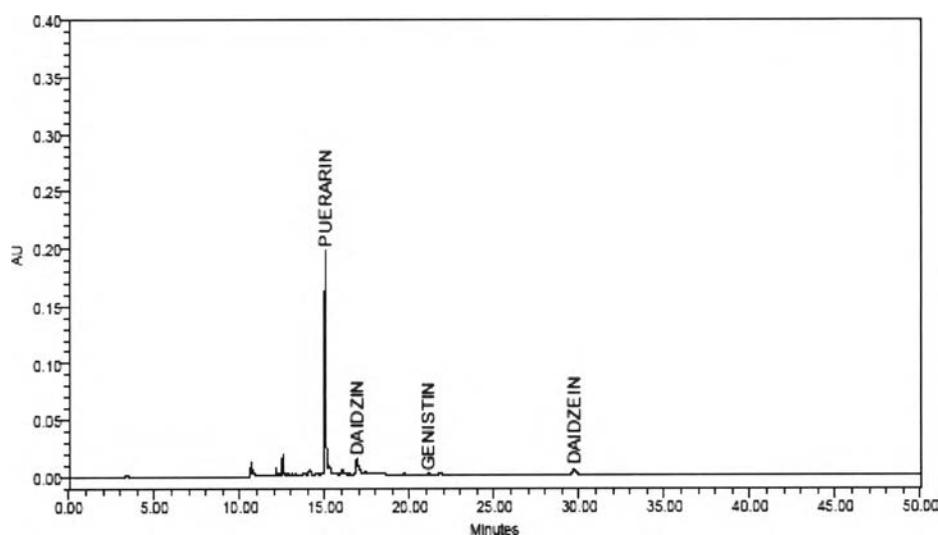
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 16:40:11
Vial:	9	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	26/5/2554 16:47:43
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.027	1184698	75.24	0.025000	mg/ml
2 DAIDZIN	16.910	153519	9.92	0.025000	mg/ml
3 GENISTIN	21.133	7251	0.47	0.025000	mg/ml
4 DAIDZEIN	29.721	76568	4.95	0.025000	mg/ml
5 GENISTIN	40.400				



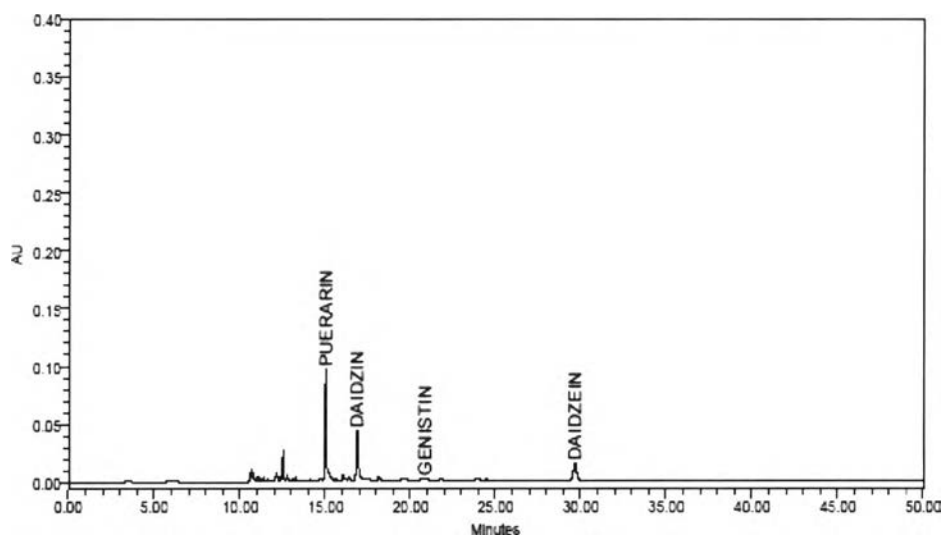
## Default Individual Report

Reported by User: System

Project Name: isoflavone.2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	10/3/2554 23:40:23
Vial:	12	Acq. Method Set:	Isoflavone_water0_2
Injection #:	1	Date Processed:	28/5/2554 18:47:38
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.021	550233	38.28	0.025000	mg/ml
2 DAIDZIN	18.883	372312	25.90	0.025000	mg/ml
3 GENISTIN	20.873	44025	3.08	0.025000	mg/ml
4 DAIDZEIN	29.879	190967	13.29	0.025000	mg/ml
5 GENISTIN	40.400				





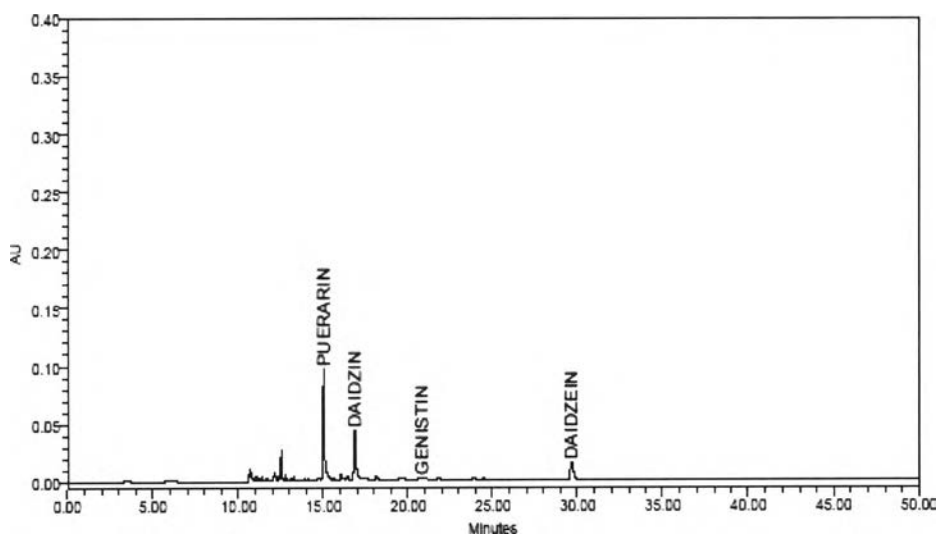
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	20/3/2554 0:47:13
Vial:	12	Acq. Method Set:	Isoflavone_water@_2
Injection #:	2	Date Processed:	26/5/2554 16:47:37
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.023	585419	40.33	0.025000	mg/ml
2 DAIDZIN	16.876	376266	25.92	0.025000	mg/ml
3 GENISTIN	20.662	43953	3.03	0.025000	mg/ml
4 DAIDZEIN	29.663	193204	13.31	0.025000	mg/ml
5 GENISTEN	40.400				



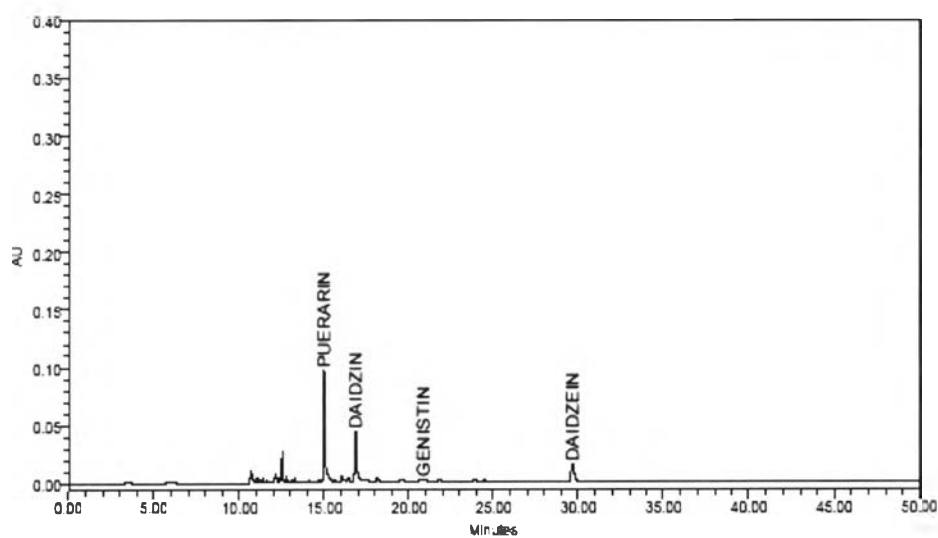
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-V Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	20/3/2554 1:48:03
Vial:	12	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	26/5/2554 16:47:30
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.008	585281	41.79	0.025000	mg/ml
2 DAIDZIN	18.873	361483	25.81	0.025000	mg/ml
3 GENISTIN	20.870	33793	2.41	0.025000	mg/ml
4 DAIDZEIN	29.664	191864	13.70	0.025000	mg/ml
5 GENISTIN	40.400				



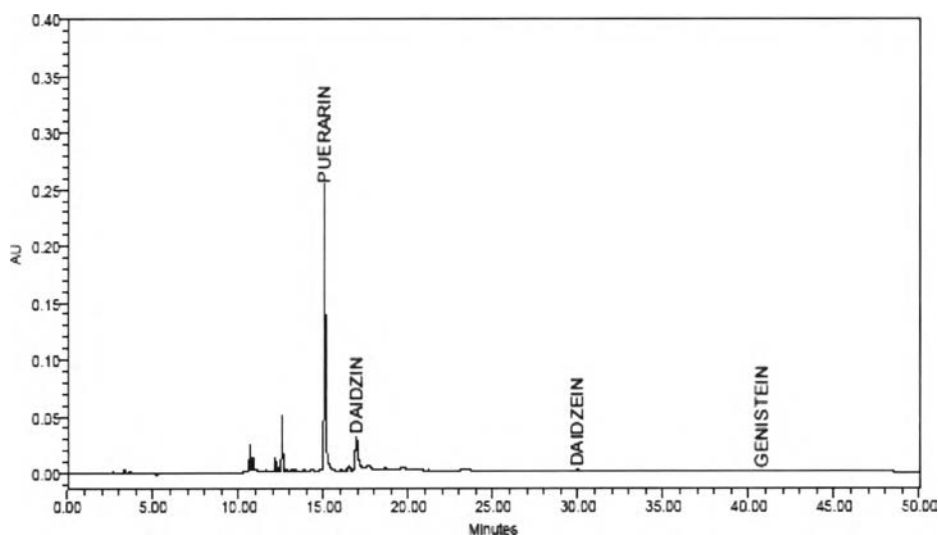
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	18/3/2554 23:25:15
Vial:	4	Acq. Method Set:	Isoflavone_water@_2
Injection #:	1	Date Processed:	26/5/2554 16:47:58
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.074	1706541	70.50	0.025000	mg/ml
2	DAIDZIN	16.863	397817	16.41	0.025000	mg/ml
3	GENISTIN	20.925				
4	DAIDZEIN	26.976	24179	1.00	0.025000	mg/ml
5	GENISTEN	40.768	3939	0.16	0.050000	mg/ml



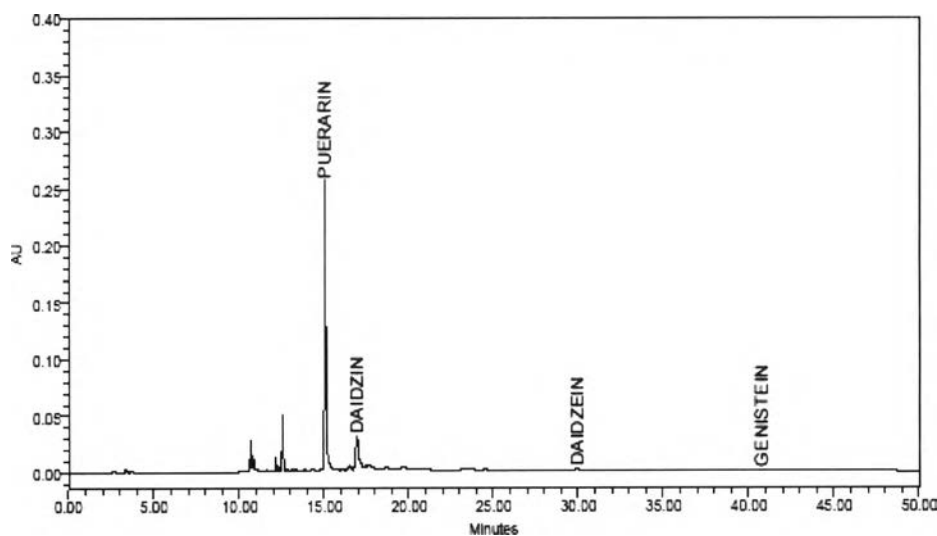
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 0:28:05
Vial:	4	Acq. Method Set:	Isoflavone_water9_2
Injection #:	2	Date Processed:	26/5/2554 18:47:57
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.071	1722221	70.63	0.025000	mg/ml
2 DAIDZIN	16.951	397713	16.31	0.025000	mg/ml
3 GENISTIN	20.925				
4 DAIDZEIN	29.977	24031	0.99	0.025000	mg/ml
5 GENISTEIN	40.780	3655	0.16	0.050000	mg/ml



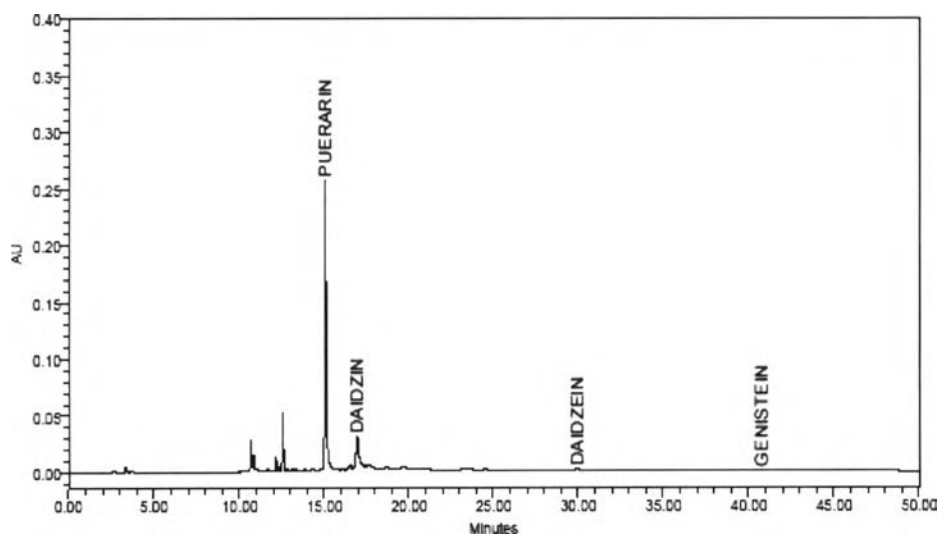
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 1:28:58
Vial:	4	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	26/5/2554 16:47:58
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	FDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.083	1728115	70.33	0.025000	mg/ml
2 DAIDZIN	16.989	399899	16.28	0.025000	mg/ml
3 GENISTIN	20.925				
4 DAIDZEIN	29.986	24876	1.00	0.025000	mg/ml
5 GENISTEIN	40.783	4847	0.20	0.050000	mg/ml



