Middle-East Journal of Scientific Research 5 (6): 454-463, 2010
ISSN 1990-9233
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# Total Phenolic Content and RAPD Analysis of Garden Balsam (*Impatiens balsamina* L.) Accessions from Malaysia

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**Abstract:** Garden Balsam (*Impatiens balsamina* L.) a family member of Balsaminaceae is widely used as an ornamental plant in landscaping. Different parts of this plant are used traditionally to treat skin diseases and skin afflictions. There are four forms of *I. balsamina*, namely red, purples, white and pink. The objective of this study is to determine the total phenolic content and to understand the genetic variation in four forms of *I. balsamina*. Ten accessions representing four forms of Garden Balsam were collected from different parts of Melaka State (Malaysia). The total phenolic contents in methanol extracts of stem, leaf and flower tissues is analyzed using HPLC and UV spectrophotometry. Twenty random primers were used for RAPD analysis of the ten accessions. Data from RAPD profile generated by RDP35 primer is used as an input in NTYSYS-pc V.2.0 to draw a dendrogram for the ten accessions. Percentage of polymorphic loci was calculated based on observed degree of DNA polymorphism as revealed in RAPD and statistical genetic diversity was calculated using Shannon diversity index (*H*). The phytochemical analysis results show that purple color flower tissues contains maxim (8 mg /g GAE) total phenolic content in average in comparison to red, pink and white color flowers tissues. The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity in methanol extracts of flower, leaf and stem tissues of ten accessions was determined. The DPPH activity was maximum (62.32 %) as average in leaf tissues. The RAPD analysis results show that there is reasonable genetic diversity between and among red, pink, purple and white forms of *I. balsamina*. The results reported here could be useful as a base for herbal product development and in breeding strategy of *I. balsamina*.

**Key words:** Antioxidants · Markers · Ornamental plants · Phenolic compounds · RAPD

## INTRODUCTION

Garden Balsam (*Impatiens balsamina* L.) called as 'Rose Balsam' is an annual plant native to Asia. This plant can grow up to 2.5 feet in height and it has been used in the landscape since Victorian times. This plant is easy to grow and due to attractive flowers widely used as an ornamental plant in landscaping. Different parts of the plant are used to treat different diseases and skin afflictions. Juice from balsam leaves is used in treatment of warts and snakebite, while the flower extracts can be applied to burns to cool the skin [1].

Traditionally, *I. balsamina* has been used as indigenous medicine in Asian countries in the treatment of rheumatism, fractures and fingernail inflammation [2, 3]. The results published by Oku and Ishiguro support the use of *I. balsamina* to treat articular rheumatism, pain and swelling [4]. Extracts of the *I. balsamina* are also known to have anti *Helicobacter pylori* activity and hence this plant could be used as a potential candidate to eradicate *H. pylori* [2, 3]. Infection with *H. pylori* is strongly associated with gastric cancer and gastric adenocarcinoma. The seeds and the dried aerial parts of Garden Balsam do contain antimicrobial compounds [5, 6].

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The petals and other organ tissues of *I. balsamina* contain phenolic compounds [7]. It is also reported that the extracts of its flower petals contain enzymes which catalyze the glycosylation of phenolic (polyphenolic) compounds [8]. Polyphenols are especially important due to their free radical scavenging activities and there are several herbal cosmetic, pharmaceutical, supplementary and herbal tea products in the market which exploits these properties of the polyphenols from different plants [9].

Phenolic compounds include a wide range of substances that possess antioxidant properties and herbal extracts from natural resources are in a great demand. Herbal extracts containing high amount of polyphenols are widely used as supplements, food and cosmetics preservatives as well as the additive ingredients in food and cosmetics to enhance the functional food value [10]. Among the natural phenolic compounds, the flavonoids and their relatives form the largest group, but phenolic quinones, lignans, xanthones, depsidones and other groups exist in considerable numbers.

Anthocyanin production, flavonols, chemical aspects of the flower color inheritance and inheritance of flower colors in *I. balsamina* is reported by other researchers [11-14]. However, to date there is no reports about the total amount of phenolic contents and their comparison in red, purple, pink and white flower producing cultivars of *I. balsamina*. In addition, the information on genetic variation among four forms of *I. balsamina* is not available. RAPD marker is an economical, simple and yet a useful technique to study the genetic variation. This technique is used in number of studies to study genetic diversity [15-17]. Hence, RAPD analysis of four forms of *I. balsamina* could shade light on their genetic variation and possibly could help in correlating chemical profiles of the four forms in addition to other applications of the DNA fingerprinting [15-17]. Therefore to correlate the total phenolic content and to determine the genetic variation in four forms of *I. balsamina*, the objectives of this study were to determine and compare the total amount of phenolic contents in red, pink, purple and white flower producing four forms of *I. balsamina*; and to determine the genetic variation in their genomes by using RAPD technique. The phytochemical analysis for total phenolic content, the scavenging of 2,2-diphenyl- 1-picrylhydrazyl (DPPH) activity in % and Gallic acid content in stem leaf and flower tissues from four forms of *I. balsamina* is reported in this paper in addition to the RAPD analysis of ten accessions representing four forms of *I. balsamina*.