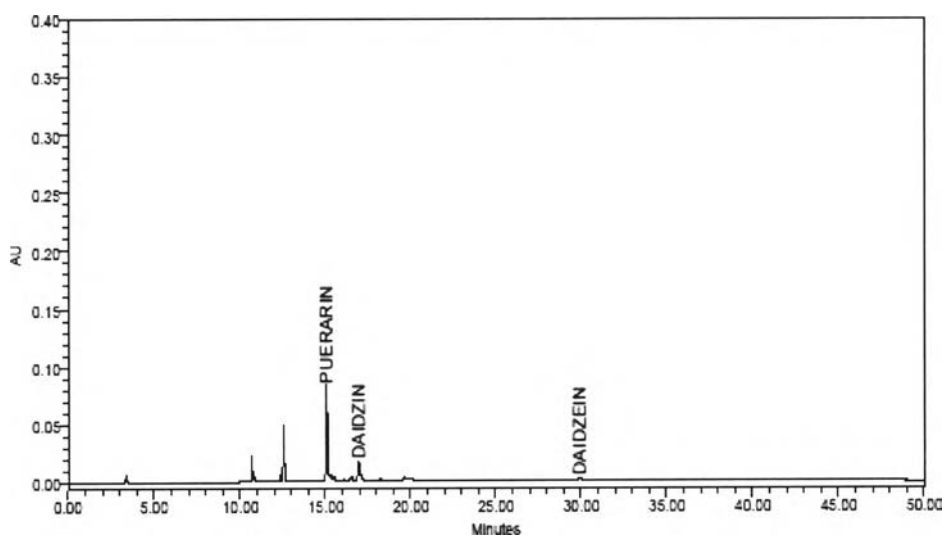
## Default Individual Report

Reported by User System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 8:33:12
Vial:	7	Acq. Method Set:	Isoflavone_water0_2
Injection #:	1	Date Processed:	28/5/2554 16:47:50
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.088	511105	56.32	0.025000	mg/ml
2 DAIDZIN	18.986	165695	18.26	0.025000	mg/ml
3 GENISTIN	20.925				
4 DAIDZEIN	29.935	28579	3.15	0.025000	mg/ml
5 GENISTEN	40.400				



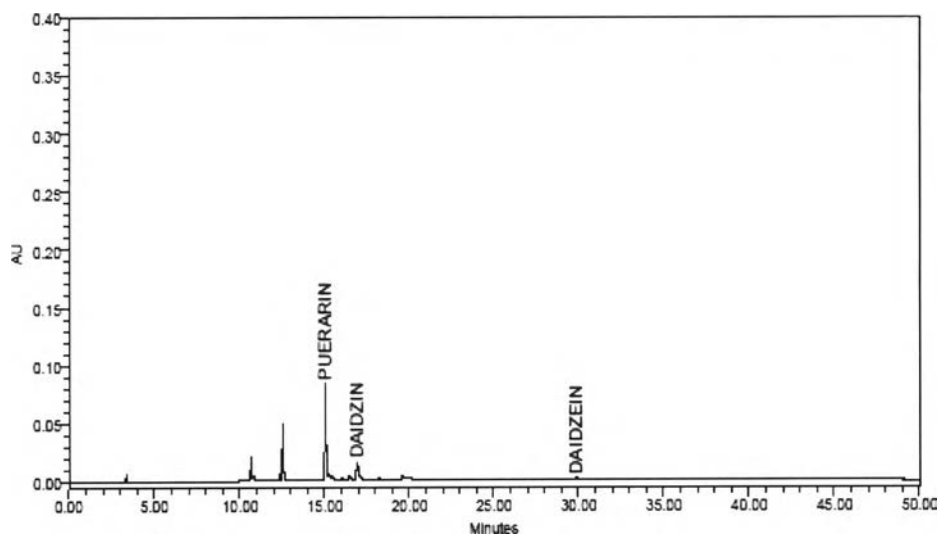
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 9:34:02
Vial:	7	Acq. Method Set:	Isoflavone_water9_2
Injection #:	2	Date Processed:	26/5/2554 16:47:49
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.062	508506	58.19	0.025000	mg/ml
2 DAIDZIN	18.947	155045	17.74	0.025000	mg/ml
3 GENISTIN	20.825				
4 DAIDZEIN	29.886	28728	3.29	0.025000	mg/ml
5 GENISTEIN	40.400				



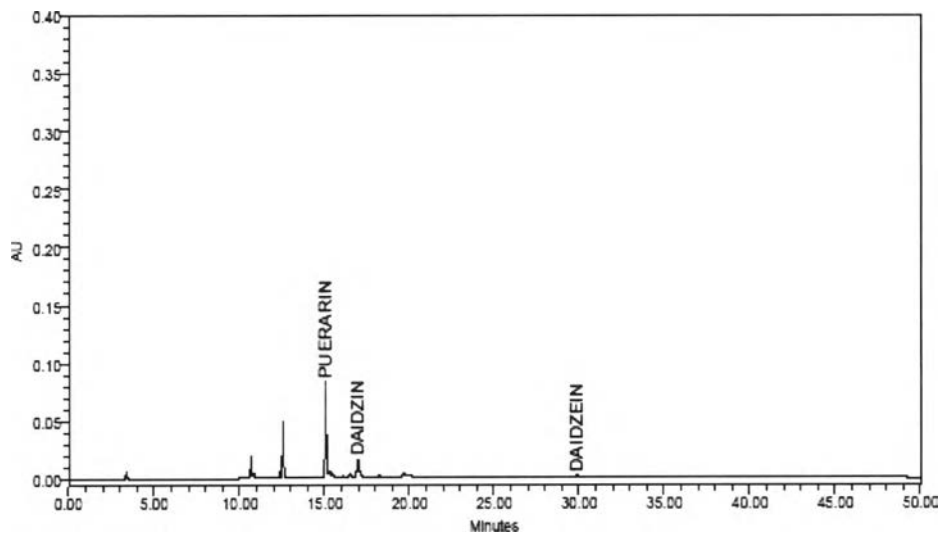
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Aug	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 10:34:53
Vial:	7	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	26/5/2554 16:47:48
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.089	506698	58.00	0.025000	mg/ml
2	DAIDZIN	16.987	154593	17.70	0.025000	mg/ml
3	GENISTIN	20.925				
4	DAIDZEIN	29.881	28879	3.31	0.025000	mg/ml
5	GENISTEN	40.400				





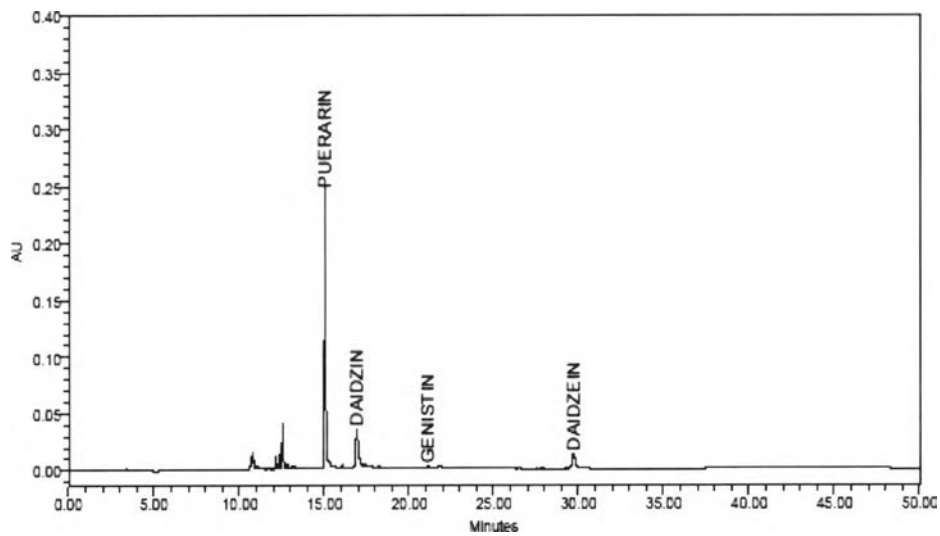
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 17:41:09
Vial:	10	Acq Method Set:	Isoflavone_water@_2
Injection #:	1	Date Processed:	26/5/2554 16:47:43
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.047	1450306	64.36	0.025000	mg/ml
2 DAIDZIN	16.832	402527	17.86	0.025000	mg/ml
3 GENISTIN	21.130	13648	0.61	0.025000	mg/ml
4 DAIDZEIN	29.726	169051	7.50	0.025000	mg/ml
5 GENISTEN	40.400				



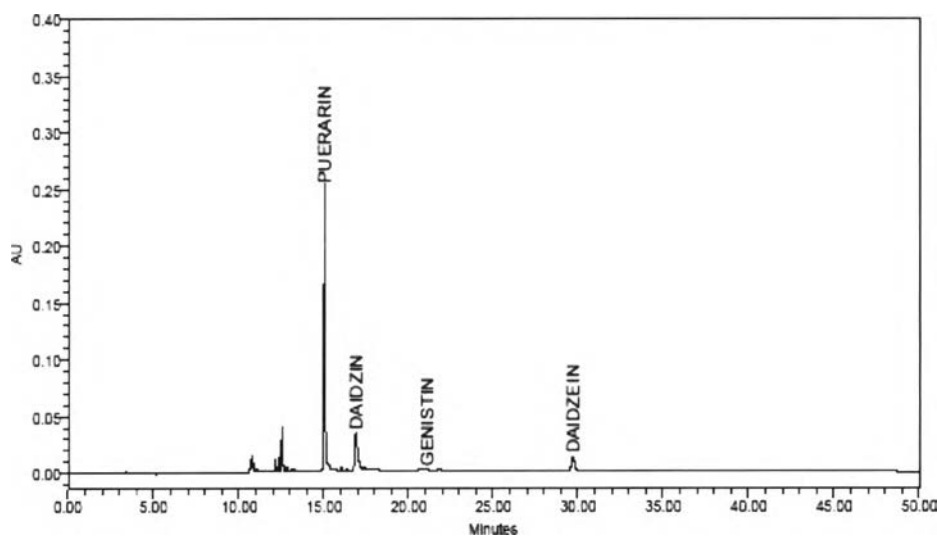
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 18:41:59
Vial:	10	Acq. Method Set:	Isoflavone_water@_2
Injection #:	2	Date Processed:	26/5/2554 18:47:42
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	Wwin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	PDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.035	1461628	87.29	0.025000	mg/ml
2 DAIDZIN	16.809	378284	17.48	0.025000	mg/ml
3 GENISTIN	21.068	13724	0.63	0.025000	mg/ml
4 DAIDZEIN	29.718	170147	7.83	0.025000	mg/ml
5 GENISTEN	40.400				



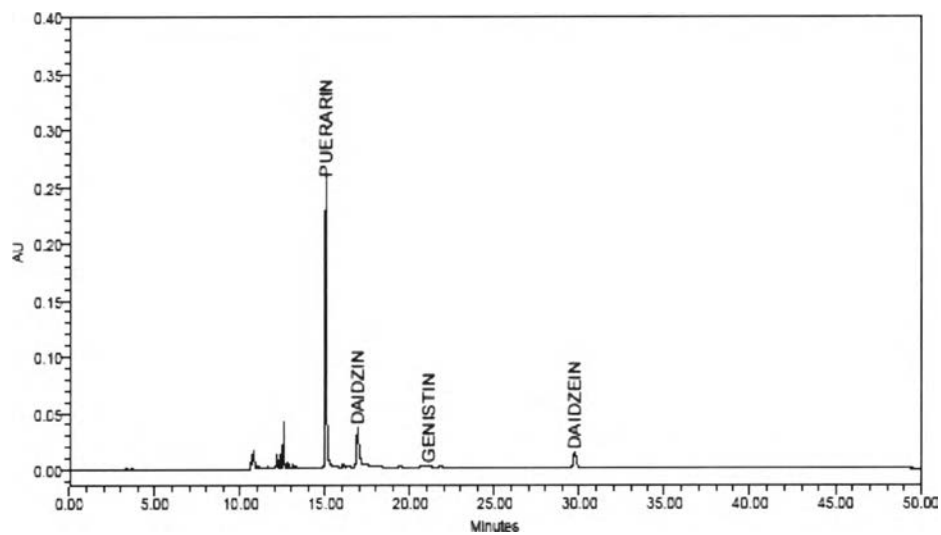
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-III Dec	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 19:42:49
Vial:	10	Acq. Method Set:	Isoflavone_water9_2
Injection #:	3	Date Processed:	28/5/2554 16:47:41
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	FDA 254.0 nm



	Peak Name	RT	Area	% Area	Amount	Units
1	PUERARIN	15.020	1486236	67.21	0.025000	mg/ml
2	DAIDZIN	16.906	396988	17.50	0.025000	mg/ml
3	GENISTIN	21.107	14076	0.64	0.025000	mg/ml
4	DAIDZEIN	29.725	174578	7.89	0.025000	mg/ml
5	GENISTEN	40.400				



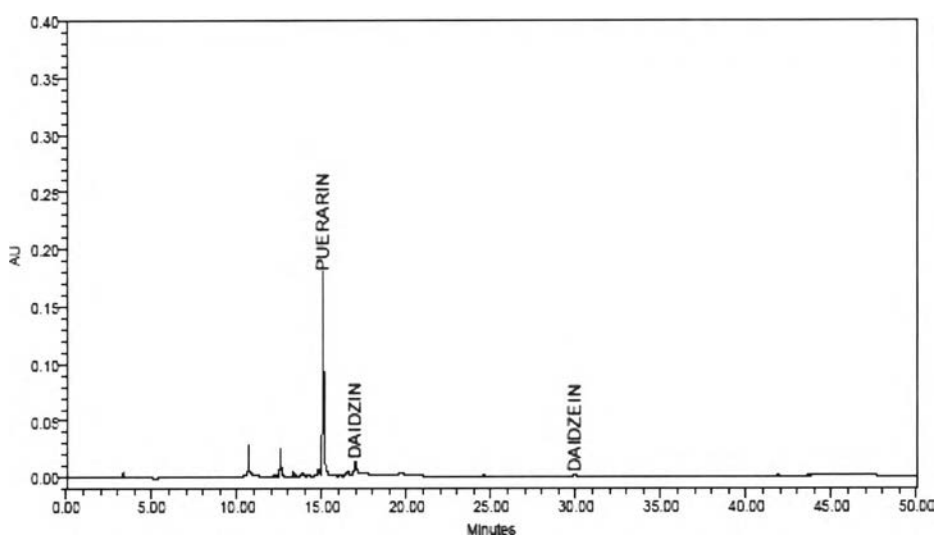
## Default Individual Report

Reported by User: System

Project Name: isoflavone2

## SAMPLE INFORMATION

Sample Name:	Tuber PM-IV Apr	Acquired By:	System
Sample Type:	Standard	Date Acquired:	19/3/2554 2:27:54
Vial:	5	Acq. Method Set:	Isoflavone_water9_2
Injection #:	1	Date Processed:	26/5/2554 18:47:55
Injection Volume:	10.00 ul	Processing Method:	STD Isoflavonoids KOONG
Run Time:	50.0 Minutes	Channel Name:	WWin Ch 1
Sample Set Name:	KOONG Tuber of PM	Proc. Chnl. Descr.:	FDA 254.0 nm



Peak Name	RT	Area	% Area	Amount	Units
1 PUERARIN	15.072	1187478	78.87	0.032500	mg/ml
2 DAIDZIN	16.970	149189	9.88	0.032500	mg/ml
3 GENISTIN	20.925				
4 DAIDZEIN	29.028	10675	0.71	0.032500	mg/ml
5 GENISTEN	40.400				



Appendix E

Table 12 The correlation of rainfall amount, temperature and isoflavonoids in PM-III by Pearson Product Moment Correlation Coefficient (Correlation is significant at the 0.05 level (2-tailed))

		PM-III				PM-IV				PM-V			
		Puerarin	Daidzin	Genistin	Daidzein	Puerarin	Daidzin	Genistin	Daidzein	Puerarin	Daidzin	Genistin	Daidzein
Rainfall	Pearson	-0.118	-0.012	0.203 <sup>*</sup>	-0.101	0.165	0.106	-0.030	-0.065	0.335 <sup>**</sup>	0.127	-0.028	0.121
	Correlation												
	Sig. (2-tailed)	0.225	0.903	0.035	0.298	0.088	0.276	0.756	0.506	0.000	0.189	0.775	0.211
	N	108	108	108	108	108	108	108	108	108	108	108	108
Temperature	Pearson	-0.454 <sup>**</sup>	-0.419 <sup>**</sup>	0.059	-0.407 <sup>**</sup>	-0.177	0.167	0.137	-0.169	0.050	0.259 <sup>**</sup>	0.083	0.066
	Correlation												
	Sig. (2-tailed)	0.000	0.000	0.543	0.000	0.067	0.083	0.159	0.081	0.609	0.007	0.394	0.497
	N	108	108	108	108	108	108	108	108	108	108	108	108



## Appendix F

Table 13 The protein spot analysis of *P. mirifica* tuber.

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
1	gij356539264	TGACG-sequence-specific DNA-binding protein TGA-2.1 [ <i>Glycine max</i> ]	37	51.17/6.09	2(0)	3
1	gij384246084	Hypothetical protein COCSUDRAFT_31020 [ <i>Coccomyxa subellipsoidea</i> C-169]	36	21.38/8.22	1(0)	6
1	gij4926870	Putative non-LTR retroelement reverse transcriptase [ <i>Arabidopsis thaliana</i> ]	31	88.36/9.61	1(0)	2
1	gij255580568	Adenosine 3'-phospho 5'-phosphosulfate transporter, putative [ <i>Ricinus communis</i> ]	28	38.69/9.03	1(0)	4
1	gij145351610	MFS family transporter: phosphate/sugar [ <i>Ostreococcus lucimarinus</i> CCE9901]	27	51.14/4.90	1(0)	6
2	gij167999209	Ribosome biogenesis protein BOP1 homolog [ <i>Physcomitrella patens</i> subsp. <i>patens</i> ]	25	71.54/6.09	1(0)	2
2	gij357473035	Kinase-like protein [ <i>Medicago truncatula</i> ]	23	88.63/6.84	1(0)	2
2	gij356535919	3-ketoacyl-CoA synthase 11-like [ <i>Glycine max</i> ]	21	58.04/9.40	1(0)	3
2	gij28564823	Putative short-root transcription factor [ <i>Oryza sativa Japonica</i> Group]	19	50.43/5.31	1(0)	4
2	gij357458239	NBS-LRR type disease resistance protein [ <i>Medicago truncatula</i> ]	19	149.49/5.95	1(0)	1
3	gij356496249	Proteasome subunit alpha type-4-like isoform 1 [ <i>Glycine max</i> ]	270	27.29/5.96	5(2)	28
3	gij2511584	Multicatalytic endopeptidase [ <i>Arabidopsis thaliana</i> ]	125	27.49/6.60	2(1)	10
3	gij77540216	Triosephosphate isomerase [ <i>Glycine max</i> ]	47	27.19/5.87	9(5)	3
3	gij384250175	GTP-binding protein lepa [ <i>Coccomyxa subellipsoidea</i> C-169]	41	137.94/6.24	2(0)	1



**Table 13** The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
4	gi 356554233	Elongation factor 1-beta 1-like [ <i>Glycine max</i> ]	49	24.20/4.53	1(0)	6
4	gi 326499532	Predicted protein [ <i>Hordeum vulgare</i> subsp. <i>vulgare</i> ]	33	47.03/8.97	1(0)	3
4	gi 412985813	CMP-N-acetylneuraminase-beta-galactosamide-alpha-2,3-sialyltransferase 1 [ <i>Bathycoccus prasinus</i> ]	22	69.82/8.92	1(0)	2
5	gi 351720808	14-3-3-like protein A [ <i>Glycine max</i> ]	212	29.05/4.72	5(2)	26
5	gi 118486542	Cysteine proteinase [ <i>Populus trichocarpa</i> ]	148	51.38/5.42	4(1)	6
6	gi 363807580	Uncharacterized protein LOC100801348 [ <i>Glycine max</i> ]	197	29.46/4.75	4(2)	19
6	gi 84468444	Putative DNA-damage-repair/toleration protein DRT102 [ <i>Trifolium pratense</i> ]	54	32.95/5.22	1(1)	4
7	gi 224037304	Putative elongation factor 1-beta, partial [ <i>Populus tremula</i> x <i>Populus alba</i> ]	71	17.32/4.32	2(0)	13
7	gi 226505926	Elongation factor 1-delta 1 [ <i>Zea mays</i> ]	71	24.77/4.39	2(0)	9
7	gi 357130500	Uncharacterized protein LOC100844455 [ <i>Brachypodium distachyon</i> ]	32	73.58/8.02	1(0)	1
8	gi 356516563	Elongation factor 1-delta-like [ <i>Glycine max</i> ]	113	25.02/4.42	2(2)	9
8	gi 356568196	Cationic peroxidase 1-like [ <i>Glycine max</i> ]	72	34.41/5.01	3(0)	7
8	gi 224037304	Putative elongation factor 1-beta, partial [ <i>Populus tremula</i> x <i>Populus alba</i> ]	56	17.32/4.32	1(1)	8
8	gi 356525022	Potassium transporter 17-like [ <i>Glycine max</i> ]	40	80.91/8.65	1(0)	1
9	gi 356568196	Cationic peroxidase 1-like [ <i>Glycine max</i> ]	179	34.41/5.01	4(1)	14



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
9	gi 1633130	Chain A, Peanut Peroxidase	119	31.16/8.06	3(1)	13
9	gi 27448344	Peroxidase 2 [ <i>Lupinus albus</i> ]	73	37.29/9.47	2(1)	8
10	gi 255630726	Unknown [ <i>Glycine max</i> ]	80	22.33/4.83	1(1)	4
10	gi 2506470	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic; AltName: Full=Starch phosphorylase L	34	113.51/5.63	1(0)	1
10	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	30	61.15/8.98	1(0)	3
11	gi 356507341	Glyceraldehyde-3-phosphate dehydrogenase-like [ <i>Glycine max</i> ]	724	54.91/8.43	14(7)	34
11	gi 356500825	Fructose-bisphosphate aldolase, cytoplasmic isozyme-like [ <i>Glycine max</i> ]	553	38.24/7.12	12(6)	34
12	gi 356507341	Glyceraldehyde-3-phosphate dehydrogenase-like [ <i>Glycine max</i> ]	850	54.91/8.43	21(10)	34
13	gi 356504480	NADP-dependent alkenal double bond reductase P1-like (Alcohol dehydrogenase)[ <i>Glycine max</i> ]	220	37.81/7.01	4(2)	12
13	gi 388506034	Unknown [ <i>Lotus japonicus</i> ]	220	35.56/5.33	4(3)	17
13	gi 255529745	Isoflavone reductase-like protein [ <i>Coffea arabica</i> ]	85	34.36/5.55	2(1)	7
13	gi 356538694	Fructose-bisphosphate aldolase 2, chloroplastic-like [ <i>Glycine max</i> ]	82	42.93/8.24	2(1)	5
14	gi 357499099	Aldose 1-epimerase family protein expressed [ <i>Medicago truncatula</i> ]	136	36.86/5.94	2(1)	9
14	gi 359806880	Uncharacterized protein LOC100809668 [ <i>Glycine max</i> ]	113	37.90/5.94	5(0)	18





**Table 13** The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
14	gi 356538694	Fructose-bisphosphate aldolase 2, chloroplastic-like [ <i>Glycine max</i> ]	91	42.93/8.24	2(1)	5
14	gi 224482653	UDP-glucose pyrophosphorylase [ <i>Annona cherimola</i> ]	70	51.29/5.81	2(0)	5
14	gi 22597178	Alcohol dehydrogenase 1 [ <i>Glycine max</i> ]	66	39.98/6.19	1(1)	2
14	gi 356519136	Serpin-ZX-like [ <i>Glycine max</i> ]	65	42.81/5.18	2(0)	6
15	gi 356536625	Glutathione reductase, chloroplastic-like [ <i>Glycine max</i> ]	948	58.52/6.85	28(14)	35
15	gi 356496197	Proliferation-associated protein 2G4-like [ <i>Glycine max</i> ]	223	42.95/6.30	7(1)	21
16	gi 356570259	Lysosomal alpha-mannosidase-like [ <i>Glycine max</i> ]	568	115.53/5.84	13(8)	10
17	gi 351724891	Enolase [ <i>Glycine max</i> ]	86	47.69/5.31	21(8)	44
17	gi 356553237	UTP--glucose-1-phosphate uridylyltransferase-like [ <i>Glycine max</i> ]	552	51.44/5.20	16(3)	36
18	gi 356568953	Beta-hexosaminidase subunit B2-like [ <i>Glycine max</i> ]	507	63.47/5.55	9(5)	16
18	gi 356518561	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	178	79.88/6.17	4(2)	6
19	gi 356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	1113	65.15/5.75	26(12)	33
19	gi 356513979	Malate synthase, glyoxysomal-like [ <i>Glycine max</i> ]	750	62.72/5.88	23(7)	26
19	gi 357520877	Malic enzyme [ <i>Medicago truncatula</i> ]	755	65.30/5.98	20(12)	23
20	gi 356558946	Subtilisin-like protease-like [ <i>Glycine max</i> ]	112	81.90/6.24	3(3)	3
20	gi 974782	Cobalamine-independent methionine synthase [ <i>Solenostemon scutellarioides</i> ]	71	86.72/6.17	1(1)	1



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
21	gi 356571744	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase-like isoform 1 [ <i>Glycine max</i> ]	1083	84.22/5.97	25(14)	28
21	gi 351724907	Methionine synthase [ <i>Glycine max</i> ]	1073	84.23/5.93	25(13)	29
21	gi 309756515	Pollen allergen MetE [ <i>Amaranthus retroflexus</i> ]	650	83.68/6.12	15(9)	15
22	gi 242045024	Hypothetical protein SORBIDRAFT_02g027340 [ <i>Sorghum bicolor</i> ]	43	98.13/5.48	3(0)	3
22	gi 339013500	Chloroplast putative ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [ <i>Wolffia australiana</i> ]	38	19.75/8.84	3(0)	7
22	gi 297843214	ATBARD1/BARD1 [ <i>Arabidopsis lyrata subsp. lyrata</i> ]	37	78.73/6.36	2(0)	3
22	gi 297847694	Predicted protein [ <i>Arabidopsis lyrata subsp. lyrata</i> ]	36	87.88/5.32	1(0)	1
23	gi 351722957	Uncharacterized protein LOC100500058 precursor [ <i>Glycine max</i> ]	113	17.23/7.67	12(1)	21
23	gi 1709761	Proteasome subunit alpha type-4	69	27.43/6.17	1(1)	4
23	gi 7547630	Pathogen- and wound-inducible antifungal protein CBP20 precursor [ <i>Nicotiana tabacum</i> ]	49	22.15/8.38	1(0)	6
23	gi 15226197	Leucine-rich repeat transmembrane protein kinase-like protein [ <i>Arabidopsis thaliana</i> ]	40	78.26/5.75	1(0)	1
24	gi 303275674	Predicted protein [ <i>Micromonas pusilla</i> CCMP1545]	34	59.81/4.67	1(0)	1
24	gi 22324427	Centromere protein-like [ <i>Oryza sativa Japonica</i> Group]	30	97.95/5.85	1(0)	1



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
24	gi 303279689	C2 domain-containing protein [ <i>Micromonas pusilla</i> CCMP1545]	30	155.28/6.50	1(0)	1
24	gi 1173630	Cysteine proteinase [ <i>Phalaenopsis</i> sp. SM9108]	27	40.14/6.23	1(0)	3
24	gi 356548369	Protein CHUP1, chloroplastic-like [ <i>Glycine max</i> ]	26	50.68/7.03	3(0)	3
25	gi 351720837	Nucleoside diphosphate kinase 1 [ <i>Glycine max</i> ]	315	16.43/5.93	20(8)	36
25	gi 13899069	Nucleoside diphosphate kinase 1 [ <i>Arabidopsis thaliana</i> ]	159	15.74/6.84	7(2)	20
25	gi 255571035	Nucleoside diphosphate kinase, putative [ <i>Ricinus communis</i> ]	155	16.30/6.30	9(2)	29
25	gi 330318624	Nucleoside diphosphate kinase 1 [ <i>Camellia sinensis</i> ]	96	16.40/6.52	6(0)	25
26	gi 24421235	Superoxide dismutase [ <i>Brassica juncea</i> ]	177	15.20/5.44	17(5)	15
26	gi 357518949	14-3-3-like protein gf14-6 [ <i>Medicago truncatula</i> ]	142	29.28/4.81	4(0)	13
27	gi 356539366	Superoxide dismutase [Cu-Zn], chloroplastic-like [ <i>Glycine max</i> ]	38	20.88/6.03	1(0)	5
28	unidentified	unidentified				
29	gi 147780897	Hypothetical protein VITISV_007503 [ <i>Vitis vinifera</i> ]	26	154.50/5.85	1(0)	2
29	gi 168036239	Predicted protein [ <i>Physcomitrella patens</i> subsp. <i>patens</i> ]	26	29.66/5.91	1(0)	5
29	gi 359490818	U-box domain-containing protein 43 [ <i>Vitis vinifera</i> ]	25	113.74/5.81	1(0)	1
29	gi 356559884	Calcium-dependent protein kinase 32-like [ <i>Glycine max</i> ]	25	56.73/6.00	1(0)	3
29	gi 356515355	Phospholipid-transporting ATPase 1-like [ <i>Glycine max</i> ]	25	137.25/6.24	1(0)	1



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
30	gi 255630726	Unknown [ <i>Glycine max</i> ]	65	22.33/4.83	7(3)	4
30	gi 255088257	Fructose-1,6-bisphosphatase [ <i>Micromonas sp. RCC299</i> ]	64	39.47/5.79	2(0)	2
30	gi 357140899	Glutathione S-transferase GSTU6-like [ <i>Brachypodium distachyon</i> ]	49	25.79/6.12	1(1)	5
31	gi 218198738	Hypothetical protein Osl_24136 [ <i>Oryza sativa Indica Group</i> ]	70	17.47/6.42	2(1)	6
31	gi 255630726	Unknown [ <i>Glycine max</i> ]	65	22.33/4.83	7(3)	4
31	gi 357140899	Glutathione S-transferase GSTU6-like [ <i>Brachypodium distachyon</i> ]	50	25.79/6.12	1(1)	5
31	gi 255088257	Fructose-1,6-bisphosphatase [ <i>Micromonas sp. RCC299</i> ]	64	39.47/5.79	2(0)	2
32	gi 387135324	UDP-glycosyltransferase 1 [ <i>Linum usitatissimum</i> ] (UGT)	63	52.81/5.83	2(0)	2
33	gi 242057465	Hypothetical protein SORBIDRAFT_03g017640 [ <i>Sorghum bicolor</i> ]	34	104.21/9.51	1(0)	2
33	gi 356577578	Hydroxyethylthiazole kinase-like [ <i>Glycine max</i> ]	33	29.46/5.51	1(0)	5
33	gi 131933	Ribulose bisphosphate carboxylase large chain; RuBisCO large subunit	28	52.42/6.29	1(0)	2
34	unidentified					
35	gi 356565676	20 kDa chaperonin, chloroplastic-like [ <i>Glycine max</i> ]	110	27.29/6.45	2(1)	7
35	gi 356556406	20 kDa chaperonin, chloroplastic-like [ <i>Glycine max</i> ]	48	26.65/7.79	1(0)	3
36	gi 968987	Ferritin [ <i>Glycine max</i> ]	58	27.92/5.87	2(0)	10
37	gi 88595637	Reverse transcriptase [ <i>Orobanche hirtiflora</i> ]	26	17.20/7.88	1(0)	10



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
37	gij168027840	Predicted protein [ <i>Physcomitrella patens subsp. patens</i> ]	26	81.71/5.90	1(0)	2
37	gij357447819	Pentatricopeptide repeat-containing protein [ <i>Medicago truncatula</i> ]	25	14.39/9.84	1(0)	13
37	gij92886071	Protein kinase domain, putative [ <i>Medicago truncatula</i> ]	22	28.67/5.77	1(0)	6
37	gij82697951	CXE carboxylesterase [ <i>Malus pumila</i> ]	22	40.68/5.24	1(0)	4
38	gij242066108	Hypothetical protein SORBIDRAFT_04g029120 [ <i>Sorghum bicolor</i> ]	29	45.50/6.67	2(0)	2
38	gij15242822	Glutamate-1-semialdehyde-2,1-aminomutase [ <i>Arabidopsis thaliana</i> ]	22	50.34/6.43	1(0)	2
38	gij159484064	Minichromosome maintenance protein 2 [ <i>Chlamydomonas reinhardtii</i> ]	21	99.65/5.17	1(0)	1
39	unidentified					
40	gij351724189	Ferritin-4, chloroplastic [ <i>Glycine max</i> ]	164	27.54/5.64	4(2)	16
41	gij351734454	Uncharacterized protein LOC100306148 [ <i>Glycine max</i> ]	411	24.53/6.82	11(3)	63
41	gij17380185	Proteasome subunit beta type-1	237	24.60/6.30	7(2)	36
41	gij11385459	Glutathione S-transferase GST 22, partial [ <i>Glycine max</i> ]	58	24.26/5.57	1(1)	3
43	gij115480968	Putative bacterial blight resistance protein Xa1-like protein [ <i>Oryza sativa Japonica</i> Group]	42	160.03/8.15	1(0)	0
43	gij125525492	Hypothetical protein Osl_01491 [ <i>Oryza sativa Indica</i> Group]	35	30.52/9.51	1(0)	4



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
43	gil159472989	Transmembrane protein [ <i>Chlamydomonas reinhardtii</i> ]	34	64.35/7.67	1(0)	1
43	gil345450064	Ribosomal protein S4 [ <i>Delosperma napiforme</i> ]	30	20.06/10.33	1(0)	7
43	gil357130858	Hexokinase-9-like [ <i>Brachypodium distachyon</i> ]	29	54.10/6.88	1(0)	2
44	gil115480968	Putative bacterial blight resistance protein Xa1-like protein [ <i>Oryza sativa Japonica Group</i> ]	43	160.03/8.15	1(0)	0
44	gil125525492	Hypothetical protein Osl_01491 [ <i>Oryza sativa Indica Group</i> ]	35	30.52/9.51	1(0)	4
44	gil6642918	Maturase [ <i>Monodia stipoides</i> ]	33	61.31/9.55	1(0)	1
45	gil356567451	Glutathione S-transferase F10-like [ <i>Glycine max</i> ]	158	24.78/5.68	4(1)	16
45	gil115480968	Putative bacterial blight resistance protein Xa1-like protein [ <i>Oryza sativa Japonica Group</i> ]	43	160.03/8.15	1(0)	0
45	gil303288379	Soluble starch synthase [ <i>Micromonas pusilla CCMP1545</i> ]	34	159.03/5.30	2(0)	3
46	gil5921724	Chalcone--flavonone isomerase; Short=Chalcone isomerase	187	23.79/5.34	5(2)	24
46	gil384252728	E set domain-containing protein [ <i>Coccomyxa subellipsoidea C-169</i> ]	36	70.58/9.30	1(0)	1
47	gil356504480	NADP-dependent alkenal double bond reductase P1-like (Alcohol dehydrogenase)[ <i>Glycine max</i> ]	244	37.13/7.01	4(3)	15
47	gil57283985	Triose-phosphate isomerase [ <i>Phaseolus vulgaris var. nanus</i> ]	123	27.18/5.87	3(1)	12
47	gil22597178	Alcohol dehydrogenase 1 [ <i>Glycine max</i> ]	52	39.98/6.19	1(1)	2



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
48	gi 224101989	Cyclic nucleotide-gated channel [ <i>Populus trichocarpa</i> ]	33	21.45/8.73	2(0)	9
48	gi 15237409	TIR-NBS-LRR class disease resistance protein [ <i>Arabidopsis thaliana</i> ]	26	137.39/7.71	1(0)	1
48	gi 225460020	TMV resistance protein N-like [ <i>Vitis vinifera</i> ]	25	133.09/5.86	1(0)	1
48	gi 217385866	L-aminocyclopropane-1-carboxylate oxidase [ <i>Momordica charantia</i> ]	25	37.28/5.84	1(0)	5
49	gi 77540216	Triosephosphate isomerase [ <i>Glycine max</i> ]	799	27.19/5.87	28(19)	56
50	gi 359806098	Uncharacterized protein LOC100799108 [ <i>Glycine max</i> ]	593	25.56/5.51	10(9)	48
50	gi 356496096	Proteasome subunit alpha type-2-A-like [ <i>Glycine max</i> ]	575	25.61/5.51	12(9)	54
51	gi 77540216	Triosephosphate isomerase [ <i>Glycine max</i> ]	406	27.19/5.87	9(4)	23
52	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	32	61.15/8.98	1(0)	3
52	gi 255080640	Aminopeptidase [ <i>Micromonas sp. RCC299</i> ]	30	62.99/7.97	1(0)	1
52	gi 384252728	E set domain-containing protein [ <i>Coccomyxa subellipsoidea C-169</i> ]	27	70.58/9.30	1(0)	1
52	gi 255074983	Multidrug/Oligosaccharidyl-lipid/Polysaccharide flippase [ <i>Micromonas sp. RCC299</i> ]	31	62.39/9.60	1(0)	1
53	gi 147834155	Hypothetical protein VITISV_003792 [ <i>Vitis vinifera</i> ]	52	91.04/9.08	4(0)	1
53	gi 384252728	E set domain-containing protein [ <i>Coccomyxa subellipsoidea C-169</i> ]	34	70.58/9.30	1(0)	1
53	gi 255080640	Aminopeptidase [ <i>Micromonas sp. RCC299</i> ]	32	62.99/7.97	1(0)	1
53	gi 307111382	Expressed protein [ <i>Chlorella variabilis</i> ]	31	55.00/9.30	1(0)	1



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pl	Peptide match	% Coverage
53	gi 51535110	Pentatricopeptide (PPR) repeat-containing protein-like [ <i>Oryza sativa Japonica</i> Group]	30	56.96/8.98	1(0)	1
54	gi 356545355	Proteasome subunit alpha type-6-like [ <i>Glycine max</i> ]	605	40.95/8.85	13(6)	33
55	gi 242071029	Hypothetical protein SORBIDRAFT_05g018540 [ <i>Sorghum bicolor</i> ]	21	179.12/8.41	1(0)	1
55	gi 5360887	CsPK4.2 [ <i>Cucumis sativus</i> ]	19	3.42/5.30	1(0)	27
55	gi 115481390	Os10g0195600 [ <i>Oryza sativa Japonica</i> Group]	19	52.19/9.07	1(0)	2
56	gi 359807345	Proteasome subunit alpha type [ <i>Glycine max</i> ]	458	27.25/5.60	12(3)	44
56	gi 255584242	Proteasome subunit alpha type, putative [ <i>Ricinus communis</i> ]	204	25.45/6.11	6(2)	24
57	gi 356555198	Probable allantoinase 1-like [ <i>Glycine max</i> ]	43	56.54/8.12	1(0)	1
57	gi 356561679	Pentatricopeptide repeat-containing protein At1g12775, mitochondrial-like [ <i>Glycine max</i> ]	38	85.11/8.75	2(0)	2
57	gi 3451071	Beta adaptin-like protein [ <i>Arabidopsis thaliana</i> ]	38	80.01/5.16	1(0)	1
58	gi 356549345	Probable allantoinase 1-like [ <i>Glycine max</i> ]	104	56.59/8.69	2(2)	3
58	gi 374676359	Allantoinase [ <i>Phaseolus vulgaris</i> ]	74	56.20/6.69	2(1)	6
59	gi 343172102	Proteasome subunit alpha type 7, partial [ <i>Silene latifolia</i> ]	404	26.91/8.41	13(5)	31
60	gi 302783779	Tocopherol O-methyltransferase [EC:2.1.1.95] [ <i>Selaginella moellendorffii</i> ]	23	38.05/6.76	1(0)	9





Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
60	gi 159473921	Serine/threonine protein kinase 4 [ <i>Chlamydomonas reinhardtii</i> ]	22	39.17/5.33	1(0)	7
60	gi 255593481	Conserved hypothetical protein [ <i>Ricinus communis</i> ]	22	54.27/6.56	1(0)	5
60	gi 357120883	Uncharacterized protein LOC100835186[ <i>Brachypodium distachyon</i> ]	22	109.10/6.37	1(0)	2
61	gi 356534698	S-formylglutathione hydrolase-like [ <i>Glycine max</i> ]	63	31.94/6.82	3(0)	13
61	gi 147795374	Hypothetical protein VITISV_008263 [ <i>Vitis vinifera</i> ]	47	40.64/6.02	2(0)	5
61	gi 3366926	NADH dehydrogenase subunit F [ <i>Boechera stricta</i> ]	36	30.50/9.50	1(0)	5
62	gi 351722222	Lipoxygenase [ <i>Glycine max</i> ]	261	96.24/6.29	6(2)	7
63	gi 147794144	Hypothetical protein VITISV_000733 [ <i>Vitis vinifera</i> ]	20	20.72/4.76	1(0)	11
63	gi 7413551	Protein phosphatase-like protein [ <i>Arabidopsis thaliana</i> ]	17	40.29/8.72	1(0)	4
64	gi 359806539	Annexin[ <i>Glycine max</i> ]	819	35.78/7.12	31(12)	33
64	gi 3176098	Annexin [ <i>Medicago truncatula</i> ]	445	35.59/7.85	17(8)	16
64	gi 356548893	Annexin-like protein RJ4-like isoform 1 [ <i>Glycine max</i> ]	339	35.71/7.75	13(3)	17
65	gi 356531939	Lactoylglutathione lyase-like [ <i>Glycine max</i> ]	343	33.45/6.14	13(4)	26
65	gi 255637721	Unknown [ <i>Glycine max</i> ]	108	31.63/5.37	5(0)	15
66	gi 359806539	Annexin[ <i>Glycine max</i> ]	471	35.78/7.12	15(7)	27
66	gi 356548893	Annexin-like protein RJ4-like isoform 1 [ <i>Glycine max</i> ]	169	35.71/7.75	4(1)	13



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
66	gij356555044	Annexin-like protein RJ4-like [ <i>Glycine max</i> ]	114	20.63/7.03	4(0)	13
67	gij356495803	Pectinesterase/pectinesterase inhibitor 18-like, partial [ <i>Glycine max</i> ]	382	65.84/9.12	8(6)	13
67	gij356496705	Peroxidase 72-like [ <i>Glycine max</i> ]	269	36.42/9.23	5(3)	24
67	gij118485585	Pectinesterase (EC=3.1.1.11)[ <i>Populus trichocarpa</i> ]	138	35.55/9.21	2(2)	7
68	gij356514703	Omega-amidase NIT2-like [ <i>Glycine max</i> ]	334	38.53/7.68	8(3)	21
68	gij343887307	Carbon-nitrogen hydrolase family protein [ <i>Citrus unshiu</i> ]	218	45.61/6.91	6(1)	13
68	gij388506034	Unknown [ <i>Lotus japonicus</i> ]	194	35.56/5.33	4(2)	17
69	gij255647044	Annexin [ <i>Glycine max</i> ]	247	25.08/6.18	7(0)	26
69	gij357489617	Annexin [ <i>Medicago truncatula</i> ]	149	36.15/7.14	4(0)	10
69	gij357164096	WPP domain-interacting protein 3-like [ <i>Brachypodium distachyon</i> ]	77	33.54/4.52	2(1)	3
69	gij357446353	Quinone oxidoreductase [ <i>Medicago truncatula</i> ]	72	34.53/6.90	1(1)	5
69	gij212292267	RACK [ <i>Phaseolus vulgaris</i> ] (Receptor for Activated C-Kinase)	58	35.58/7.60	2(0)	11
69	gij156630208	Avenin-like protein [ <i>Amblyopyrum muticum</i> ]	52	32.58/7.83	1(1)	3
70	gij255647044	Annexin [ <i>Glycine max</i> ]	464	25.08/6.18	15(2)	49
70	gij357489617	Annexin [ <i>Medicago truncatula</i> ]	270	36.15/7.14	7(2)	17
70	gij357164096	WPP domain-interacting protein 3-like [ <i>Brachypodium distachyon</i> ]	85	33.54/4.52	2(0)	3



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
71	unidentified					
72	gi 356504476	NADP-dependent alkenal double bond reductase P1-like (Alcohol dehydrogenase) [ <i>Glycine max</i> ]	724	37.95/5.93	29(13)	31
72	gi 356536264	Fructose-bisphosphate aldolase 3, chloroplastic-like [ <i>Glycine max</i> ]	291	41.39/7.63	5(2)	15
73	gi 351726399	Isoflavone reductase homolog 2 [ <i>Glycine max</i> ]	252	33.92/5.60	5(3)	16
73	gi 388514585	Uncharacterized protein [ <i>Medicago truncatula</i> ]	160	22.22/6.97	3(2)	22
73	gi 356538694	Fructose-bisphosphate aldolase 2, chloroplastic-like [ <i>Glycine max</i> ]	70	42.93/8.24	2(1)	5
74	gi 351725233	Uncharacterized protein LOC100527288 precursor [ <i>Glycine max</i> ]	226	22.10/9.39	4(3)	22
74	gi 356543209	Patellin-5-like [ <i>Glycine max</i> ]	169	69.52/4.87	4(1)	9
74	gi 224145127	Predicted protein [ <i>Populus trichocarpa</i> ]	106	51.22/5.88	3(1)	6
74	gi 356560200	Pectinesterase/pectinesterase inhibitor 3-like [ <i>Glycine max</i> ]	88	59.99/8.28	3(0)	6
74	gi 166715094	Beta-glucosidase 1 [ <i>Arabidopsis thaliana</i> ]	52	9.90/7.92	2(0)	12
75	gi 255641338	Unknown [ <i>Glycine max</i> ]	452	35.22/5.23	11(5)	33
75	gi 356542914	Epoxide hydrolase 2-like [ <i>Glycine max</i> ]	427	35.19/5.24	11(2)	32
75	gi 258678568	Alcohol dehydrogenase [ <i>Elymus tsukushiensis</i> ]	96	24.46/5.57	2(1)	9
75	gi 356554862	Proline iminopeptidase-like [ <i>Glycine max</i> ]	95	44.45/5.97	2(0)	4



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
76	gi 356500825	Fructose-bisphosphate aldolase, cytoplasmic isozyme-like [ <i>Glycine max</i> ]	569	38.24/7.12	16(7)	33
76	gi 363807310	Fructose-bisphosphate aldolase (EC=4.1.2.13) [ <i>Glycine max</i> ]	454	38.32/7.12	13(4)	26
76	gi 356507341	Glyceraldehyde-3-phosphate dehydrogenase-like [ <i>Glycine max</i> ]	440	54.91/8.43	10(5)	21
77	gi 27462762	Malate dehydrogenase [ <i>Lupinus albus</i> ]	681	35.57/6.10	12(10)	31
77	gi 10334493	Cytosolic malate dehydrogenase [ <i>Cicer arietinum</i> ]	670	35.48/5.92	13(10)	36
78	gi 356500825	Fructose-bisphosphate aldolase, cytoplasmic isozyme-like [ <i>Glycine max</i> ]	752	38.24/7.12	17(11)	38
78	gi 363807310	Fructose-bisphosphate aldolase (EC=4.1.2.13) [ <i>Glycine max</i> ]	622	38.32/7.12	16(10)	31
78	gi 356507341	Glyceraldehyde-3-phosphate dehydrogenase-like [ <i>Glycine max</i> ]	393	54.91/8.43	8(4)	21
79	gi 5929964	Malate dehydrogenase [ <i>Glycine max</i> ]	393	36.12/8.23	7(5)	22
79	gi 388515609	Malate dehydrogenase (EC=1.1.1.37) [ <i>Lotus japonicus</i> ]	263	35.79/8.85	4(3)	14
80	gi 356525744	Phosphoglycerate kinase, cytosolic-like [ <i>Glycine max</i> ]	880	42.37/6.28	17(11)	37
80	gi 3738259	Cytosolic phosphoglycerate kinase 1 [ <i>Populus nigra</i> ]	448	42.64/5.70	12(5)	22
81	gi 356557030	Phosphoglycerate kinase, cytosolic-like [ <i>Glycine max</i> ]	842	42.41/5.96	20(13)	47
81	gi 357451633	Phosphoglycerate kinase [ <i>Medicago truncatula</i> ]	673	42.60/5.61	17(11)	32
81	gi 224109062	Phosphoglycerate kinase (EC=2.7.2.3) [ <i>Populus trichocarpa</i> ]	519	42.65/5.70	12(8)	24
82	gi 356557030	Phosphoglycerate kinase, cytosolic-like [ <i>Glycine max</i> ]	880	42.41/5.96	19(12)	48



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
82	gi 356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	521	65.15/5.75	13(4)	17
83	gi 356558578	Actin-101-like [ <i>Glycine max</i> ]	867	41.60/5.31	21 (9)	47
83	gi 357528267	Actin 1 [ <i>x Doritaenopsis hybrid cultivar</i> ]	636	38.83/5.39	18(7)	41
84	gi 356573281	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic-like [ <i>Glycine max</i> ]	1131	104.087/5.08	30(15)	24
84	gi 224077696	Phosphorylase (EC=2.4.1.1) [ <i>Populus trichocarpa</i> ]	476	107.58/5.37	13(3)	10
85	gi 356496197	Proliferation-associated protein 2G4-like [ <i>Glycine max</i> ]	763	42.95/6.330	21(7)	39
85	gi 356543209	Patellin-5-like [ <i>Glycine max</i> ]	174	69.52/4.87	4(2)	10
86	gi 356575611	ATP synthase subunit beta, mitochondrial-like [ <i>Glycine max</i> ]	1665	59.81/5.80	47(39)	53
86	gi 356505318	Enolase-like [ <i>Glycine max</i> ]	1124	47.63/5.49	29(15)	48
87	gi 356505318	Enolase-like [ <i>Glycine max</i> ]	1220	47.63/5.49	31(18)	52
87	gi 356575611	ATP synthase subunit beta, mitochondrial-like [ <i>Glycine max</i> ]	1002	59.81/5.80	19(16)	40
87	gi 356571429	Leucine aminopeptidase 1-like isoform 1 [ <i>Glycine max</i> ]	615	60.80/6.38	10(9)	21
88	gi 336390551	Adenosylhomocysteinase [ <i>Glycine max</i> ]	1034	53.19/5.69	28(14)	46
88	gi 224128996	Adenosylhomocysteinase/s-adenosyl-L-homocysteine hydrolase [ <i>Populus trichocarpa</i> ]	862	819/5.62	24(10)	38

Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
89	gi 356573281	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic-like [ <i>Glycine max</i> ]	1091	104.08/5.08	24(10)	26
90	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	32	61.15/8.98	1(0)	3
90	gi 255080640	Aminopeptidase [ <i>Micromonas sp.</i> RCC299]	30	62.99/7.97	1(0)	1
90	gi 384252728	E set domain-containing protein [ <i>Coccomyxa subellipsoidea</i> C-169]	27	70.58/9.30	1(0)	1
90	gi 255074983	Multidrug/Oligosaccharidyl-lipid/Polysaccharide flippase [ <i>Micromonas sp.</i> RCC299]	31	62.39/9.60	1(0)	1
91	gi 356559573	Glutathione reductase, cytosolic-like [ <i>Glycine max</i> ]	866	53.96/5.63	22(9)	39
91	gi 94494355	Cytosolic glutathione reductase [ <i>Phaseolus vulgaris</i> ]	567	54.73/6.32	17(5)	29
91	gi 356528645	Succinate-semialdehyde dehydrogenase, mitochondrial-like [ <i>Glycine max</i> ]	497	57.60/7.49	10(6)	16
92	gi 351726214	Calreticulin-1 precursor [ <i>Glycine max</i> ]	639	48.14/4.43	19(6)	34
93	gi 356576411	Chaperonin CPN60-2, mitochondrial-like isoform 1 [ <i>Glycine max</i> ]	755	61.21/5.75	19(8)	35
93	gi 306561655	Betaine aldehyde dehydrogenase [ <i>Glycine max</i> ]	407	54.63/407	11(4)	16
94	gi 356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	1263	65.15/5.75	32(19)	42
94	gi 357520877	Malic enzyme [ <i>Medicago truncatula</i> ]	730	65.30/5.98	19(9)	25
95	gi 356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	1235	65.15/5.75	33(19)	37
95	gi 357520877	Malic enzyme [ <i>Medicago truncatula</i> ]	755	65.30/5.98	20(12)	23



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
96	gi 356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	891	65.15/5.75	23(7)	36
96	gi 357520877	Malic enzyme [ <i>Medicago truncatula</i> ]	520	65.30/5.98	12(4)	19
97	gi 297839993	Hypothetical protein ARALYDRAFT_892955 [ <i>Arabidopsis lyrata subsp. lyrata</i> ]	392	73.00/5.07	7(5)	9
97	gi 2642238	Endoplasmic reticulum HSC70-cognate binding protein precursor [ <i>Glycine max</i> ]	386	73.59/5.15	7(5)	10
97	gi 13398537	BiP-isoform D [ <i>Glycine max</i> ]	357	54.23/6.95	7(4)	13
97	gi 38325811	Heat shock protein 70-1 [ <i>Nicotiana tabacum</i> ]	197	70.97/5.02	3(2)	3
98	gi 225456004	Stromal 70 kDa heat shock-related protein, chloroplastic-like [ <i>Vitis vinifera</i> ]	353	75.59/5.31	8(3)	13
99	gi 115460074	Os04g0578000 [ <i>Oryza sativa Japonica Group</i> ]	25	54.31/6.83	1(0)	4
99	gi 15230977	F-box protein [ <i>Arabidopsis thaliana</i> ]	24	45.05/5.65	1(0)	4
99	gi 359494882	Cycloartenol synthase 2-like [ <i>Vitis vinifera</i> ]	22	104.84/8.59	1(0)	1
99	gi 302854306	Metalloproteinase, extracellular matrix glycoprotein VMP2 [ <i>Volvox carterif. nagariensis</i> ]	22	63.21/6.40	1(0)	3
100	gi 255630726	Unknown [ <i>Glycine max</i> ]	70	22.33/4.83	1(1)	4
100	gi 109892850	Putative cytochrome c oxidase subunitII PS17	53	1.707/9.62	2(0)	100
100	gi 51242645	Nitrite reductase [ <i>Pseudochlorella pringsheimii</i> ]	43	71.09/6.73	3(0)	7
100	gi 308798625	Putative acyl-CoA dehydrogenase (ISS) [ <i>Ostreococcus tauri</i> ]	40	31.83/9.32	1(0)	0



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
101	gij356532954	Actin-depolymerizing factor 2-like [ <i>Glycine max</i> ]	281	16.00/281	7(5)	42
101	gij356512709	Uncharacterized protein LOC100797102 [ <i>Glycine max</i> ]	174	20.67/6.04	15(3)	14
101	gij24421235	Superoxide dismutase [ <i>Brassica juncea</i> ]	159	15.20/5.44	3(1)	15
101	gij134598	Superoxide dismutase [Cu-Zn] 4AP	124	15.06/5.64	3(1)	15
102	gij84028521	Actin depolymerizing factor-like protein [ <i>Arachis hypogaea</i> ]	152	16.08/6.15	3(1)	23
102	gij388509416	Unknown [ <i>Lotus japonicus</i> ]	137	14.18/5.53	2(2)	19
102	gij357476151	Receptor-like protein kinase [ <i>Medicago truncatula</i> ]	64	73.48/5.73	1(1)	1
102	gij255088257	Fructose-1,6-bisphosphatase [ <i>Micromonas sp. RCC299</i> ]	64	39.47/5.79	2(0)	2
103	gij356558075	Triosephosphate isomerase, cytosolic-like [ <i>Glycine max</i> ]	138	27.18/5.87	4(3)	16
103	gij30983936	Triose-phosphate isomerase [ <i>Gossypium barbadense</i> ]	90	11.33/6.92	2(2)	19
103	gij218198738	Hypothetical protein Osl_24136 [ <i>Oryza sativa Indica Group</i> ]	82	17.47/6.42	2(1)	6
103	gij21807	Heat shock protein 17.3 [ <i>Triticum aestivum</i> ]	62	17.34/5.58	2(1)	6
103	gij357140899	Glutathione S-transferase GSTU6-like [ <i>Brachypodium distachyon</i> ]	52	25.79/6.12	1(1)	5
104	gij255630726	Unknown [ <i>Glycine max</i> ]	54	22.33/4.83	3(1)	4
104	gij357489855	Hypothetical protein MTR_5g065190 [ <i>Medicago truncatula</i> ]	48	23.99/6.28	8(0)	4
104	gij297803014	Transcription factor/ transcription regulator [ <i>Arabidopsis lyrata subsp. lyrata</i> ]	43	17.69/9.32	2(0)	14





Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
104	gij384250236	AIR synthase-related protein [ <i>Coccomyxa subellipsoidea</i> C-169]	38	144.02/5.01	1(0)	0
104	gij255074983	Multidrug/Oligosaccharidyl-lipid/Polysaccharide flippase [ <i>Micromonas</i> sp. RCC299]	35	62.39/9.60	1(0)	1
105	gij356504476	NADP-dependent alkenal double bond reductase P1-like (Alcohol dehydrogenase) [ <i>Glycine max</i> ]	652	37.91/5.81	14(8)	34
105	gij61969078	Putative ferredoxin-NADP reductase [ <i>Solanum peruvianum</i> ]	95	35.16/7.71	2(1)	11
105	gij356528948	V-type proton ATPase subunit E-like [ <i>Glycine max</i> ]	280	26.75/5.87	7(2)	28
105	gij255648265	Unknown [ <i>Glycine max</i> ]	185	37.95/5.93	7(1)	27
106	gij356513119	3-hydroxyisobutyryl-CoA hydrolase-like protein 3, mitochondrial-like isoform 1 [ <i>Glycine max</i> ]	530	44.57/6.28	11(4)	33
106	gij373432589	Malate dehydrogenase, cytoplasmic-like [ <i>Glycine max</i> ]	354	35.18/5.91	7(4)	31
107	gij60101357	Glutamine synthetase [ <i>Vigna radiata</i> ]	707	30.69/6.20	14(8)	49
107	gij417060	Glutamine synthetase nodule isozyme	621	39.08/5.73	14(7)	40
107	gij10946357	Cytosolic glutamine synthetase GSbeta1 [ <i>Glycine max</i> ]	578	38.97/5.48	12(6)	36
109	gij356543209	Patellin-5-like [ <i>Glycine max</i> ]	592	69.52/4.87	19(11)	18
109	gij84453208	Putative cytosolic factor [ <i>Trifolium pratense</i> ]	340	67.83/4.72	10(5)	8
109	gij351724907	Methionine synthase [ <i>Glycine max</i> ]	318	84.23/4.81	6(4)	5



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
110	gij171854980	Protein disulfide isomerase [ <i>Glycine max</i> ]	301	58.55/5.13	6(4)	11
111	gij359475987	Endoribonuclease Dicer homolog 3a-like [ <i>Vitis vinifera</i> ]	40	184.26/6.17	1(0)	0
111	gij159469854	Predicted protein [ <i>Chlamydomonas reinhardtii</i> ]	36	26.86/8.35	1(0)	2
111	gij412993187	Dihydropteroate synthase [ <i>Bathycoccus prasinus</i> ]	36	68.09/8.25	1(0)	1
111	gij357118922	Pectate lyase-like [ <i>Brachypodium distachyon</i> ]	33	48.98/9.27	1(0)	1
112	gij356505318	Enolase-like [ <i>Glycine max</i> ]	1439	47.63/5.49	33(17)	52
112	gij356575611	ATP synthase subunit beta, mitochondrial-like [ <i>Glycine max</i> ]	1332	59.81/5.80	26(19)	43
113	gij356576411	Chaperonin CPN60-2, mitochondrial-like isoform 1 [ <i>Glycine max</i> ]	973	61.21/5.75	19(11)	36
113	gij306561655	Betaine aldehyde dehydrogenase [ <i>Glycine max</i> ]	602	54.63/5.28	18(7)	34
114	gij356535474	Chaperonin CPN60-2, mitochondrial-like isoform 1 [ <i>Glycine max</i> ]	1950	61.39/5.75	51(25)	56
115	gij356554826	Heat shock 70 kDa protein, mitochondrial-like [ <i>Glycine max</i> ]	1665	72.43/5.78	44(22)	41
116	gij363806992	V-type proton ATPase catalytic subunit A-like [ <i>Glycine max</i> ]	1582	68.74/5.41	34(14)	56
116	gij356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	1340	65.15/5.75	31(17)	48
117	unidentified					
118	gij356505318	Enolase-like [ <i>Glycine max</i> ]	1452	47.63/5.49	40(21)	52
118	gij312281815	mRNA, clone: RTFL01-03-H18 [ <i>Thellungiella halophila</i> ]	649	47.61/5.55	20(8)	31



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
118	gi 356571429	Leucine aminopeptidase 1-like isoform 1 [ <i>Glycine max</i> ]	511	60.80/6.38	10(8)	19
119	gi 356512590	Rab GDP dissociation inhibitor alpha-like [ <i>Glycine max</i> ]	538	49.63/5.41	14(5)	31
119	gi 363814290	Uncharacterized protein LOC100805858 precursor [ <i>Glycine max</i> ]	465	55.32/5.57	14(8)	19
120	gi 255642137	Unknown [ <i>Glycine max</i> ]	693	49.56/5.48	17(5)	38
120	gi 356555230	Succinate-semialdehyde dehydrogenase, mitochondrial-like [ <i>Glycine max</i> ]	335	57.61/8.44	9(3)	12
121	gi 356538311	3-isopropylmalate dehydrogenase, chloroplastic-like [ <i>Glycine max</i> ]	618	43.56/5.75	12(8)	28
121	gi 356520651	Glutamine synthetase PR-2-like [ <i>Glycine max</i> ]	488	39.12/5.32	9(6)	34
122	gi 356538327	Aconitate hydratase 1-like [ <i>Glycine max</i> ]	335	98.38/5.75	13(3)	13
123	gi 356571744	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase-like isoform 1 [ <i>Glycine max</i> ]	602	84.22/5.97	13(6)	20
123	gi 219522337	Methionine synthase [ <i>Cicer arietinum</i> ]	429	84.32/6.01	9(5)	13
123	gi 351722222	Lipoxygenase [ <i>Glycine max</i> ]	428	96.24/6.29	10(5)	10
124	gi 126411	Lipoxygenase	738	96.76/5.78	20(9)	15
124	gi 351724907	Methionine synthase [ <i>Glycine max</i> ]	689	84.23/5.93	17(6)	25
124	gi 356508448	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase-like [ <i>Glycine max</i> ]	442	88.61/6.41	10(4)	13



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
125	gi 356571744	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase-like isoform 1 [ <i>Glycine max</i> ]	1685	84.22/5.97	44(26)	37
125	gi 351724907	Methionine synthase [ <i>Glycine max</i> ]	1607	84.23/5.93	41(24)	39
126	gi 356558946	Subtilisin-like protease-like [ <i>Glycine max</i> ]	775	81.90/6.24	28(11)	19
127	gi 356570259	Lysosomal alpha-mannosidase-like [ <i>Glycine max</i> ]	639	115.53/5.84	14(8)	12
127	gi 356574603	Phosphomannomutase/phosphoglucomutase-like [ <i>Glycine max</i> ]	474	64.16/6.67	13(4)	19
127	gi 296082270	Unnamed protein product [ <i>Vitis vinifera</i> ]	212	113.63/5.66	5(2)	5
128	gi 356512590	Rab GDP dissociation inhibitor alpha-like [ <i>Glycine max</i> ]	895	49.63/5.41	24(10)	47
128	gi 356521227	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial-like [ <i>Glycine max</i> ]	616	57.58/6.53	12(7)	24
129	gi 356543900	Ketol-acid reductoisomerase, chloroplastic-like [ <i>Glycine max</i> ]	859	63.34/6.85	24(14)	30
130	gi 356551144	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic-like [ <i>Glycine max</i> ]	865	109.97/5.54	29(7)	17
131	gi 356551144	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic-like [ <i>Glycine max</i> ]	1102	109.97/5.54	29(12)	19



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pi	Peptide match	% Coverage
132	gij356558908	NADP-dependent malic enzyme-like [ <i>Glycine max</i> ]	813	65.15/5.75	14(10)	27
132	gij356524774	LL-diaminopimelate aminotransferase, chloroplastic-like [ <i>Glycine max</i> ]	631	49.89/6.67	16(8)	35
132	gij255641490	Unknown [ <i>Glycine max</i> ]	477	45.81/5.95	11(7)	25
132	gij356555724	Fumarylacetoacetase-like [ <i>Glycine max</i> ]	442	45.79/5.84	10(6)	21
133	gij356513119	3-hydroxyisobutyryl-CoA hydrolase-like protein 3, mitochondrial-like isoform 1 [ <i>Glycine max</i> ]	654	44.57/6.28	17(7)	34
133	gij27462762	Malate dehydrogenase [ <i>Lupinus albus</i> ]	246	35.57/6.10	5(3)	14
133	gij22597178	Alcohol dehydrogenase 1 [ <i>Glycine max</i> ]	153	39.98/6.19	5(1)	7
134	gij356526581	6-phosphogluconate dehydrogenase, decarboxylating-like isoform 1 [ <i>Glycine max</i> ]	604	53.53/6.11	18(6)	34
134	gij217330650	Actin related protein 1 [ <i>Acyrtosiphon pisum</i> ]	257	41.76/5.29	6(4)	14
134	gij356497044	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3-like [ <i>Glycine max</i> ]	196	51.03/6.20	4(2)	6
135	gij479386	Isocitrate dehydrogenase (NADP) (EC 1.1.1.42) - soybean	1456	50.19/6.25	24(11)	47
136	gij363806950	Aldose 1-epimerase (EC=5.1.3.3) [ <i>Glycine max</i> ]	234	36.93/6.12	8(2)	20
136	gij357499099	Aldose 1-epimerase family protein expressed [ <i>Medicago truncatula</i> ]	184	36.86/5.94	6(2)	15
136	gij4731376	Isoflavone reductase homolog Bet v 6.0101 [ <i>Betula pendula</i> ]	65	33.13/7.82	2(0)	8



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
137	unidentified					
138	gij356497044	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3-like [ <i>Glycine max</i> ]	458	51.03/6.20	20(10)	16
138	gij255641587	Unknown [ <i>Glycine max</i> ]	372	48.91/5.82	13(7)	12
138	gij356552986	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex 2, mitochondrial-like [ <i>Glycine max</i> ]	281	49.83/9.18	7(2)	19
138	gij356496197	Proliferation-associated protein 2G4-like [ <i>Glycine max</i> ]	281	42.95/6.30	7(1)	24
138	gij356511468	Monodehydroascorbate reductase, chloroplastic-like [ <i>Glycine max</i> ]	246	51.86/8.55	6(1)	16
139	gij356496197	Proliferation-associated protein 2G4-like [ <i>Glycine max</i> ]	479	42.95/6.30	12(4)	31
139	gij356559573	Glutathione reductase, cytosolic-like [ <i>Glycine max</i> ]	466	53.96/5.63	11(4)	25
139	gij356511468	Monodehydroascorbate reductase, chloroplastic-like [ <i>Glycine max</i> ]	410	51.86/8.55	11(2)	27
139	gij356543209	Patellin-5-like [ <i>Glycine max</i> ]	406	69.52/4.87	10(8)	14
140	gij351720808	14-3-3-like protein A [ <i>Glycine max</i> ]	314	29.05/4.72	5(4)	25
141	gij108946919	Putative NBS-LRR disease resistance protein [ <i>Malus x domestica</i> ]	24	17.99/8.50	1(0)	12
141	gij148909375	Unknown [ <i>Picea sitchensis</i> ]	22	37.84/8.33	1(0)	2
141	gij356534232	Mitogen activated protein kinase kinase kinase 3-like [ <i>Glycine max</i> ]	20	69.27/6.19	1(0)	1



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
141	gi 90403745	RNA polymerase beta chain [ <i>Nepenthes sp. 'Kosobe'</i> ]	18	98.24/9.72	1(0)	1
142	gi 159478785	Predicted protein [ <i>Chlamydomonas reinhardtii</i> ]	27	48.61/8.47	1(0)	3
142	gi 357509503	Adipocyte plasma membrane-associated protein [ <i>Medicago truncatula</i> ]	27	44.93/9.19	1(0)	3
143	gi 218198738	Hypothetical protein Osl_24136 [ <i>Oryza sativa Indica Group</i> ]	69	17.40/6.42	2(0)	6
143	gi 357140899	Glutathione S-transferase GSTU6-like [ <i>Brachypodium distachyon</i> ]	54	25.79/6.12	1(1)	5
143	gi 308809894	DNA replication licensing factor, MCM5 component (ISS) [ <i>Ostreococcus tauri</i> ]	49	144.15/8.14	4(0)	1
142	gi 159478785	Predicted protein [ <i>Chlamydomonas reinhardtii</i> ]	27	48.61/8.47	1(0)	3
142	gi 357509503	Adipocyte plasma membrane-associated protein [ <i>Medicago truncatula</i> ]	27	44.93/9.19	1(0)	3
143	gi 218198738	Hypothetical protein Osl_24136 [ <i>Oryza sativa Indica Group</i> ]	69	17.40/6.42	2(0)	6
143	gi 357140899	Glutathione S-transferase GSTU6-like [ <i>Brachypodium distachyon</i> ]	54	25.79/6.12	1(1)	5
143	gi 308809894	DNA replication licensing factor, MCM5 component (ISS) [ <i>Ostreococcus tauri</i> ]	49	144.15/8.14	4(0)	1
144	gi 356539366	Superoxide dismutase [Cu-Zn], chloroplastic-like [ <i>Glycine max</i> ]	38	20.88/6.03	1(0)	5
145	gi 130829	Pathogenesis-related protein 1; AltName: Full=PR2; AltName: Full=PvPR1	264	16.52/4.83	7(7)	29
145	gi 308154384	PR10.61 [ <i>Oxytropis splendens</i> ]	257	15.70/5.13	11(1)	17
145	gi 351724911	Uncharacterized protein LOC100527731 [ <i>Glycine max</i> ]	206	16.75/4.73	11(5)	27
146	gi 82407319	Chain A, Native Crystal Structure Of Spe16	134	16.37/4.91	6(2)	18



Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
146	gi 159471898	Predicted protein [ <i>Chlamydomonas reinhardtii</i> ]	49	28.39/5.42	1(0)	3
146	gi 308803158	ABC transporter, putative (ISS) [ <i>Ostreococcus tauri</i> ]	45	71.11/8.40	1(0)	1
147	gi 147853041	Hypothetical protein VITISV_021317 [ <i>Vitis vinifera</i> ]	33	96.47/6.29	2(0)	1
147	gi 297850844	Kinase family protein [ <i>Arabidopsis lyrata subsp. lyrata</i> ]	29	43.73/6.03	1(0)	3
147	gi 79416713	RNA-directed DNA polymerase (reverse transcriptase) [ <i>Arabidopsis thaliana</i> ]	27	84.82/6.94	1(0)	1
148	unidentified					
149	gi 293332265	Putative leucine-rich repeat protein kinase family protein [ <i>Zea mays</i> ]	42	78.52/8.66	1(0)	1
149	gi 308805745	Mg <sup>2+</sup> -dependent phosphodiesterase TTRAP (ISS) [ <i>Ostreococcus tauri</i> ]	32	43.38/7.67	1(0)	3
149	gi 619745	Cytochrome p450 dependent monooxygenase [ <i>Arabidopsis thaliana</i> ]	22	57.39/8.49	1(0)	3
150	gi 3122071	Elongation factor 1-alpha; Short=EF-1-alpha	612	49.39/9.11	15(6)	22
151	gi 356560200	Pectinesterase/pectinesterase inhibitor 3-like [ <i>Glycine max</i> ]	269	59.99/8.28	4(4)	9
151	gi 351725233	Uncharacterized protein LOC100527288 precursor [ <i>Glycine max</i> ]	56	22.10/9.39	2(0)	12
151	gi 21060	Pectinesterase [ <i>Phaseolus vulgaris</i> ]	46	23.80/9.52	1(0)	4
151	gi 29126356	Retrotransposon protein, putative, Ty3-gypsy sub-class [ <i>Oryza sativa Japonica Group</i> ]	45	153.85/8.66	2(0)	1
152	gi 3769330	Alpha-amylase [ <i>Phaseolus vulgaris</i> ]	490	46.82/5.45	15(5)	24

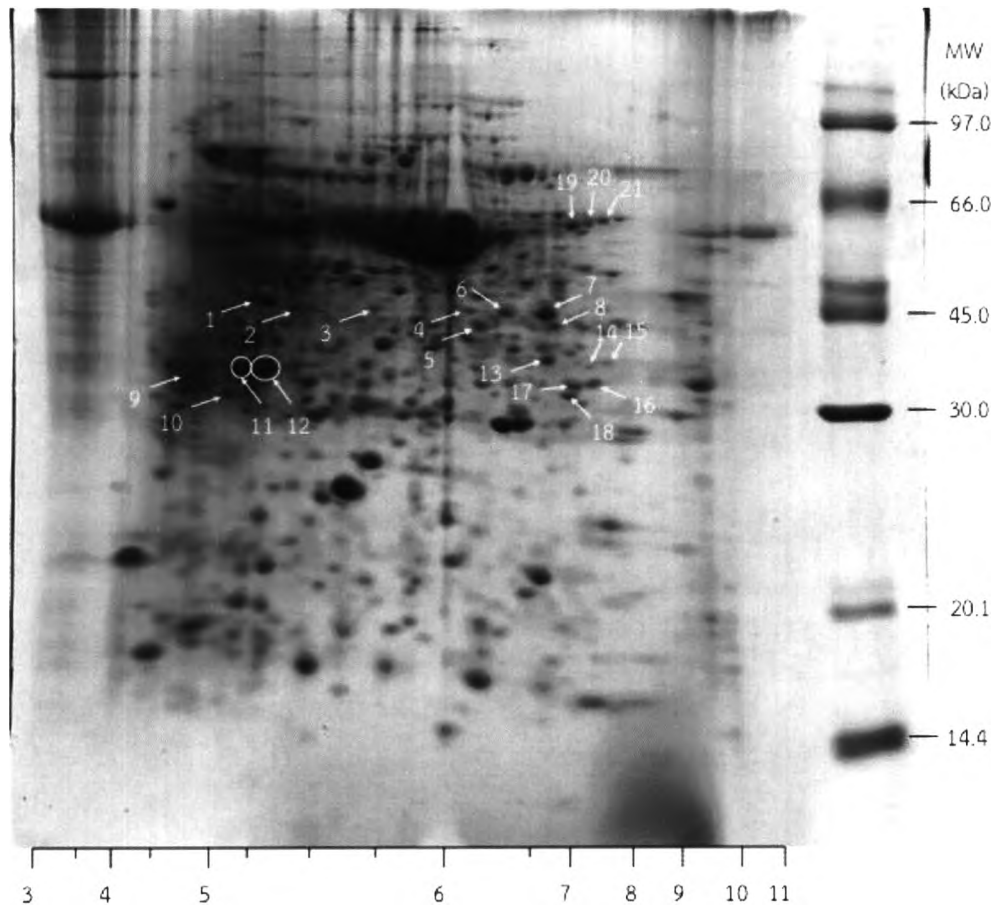




Table 13 The protein spot analysis of *P. mirifica* tuber (continued)

No.	Protein Name(s)	Protein name	Score	MW/pI	Peptide match	% Coverage
152	gi 255636611	Unknown [ <i>Glycine max</i> ]	468	47.42/5.76	16(4)	29
153	gi 356564192	Alpha-amylase-like [ <i>Glycine max</i> ]	680	46.12/5.41	23(6)	34
153	gi 255636611	Unknown [ <i>Glycine max</i> ]	658	47.42/5.76	24(6)	31
153	gi 356505318	Enolase-like [ <i>Glycine max</i> ]	352	47.63/5.49	8(3)	22
154	gi 312281815	mRNA, clone: RTFL01-03-H18 [ <i>Theilungiella halophila</i> ]	100	47.61/5.55	3(1)	8
154	gi 356551144	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic-like [ <i>Glycine max</i> ]	100	109.97/5.54	3(1)	3
154	gi 5002348	Peroxidase 5 precursor [ <i>Phaseolus vulgaris</i> ]	52	35.49/4.45	1(0)	6

## Appendix G



**Figure 37** The representative gel of *P. mirifica* (PM-V) during winter season with selected spot. The protein spots were selected from the range of isoelectric point (pI) and molecular weight (Mw) of the interesting protein



## Appendix H

Table 14 The protein analysis of *P. mirifica* leaves

No.	Protein Name(s)	Description	Score	MW/pI	Peptide match	% Coverage
1	gi 356495988	Phosphoribulokinase, chloroplastic-like [ <i>Glycine max</i> ]	453	45.24/5.89	8(2)	38
2	gi 351724415	protein disulfide isomerase-like protein precursor [ <i>Glycine max</i> ]	203	40.33/5.73	7(3)	17
2	gi 27462762	malate dehydrogenase [ <i>Lupinus albus</i> ]	157	35.57/6.10	4(1)	15
2	gi 109892850	Putative cytochrome c oxidase subunit II PS17	45	1.71/9.62	2(0)	100
2	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	44	61.15/8.98	1(0)	3
2	gi 359473485	Transcription factor bHLH49-like [ <i>Vitis vinifera</i> ]	41	64.67/5.50	1(0)	3
3	gi 27462762	Malate dehydrogenase [ <i>Lupinus albus</i> ]	327	35.57/6.10	9(2)	28
3	gi 351721288	Glyceraldehyde-3-phosphate dehydrogenase A subunit [ <i>Glycine max</i> ]	80	43.19/8.42	2(0)	8
4	gi 5929964	Malate dehydrogenase [ <i>Glycine max</i> ]	215	36.12/8.23	6(2)	19
4	gi 359473485	Ttranscription factor bHLH49-like [ <i>Vitis vinifera</i> ]	47	64.67/5.50	2(0)	3
4	gi 15242338	TIR-NBS-LRR class disease resistance protein [ <i>Arabidopsis thaliana</i> ]	45	139.64/6.70	1(0)	0

Table 14 The protein analysis of *P. mirifica* leaves (continued)

no.	Protein Name(s)	Description	Score	MW/pI	Peptide match	% Coverage
5	gi 351724481	Fructose-bisphosphate aldolase [ <i>Glycine max</i> ]	229	38.37/6.77	7(2)	26
5	gi 255647134	Unknown [ <i>Glycine max</i> ]	185	43.61/6.61	6(0)	11
5	gi 356504466	Haloalkane dehalogenase-like [ <i>Glycine max</i> ]	45	44.49/8.43	1(0)	2
6	gi 351721288	Glyceraldehyde-3-phosphate dehydrogenase A subunit [ <i>Glycine max</i> ]	362	43.19/8.42	10(4)	27
7	gi 351721288	Glyceraldehyde-3-phosphate dehydrogenase A subunit [ <i>Glycine max</i> ]	741	43.19/8.42	18(9)	38
8	gi 356516587	Glyceraldehyde-3-phosphate dehydrogenase-like [ <i>Glycine max</i> ]	204	36.71/6.72	6(1)	20
9	gi 1168196	14-3-3-like protein	364	29.31/4.71	11(2)	41
10	gi 356531866	Phosphoglycolate phosphatase-like [ <i>Glycine max</i> ]	257	40.85/8.09	8(2)	29
10	gi 8885622	N-glyceraldehyde-2-phosphotransferase-like [ <i>Arabidopsis thaliana</i> ]	184	31.71/5.14	6(1)	21
10	gi 356508764	Protein CbbY-like isoform 1 [ <i>Glycine max</i> ]	110	35.40/6.33	2(1)	7
10	gi 2494261	Full=Elongation factor Tu, chloroplastic; Short=EF-Tu	49	52.06/6.21	2(0)	5
11	unidentified					

Table 14 The protein analysis of *P. mirifica* leaves (continued)

no.	Protein Name(s)	Description	Score	MW/pi	Peptide match	% Coverage
12	gi 356559442	Oxygen-evolving enhancer protein 1, chloroplastic-like [ <i>Glycine max</i> ]	270	35.04/6.66	6(2)	26
12	gi 152143640	Chloroplast photosynthetic water oxidation complex 33kDa subunit precursor [ <i>Morus nigra</i> ]	201	28.25/5.48	4(1)	16
12	gi 145356482	psbO, PSII-O, OEE1, photosystem II polypeptide, oxygen evolving enhancer 1 [ <i>Ostreococcus lucimarinus</i> CCE9901]	45	31.88/7.60	1(0)	2
12	gi 1619903	Thiol protease isoform B, partial [ <i>Glycine max</i> ]	43	34.95/7.60	1(0)	6
12	gi 356553413	Cysteine proteinase 15A-like [ <i>Glycine max</i> ]	43	40.22/6.82	1(0)	5
12	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	39	61.15/8.98	1(0)	3
13	unidentified					
14	unidentified					
15	unidentified					
16	gi 89145876	Xyloglucan endotransglycosylase precursor [ <i>Glycine max</i> ]	99	19.48/6.56	2(1)	12
16	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	54	61.15/8.98	1(1)	3
16	gi 255605063	Xyloglucan: xyloglucosyl transferase, putative [ <i>Ricinus communis</i> ]	51	6.34/8.80	1(1)	25

Table 14 The protein analysis of *P. mirifica* leaves (continued)

no.	Protein Name(s)	Description	Score	MW/pI	Peptide match	% Coverage
16	gi 356523789	Uncharacterized protein LOC100800881 [ <i>Glycine max</i> ]	41	199.08/8.77	1(0)	1
16	gi 116347	Endochitinase; Flags: Precursor	35	35.42/8.43	1(0)	3
17	gi 116347	Endochitinase; Flags: Precursor	58	35.42/8.43	3(0)	6
17	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	47	61.15/8.98	1(0)	3
17	gi 255080640	Aminopeptidase [ <i>Micromonas sp.</i> RCC299]	36	62.99/7.97	1(0)	1
17	gi 30526289	29 kDa chitinase-like thermal hysteresis protein [ <i>Solanum dulcamara</i> ]	36	28.83/8.88	1(0)	4
17	gi 13620976	2-dehydro-3-deoxyphosphooctonate aldolase [ <i>Arabidopsis thaliana</i> ]	35	31.60/6.33	1(0)	4
18	gi 356502736	Proteasome subunit alpha type-7-like isoform 1 [ <i>Glycine max</i> ]	196	27.09/6.98	5(1)	25
18	gi 2511580	Multicatalytic endopeptidase [ <i>Arabidopsis thaliana</i> ]	129	25.83/7.74	4(0)	22
18	gi 359473485	Transcription factor bHLH49-like [ <i>Vitis vinifera</i> ]	53	64.67/5.50	2(0)	3
18	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	44	61.15/8.98	1(0)	3
18	gi 15230186	F-box/kelch-repeat protein [ <i>Arabidopsis thaliana</i> ]	32	42.65/5.50	1(0)	5
19	gi 225433510	Serine hydroxymethyltransferase 1 [ <i>Vitis vinifera</i> ]	448	51.87/7.17	7(5)	18

Table 14 The protein analysis of *P. mirifica* leaves (continued)

no.	Protein Name(s)	Description	Score	MW/pi	Peptide match	% Coverage
19	gi 389548688	Serine hydroxymethyltransferase [ <i>Glycine max</i> ]	446	51.73/7.59	9(4)	19
19	gi 255642128	Unknown [ <i>Glycine max</i> ]	425	48.25/8.02	8(4)	21
19	gi 15235745	Glycine hydroxymethyltransferase [ <i>Arabidopsis thaliana</i> ]	221	57.36/8.13	6(3)	13
20	gi 351722703	Serine hydroxymethyltransferase 5 [ <i>Glycine max</i> ]	406	57.34/8.83	11(3)	23
20	gi 351722183	Serine hydroxymethyltransferase 3 [ <i>Glycine max</i> ]	399	57.35/8.03	11(3)	21
20	gi 357485333	Serine hydroxymethyltransferase [ <i>Medicago truncatula</i> ]	350	57.37/8.37	10(2)	20
21	gi 351722703	Serine hydroxymethyltransferase 5 [ <i>Glycine max</i> ]	346	57.34/8.83	9(1)	22
21	gi 15235745	Glycine hydroxymethyltransferase [ <i>Arabidopsis thaliana</i> ]	294	57.36/8.13	8(1)	17
21	gi 351722183	Serine hydroxymethyltransferase 3 [ <i>Glycine max</i> ]	284	57.35/8.03	7(1)	14
22	gi 1771248	Ribulose 1,5-biphosphate carboxylase large subunit [ <i>Maackia amurensis</i> ]	82	50.37/6.33	2(0)	3
22	gi 15226437	D-arabinono-1,4-lactone oxidase family protein [ <i>Arabidopsis thaliana</i> ]	66	66.24/8.47	1(1)	2
22	gi 11990288	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [ <i>Pravinaria leucocarpa</i> ]	66	51.76/6.04	2(0)	3



Table 14 The protein analysis of *P. mirifica* leaves (continued)

no.	Protein Name(s)	Description	Score	MW/pI	Peptide match	% Coverage
23	gi 356522011	Aminomethyltransferase, mitochondrial-like [ <i>Glycine max</i> ]	397	44.31/8.68	12(3)	28
24	gi 356562997	Aminomethyltransferase, mitochondrial-like [ <i>Glycine max</i> ]	322	44.26/8.34	8(4)	20
24	gi 349592191	Glycine decarboxylase complex subunit T [ <i>Cicer arietinum</i> ]	157	14.84/5.21	5(2)	27
24	gi 5817720	Maturase-like protein (chloroplast) [ <i>Adesmia volckmannii</i> ]	53	61.15/8.98	1(1)	3
24	gi 359473485	Transcription factor bHLH49-like [ <i>Vitis vinifera</i> ]	52	64.67/5.50	2(0)	3



## Appendix I

Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold).

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
1	0.16610	0.20313	0.21485	-1.22294	-1.29349
2	0.10478	0.12575	0.06150	-1.20005	+1.70386
3	0.35272	0.55999	0.39832	-1.58764	-1.12928
4	0.15310	0.12392	0.16969	+1.23546	-1.10834
5	0.16152	0.13417	0.26286	+1.20392	-1.62737
6	0.12068	0.18110	0.31168	-1.50068	-2.58266
7	0.11365	0.09180	0.22462	+1.23799	-1.97644
8	0.10792	0.09860	0.29936	+1.09456	-2.77380
9	0.13295	0.16411	0.48337	-1.23440	-3.63588
10	0.12821	0.08941	0.04679	+1.43394	+2.74022
11	0.11834	0.16523	0.11495	-1.39623	+1.02953
12	0.09575	0.10145	0.10841	-1.05953	-1.13225
13	0.12927	0.02417	0.03570	+5.34858	+3.62055
14	0.13409	0.07962	0.08355	+1.68422	+1.60505
15	0.09363	0.05181	0.08563	+1.80732	+1.09346
16	0.08271	0.08186	0.11987	+1.01042	-1.44931
17	0.48034	0.16112	0.35581	+2.98129	+1.34996
18	0.08248	0.13338	0.10703	-1.61708	-1.29769
19	0.16127	0.10746	0.18845	+1.50077	-1.16857
20	0.07564	0.06681	0.05222	+1.13217	+1.44844



Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
21	0.09938	0.03784	0.04012	+2.62656	+2.47714
22	0.70818	0.35501	0.65092	+1.99480	+1.08797
23	1.35692	0.98127	1.22852	+1.38282	+1.10452
24	0.47758	0.33535	0.46227	+1.42412	+1.03311
25	0.39953	0.19796	0.19949	+2.01821	+2.00279
26	1.90398	2.39443	3.02036	-1.25759	-1.58634
27	0.24184	1.38729	0.94325	-5.73647	-3.90035
28	0.31168	0.10163	0.11511	+3.06694	+2.70765
29	0.48487	0.96093	0.76759	-1.98185	-1.58309
30	5.13381	1.11891	1.55159	+4.58822	+3.30874
31	13.52980	6.57165	7.14930	+2.05881	+1.89246
32	6.21688	3.22031	1.14223	+5.44276	+1.93052
33	0.37764	2.33790	1.43035	-6.19078	-3.78758
34	0.63769	1.14147	0.84513	-1.79001	-1.32530
35	0.11828	0.09602	0.10504	+1.23191	+1.12614
36	0.12659	0.05398	0.09806	+2.34520	+1.29088
37	0.13296	0.09416	0.08675	+1.41201	+1.53273
38	0.09877	0.06774	0.19726	+1.45799	-1.99712
39	0.52958	0.16273	0.19450	+3.25429	+2.72278
40	0.18887	0.07143	0.09617	+2.64406	+1.96382
41	0.24959	0.11435	0.08671	+2.18263	+2.87840
42	0.30188	0.45691	0.31443	-1.51358	-1.04160



Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
43	0.14146	0.19221	0.19233	-1.35878	-1.35958
44	0.11636	0.19536	0.13556	-1.67891	-1.16495
45	0.22048	0.09433	0.07563	+2.33725	+2.91540
46	0.30671	0.09420	0.17516	+3.25599	+1.75103
47	0.65565	0.32681	0.19369	+2.00620	+3.38501
48	0.19197	0.23308	0.15154	-1.21415	+1.26678
49	1.10348	0.40025	0.30177	+2.75700	+3.65667
50	0.24419	0.19472	0.15711	+1.25402	+1.55420
51	0.20958	0.11326	0.08453	+1.85049	+2.47942
52	0.21718	0.20832	0.14108	+1.04255	+1.53942
53	0.16929	0.05389	0.50947	+3.14163	-3.00943
54	0.26213	0.16948	0.14618	+1.54664	+1.79318
55	0.18802	0.08975	0.04842	+2.09481	+3.88332
56	0.22426	0.19779	0.12910	+1.13384	+1.73715
57	0.06791	0.02157	0.01874	+3.14855	+3.62403
58	0.08811	0.09085	0.08134	-1.03109	+1.08327
59	0.24736	0.11450	0.10226	+2.16044	+2.41895
60	0.14979	0.10360	0.10085	+1.44588	+1.48525
61	0.08258	0.03044	0.03317	+2.71319	+2.48989
62	0.14684	0.03837	0.03662	+3.82727	+4.00929
63	0.17199	0.07564	0.06214	+2.27360	+2.76750
64	0.96286	0.33260	0.16576	+2.89500	+5.80895

Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
65	0.32254	0.28272	0.22423	+1.14086	+1.43848
66	0.21254	0.16992	0.07695	+1.25088	+2.76216
67	0.19241	0.15061	0.24088	+1.27755	-1.25194
68	0.13881	0.08156	0.08005	+1.70179	+1.73388
69	0.31786	0.17532	0.13615	+1.81301	+2.33466
70	0.30840	0.11795	0.09500	+2.61480	+3.24622
71	0.13793	0.04160	0.02695	+3.31592	+5.11802
72	0.53343	0.05178	0.06030	+10.30199	+8.84617
73	0.38565	0.08177	0.13378	+4.71603	+2.88272
74	0.16554	0.22292	0.22426	-1.34666	-1.35474
75	0.11035	0.08634	0.10302	+1.27802	+1.07107
76	0.08805	0.08504	0.08012	+1.03539	+1.09904
77	0.24471	0.09863	0.10057	+2.48107	+2.43337
78	1.07698	0.63957	0.50418	+1.68390	+2.13609
79	0.17001	0.07329	0.05499	+2.31969	+3.09182
80	0.07591	0.09316	0.06990	-1.22719	+1.08608
81	0.17675	0.15668	0.14208	+1.12804	+1.24396
82	0.20010	0.09184	0.16121	+2.17873	+1.24118
83	0.27690	0.14011	0.53724	+1.97635	-1.94021
84	0.93128	0.67508	0.21656	+1.37951	+4.30038
85	0.10095	0.06355	0.04398	+1.58840	+2.29534
86	0.51407	0.46634	0.48401	+1.10235	+1.06210



**Table 15** The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
87	0.84954	0.72876	0.82746	+1.16574	+1.02669
88	0.18494	0.06872	0.08271	+2.69118	+2.23603
89	0.22600	0.27052	0.08368	-1.19702	+2.70085
90	0.19745	0.23897	0.19239	-1.21024	+1.02633
91	0.18471	0.15678	0.14797	+1.17819	+1.24832
92	0.09753	0.16115	0.08976	-1.65221	+1.08656
93	0.19400	0.06603	0.06705	+2.93805	+2.89334
94	0.28039	0.12870	0.10358	+2.17871	+2.70688
95	0.42116	0.24285	0.35707	+1.73427	+1.17951
96	0.31516	0.09962	0.13031	+3.16351	+2.41862
97	1.48345	0.67070	0.78020	+2.21181	+1.90137
98	0.62149	0.49426	0.49543	+1.25740	+1.25445
99	0.17152	0.17906	0.09070	-1.04397	+1.89096
100	3.29596	7.46976	10.48000	-2.26634	-3.17965
101	1.16719	0.86404	0.85710	+1.35086	+1.36178
102	1.95044	0.29132	0.52725	+6.69516	+3.69930
103	2.21075	1.93851	3.03040	+1.14044	-1.37076
104	5.25685	14.80850	11.08990	-2.81699	-2.10961
105	0.41499	0.23295	0.30338	+1.78141	+1.36788
106	0.09417	0.04150	0.05135	+2.26905	+1.83400
107	0.12715	0.03099	0.03832	+4.10325	+3.31825
108	0.21476	0.11995	0.11779	+1.79036	+1.82319

Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
109	1.13657	1.05697	1.24348	+1.07531	-1.09406
110	0.43078	0.17439	0.20706	+2.47019	+2.08049
111	0.35467	0.08851	0.11474	+4.00688	+3.09108
112	0.29615	0.31075	0.29468	-1.04930	+1.00497
113	0.15161	0.04601	0.04775	+3.29555	+3.17507
114	0.15302	0.09099	0.09363	+1.68168	+1.63432
115	0.61853	0.32138	0.32289	+1.92462	+1.91562
116	0.30891	0.27491	0.19212	+1.12367	+1.60789
117	0.33419	0.21124	0.22481	+1.58204	+1.48652
118	0.22484	0.21194	0.22421	+1.06089	+1.00282
119	0.28163	0.20496	0.19478	+1.37404	+1.44591
120	0.39371	0.28974	0.27149	+1.35881	+1.45018
121	4.07915	2.16158	1.81349	+1.88711	+2.24934
122	0.16612	0.12575	0.05347	+1.32102	+3.10650
123	0.63978	0.36179	0.44857	+1.76837	+1.42627
124	0.09762	0.04060	0.02882	+2.40444	+3.38787
125	0.13200	0.03594	0.03464	+3.67234	+3.81040
126	0.66779	0.27177	0.25348	+2.45720	+2.63452
127	0.25705	0.04853	0.06814	+5.29650	+3.77228
128	0.09337	0.08330	0.11054	+1.12099	-1.18381
129	0.12193	0.07118	0.07280	+1.71298	+1.67471
130	0.14749	0.07614	0.08265	+1.93690	+1.78446

Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
131	0.19544	0.06021	0.06003	+3.24598	+3.25545
132	0.21298	0.07854	0.04972	+2.71188	+4.28362
133	0.13487	0.53021	0.48602	-3.93131	-3.60362
134	0.19567	0.07984	0.07950	+2.45075	+2.46142
135	0.07656	0.05615	0.09102	+1.36337	-1.18888
136	0.09602	0.04293	0.03873	+2.47903	+2.23692
137	0.30910	0.08500	0.14389	+3.63660	+2.14819
138	0.34756	0.04915	0.19595	+7.07144	+1.77374
139	0.16899	0.15355	0.15764	+1.10052	+1.07199
140	0.33207	0.15280	0.22492	+2.17319	+1.47640
141	0.09229	0.06759	0.04698	+1.36544	+1.96453
142	0.18468	0.29521	0.25736	-1.59847	-1.39354
143	0.08827	0.06014	0.31926	+1.46780	-3.61676
144	0.74124	2.08308	1.64215	-2.81028	-2.21542
145	16.32410	21.50120	20.62100	-1.31714	-1.26322
146	0.57588	0.40349	0.25412	+1.42725	+2.26622
147	1.62338	0.89491	1.19876	+1.81401	+1.35422
148	0.70658	0.29669	0.44821	+2.38156	+1.57643
149	0.27273	0.36695	0.34329	-1.34549	-1.25873
150	0.42330	0.12062	0.24924	+3.50946	+1.69839
151	0.26488	0.45665	0.58351	-1.72396	-2.20289
152	0.20872	0.23349	0.16207	-1.11871	+1.28780

Table 15 The fold comparison of protein in *P. mirifica* tuber in seasonal change. (+) Protein density in winter season is greater than (fold), (-) Protein density in winter season is less than (fold) (continued)

Match ID	Protein density			The comparison between winter (Fold)	
	Winter	Summer	Rainy	Summer	Rainy
153	0.15495	0.15125	0.19965	+1.02442	-1.28850
154	0.33278	0.21677	0.24640	+1.53517	+1.35054







### Appendix J

Table 16 The correlation between isoflavonoid contents and identified proteins in *P. mirifica* tuber ( $P < 0.05$ )

Name		CHI	UGT	P450	IFR	Puerarin	Daidzin	Genistin	Daidzein	Genistein
CHI	Pearson Correlation	1	-.957**	-.905**	.948**	-.511	-.624*	-.612*	.117	.683**
	Sig. (2-tailed)		.000	.000	.000	.052	.013	.015	.679	.005
	N	15	15	15	15	15	15	15	15	15
UGT	Pearson Correlation	-.957**	1	.866**	-.868**	.592*	.757**	.766**	-.219	-.729**
	Sig. (2-tailed)	.000		.000	.000	.020	.001	.001	.434	.002
	N	15	15	15	15	15	15	15	15	15
P450	Pearson Correlation	-.905**	.866**	1	-.913**	.417	.564*	.547*	-.041	-.645**
	Sig. (2-tailed)	.000	.000		.000	.122	.028	.035	.886	.009
	N	15	15	15	15	15	15	15	15	15
IFR	Pearson Correlation	.948**	-.868**	-.913**	1	-.391	-.450	-.429	.072	.703**
	Sig. (2-tailed)	.000	.000	.000		.149	.092	.111	.800	.003
	N	15	15	15	15	15	15	15	15	15

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).



Table16 The correlation between isoflavonoid contents and identified proteins in *P. mirifica* tuber ( $P < 0.05$ )

Name		CHI	UGT	P450	IFR	Puerarin	Daidzin	Genistin	Daidzein	Genistein
Puerarin	Pearson Correlation	-.511	.592*	.417	-.391	1	.901**	.703**	-.226	-.338
	Sig. (2-tailed)	.052	.020	.122	.149		.000	.003	.419	.218
	N	15	15	15	15	15	15	15	15	15
Daidzin	Pearson Correlation	-.624*	.757**	.564*	-.450	.901**	1	.920**	-.217	-.423
	Sig. (2-tailed)	.013	.001	.028	.092	.000		.000	.438	.116
	N	15	15	15	15	15	15	15	15	15
Genistin	Pearson Correlation	-.612*	.766**	.547*	-.429	.703**	.920**	1	-.028	-.351
	Sig. (2-tailed)	.015	.001	.035	.111	.003	.000		.921	.200
	N	15	15	15	15	15	15	15	15	15
Daidzein	Pearson Correlation	.117	-.219	-.041	.072	-.226	-.217	-.028	1	.434
	Sig. (2-tailed)	.679	.434	.886	.800	.419	.438	.921		.106
	N	15	15	15	15	15	15	15	15	15
Genistein	Pearson Correlation	.683**	-.729**	-.645**	.703**	-.338	-.423	-.351	.434	1
	Sig. (2-tailed)	.005	.002	.009	.003	.218	.116	.200	.106	
	N	15	15	15	15	15	15	15	15	15

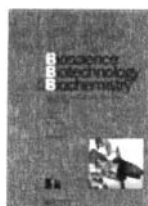
\*\* . Correlation is significant at the 0.01 level (2-tailed). \* . Correlation is significant at the 0.05 level (2-tailed).

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### **Pueraria mirifica leaves, an alternative potential isoflavonoid source**

Jutarmas Jungsukcharoen<sup>a</sup>, Binar Asrining Dhiani<sup>b</sup>, Wichai Cherdshewasart<sup>c</sup>, Nawaporn Vinayavekhin<sup>d</sup>, Polkit Sangvanich<sup>e</sup> & Chuenchit Boonchird

<sup>a</sup> Faculty of Science, Program in Biotechnology, Chulalongkorn University, Bangkok, Thailand

<sup>b</sup> Faculty of Science, Department of Biotechnology, Mahidol University, Bangkok, Thailand

<sup>c</sup> Faculty of Science, Department of Biology, Chulalongkorn University, Bangkok, Thailand

<sup>d</sup> Faculty of Science, Department of Chemistry, Chulalongkorn University, Bangkok, Thailand

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## *Pueraria mirifica* leaves, an alternative potential isoflavonoid source

Jutarmas Jungsukeharoen<sup>1</sup>, Binar Asrining Dhiani<sup>2</sup>, Wichai Cherdshewasart<sup>3</sup>,  
Nawaporn Vinayavekhin<sup>4</sup>, Polkit Sangvanich<sup>4</sup> and Chuenchit Boonchird<sup>2,\*</sup>

<sup>1</sup>Faculty of Science, Program in Biotechnology, Chulalongkorn University, Bangkok, Thailand; <sup>2</sup>Faculty of Science, Department of Biotechnology, Mahidol University, Bangkok, Thailand; <sup>3</sup>Faculty of Science, Department of Biology, Chulalongkorn University, Bangkok, Thailand; <sup>4</sup>Faculty of Science, Department of Chemistry, Chulalongkorn University, Bangkok, Thailand

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We investigated the major leaf isoflavonoid contents of *Pueraria mirifica* from three different cultivars (PM-III, PM-IV, and PM-V) using reverse RP-HPLC analysis. The proportions and net levels of puerarin, daidzin, genistin, and daidzein in *P. mirifica* leaves were found to depend on the plant cultivar and to correlate with cultivation temperature and rainfall amount. The crude leaf-extracts were tested using the Yeast Estrogen Screen (YES) assay with both human estrogen receptors (hER $\alpha$  and hER $\beta$ ). Their estrogenic activity was higher when determined by the YES system containing hER $\beta$  than that with hER $\alpha$  and was also higher when the  $\Delta$ snq2 than the wildtype yeast was employed. The results open the possibility of selecting and cultivating certain *P. mirifica* cultivars at a farm scale to produce a sufficient supply of leaf material to act as a starting source for the commercial scale extraction of these major isoflavonoids.

**Key words:** *Pueraria mirifica*; isoflavonoid; YES assay; estrogenic activity

Isoflavonoids, one of the common types of phytoestrogens, are found in relative abundance in many forms of edible legume seeds<sup>(1-3)</sup> legume-derived foods<sup>(4,5)</sup> and dietary supplements.<sup>(6)</sup> They are of increasing interest to the food industry due to their potential health benefits, including for the potential chemoprevention of cancers.<sup>(6)</sup> The recent establishment of several phytoestrogen databases illustrates the growing interest of the food industry in phytoestrogens from natural sources and their bioactivities.<sup>(7)</sup>

Soy isoflavonoids have been well studied, with most of the results being obtained from daidzein and genistein,<sup>(7)</sup> which appear to harbor anti-cancer properties.<sup>(8)</sup> However, a new source of isoflavonoids would promote

the alternative production and consumption of such chemicals from those new plant sources and relieve the pressure on the currently limited amount of available plant material and risks associated with sole large scale monoculture production<sup>(9)</sup> or the *in vitro* culture of plants.<sup>(10)</sup>

*Pueraria mirifica* Ary Shaw et Suvatubhandu is a Thai indigenous legume herb with a long-term folk medicinal consumption among Thai women for menopausal treatment. The tuberous materials of the plant revealed strong estrogenic effects in the MCF-7 proliferation antiproliferation,<sup>(11)</sup> uterotrophic,<sup>(12,14)</sup> and Yeast Estrogen Screen (YES)<sup>(15)</sup> test assays. Among the phytoestrogen-rich plant materials, the tubers of kudzu (*Pueraria lobata*) are widely used in traditional Chinese, Japanese, and Korean medicines,<sup>(11)</sup> whereas in traditional Thai medicines the related *P. mirifica* is used and has been extensively studied. *P. lobata* has been analyzed for the purpose of the potential development of the plant products or chemicals for the benefit of the food industry,<sup>(11)</sup> whereas *P. mirifica* has been subjected to long-term studies to establish products for menopausal treatment.<sup>(8,10)</sup> At present, these two plants are used as the main botanical ingredients in cosmetic and dietary supplement products.

The estrogenic activity in *P. mirifica* was found to be stronger than that in *P. lobata* in the MCF-7 antiproliferation, uterotrophic, and ovariectomized rat assays.<sup>(10,20)</sup> However, the analysis of *P. mirifica* tubers collected from wild plant populations over a vast area in Thailand in comparison with that of *P. lobata* collected from China revealed that neither daidzin nor genistin, which are the isoflavonoid glycosides derived from daidzein and genistein, respectively, were the major isoflavonoids in these plants, but rather puerarin was.<sup>(20)</sup> Accordingly, it is of interest to find out which isoflavonoids or other phytoestrogens, if any, might be responsible for the beneficial effects observed in these

\*Corresponding author. Email: chuenchit.boonchird@mahidol.ac.th

**Abbreviations:** RP-HPLC, reverse phase high-performance liquid chromatography; *P. mirifica*, *Pueraria mirifica*; YES, yeast estrogen screen; hER, human estrogen receptors; E<sub>2</sub>, 17 $\beta$ -estradiol; RT, room temperature; DMSO, dimethyl sulfoxide; SDA, medium supplemented with adenine; RP, Relative Retention; SNQ2, Sensitivity to 4-Nitro-Quinolone-N-oxide; oNPC, ortho-nitrophenyl- $\beta$ -galactosidase; SEM, standard error mean; LOD, limit of detection; NA, not applicable.

## VITA

Miss Jutarnas Jungsukcharoen was born on June 19, 1984 in Chonburi Province, Thailand. She received the Bachelor Degree of Science (Biotechnology), Faculty of Engineering and Industrial Technology, Silpakorn University in 2005 and the Master degree of Science (Biotechnology), Faculty of Science, Chulalongkorn University in 2007. She had participated for outstanding oral presentation in Pure and Applied Chemistry Conference (PACCON) in 2008. She continued her academic education for Doctor of Philosophy at Program in Biotechnology, Faculty of Science, Chulalongkorn University since 2008. She had poster presented at 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research in Antalya, Turkey (4-9 September 2011). In 2014, She published the article title "Pueraria mirifica leaves, an alternative potential isoflavonoid source" in Bioscience, Biotechnology, and Biochemistry.