## MATERIALS AND METHODS

**Plant Materials and Chemicals:** Ten accessions of *I. balsamina* representing four different forms (varieties) were collected from Melaka State of Malaysia (Table 1). The fresh leaves were collected from all accessions to be used in RAPD analysis. For phytochemical analysis, leaves, stems and flowers were cut into small pieces and dried in oven at 50°C. Fine powder was made from samples using mortar and pestle for effective chemical extraction. Sample tissue powder was stored separately into air tight containers.

Gallic acid (authentic sample), phenol reagent, DPPH reagent, HPLC grade methanol, methanol and ethanol were purchased from Acros Organics-USA, Kanto Chemical Co-Japan, Sigma-Germany, J.T. Baker-USA, Scharlau-Spain and Fluka Analytical-Ukraine respectively. Reagents used in HPLC analysis were diluted into HPLC grade methanol and filtered through ultra membrane filter (Fisher Scientific) before HPLC analysis. All mobile phase solvents were degassed before their use in HPLC.

Table 1: *Impatiens balsamina* accessions code, flower color and location from where accession were collected in this study

| Accession | Voucher ID# | Collected from | Collected in Month | Flower Color |
|---|---|---|---|---|
| PNK1 | P001 | Ayer Keroh | June 2009 | Pink |
| PW1 | P001 | Ayer Keroh | June 2009 | Pink |
| PPL1 | L001 | Ayer Keroh | June 2009 | Purple |
| PPL2 | L001 | Ayer Keroh | June 2009 | Purple |
| DR1 | R001 | Ayer Keroh | June 2009 | Red |
| LR2 | R001 | Ayer Keroh | June 2009 | Red |
| RR3 | R001 | Ayer Keroh | June 2009 | Red |
| UK1 | W001 | Ayer Keroh | June 2009 | White |
| WP1 | W001 | Malim | June 2009 | White |
| WP2 | W001 | Ayer Keroh | June 2009 | White |

#Reference voucher code for respective accession in our herbarium collection

**Preparation of Plant Extracts:** Samples were prepared by mixing 1 g powder of sample with 50 ml methanol and by shacking it for 24 h constantly. The extracts were filtered by using Whatman filter paper. Methanol was separated by using rotary evaporator and extracts were re-dissolved in the corresponding solvent at a concentration of 50 mg/ml and analyzed for total phenolic content.

**Determination of Total Phenolic Content:** Total phenols content in the methanolic extract of *I. balsamina* leaf, stem and flower samples were estimated by a colorimetric assay as described by Moreira *et al* [18] with some minor modifications. The reaction of 1 ml methanolic extract solution mixed with 0.5 ml of the of 10 % $Na_2CO_3$ was kept in the dark at room temperature for 1h and the absorbance was read at 725nm by using UV spectrophotometer. Authentic Gallic acid was used for constructing the standard curve. The total phenols content in samples was expressed as mg of Gallic acid equivalents/g of extract (GAEs).

**Scavenging of DPPH Radicals:** The scavenging of DPPH radical was assayed following the method of Hatano *et al.* [19]. Solutions with different extract concentrations were prepared. One ml of extract was dissolved in DPPH solution. The mixture was shaken vigorously and left to stand for 15 min in the dark at room temperature. The reduction of the DPPH-radical was measured by continuously monitoring the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as a percentage of DPPH discolorations using following equation:

$$\%\ \text{scavenging effect} = \frac{(\text{Abs blk - Abs spl})}{\text{Abs blk}} \times 100\%$$

**Where:**

Abs blk is the absorbance of the DPPH solution and Abs spl is the absorbance of solution when the sample extract has been added at a particular level.

**Preparation of Samples for HPLC Analysis:** The phenolic acid was extracted using method described by Shalini and Srivastava [20] and crude extracts of *I. balsamina* stem, flower and leaf powder samples were prepared. One gram of each sample powder was suspended in 5 ml methanol-water (80:20; v/v). The samples were subjected to ultrasonification for 15 min at 4°C followed by centrifugation at 12,500 x g for 15 min. The residue was reextracted twice with the same extracting solution and the supernatant was pooled together prior to evaporation under vacuum. Dried extracts were re-suspended in 1.0 ml high performance liquid chromatography (HPLC)-grade methanol by vortexing and filtered through ultra membrane filter before HPLC analysis.

**HPLC Analysis:** Quantitative analysis of phenolic acid in the samples was performed according to the method explained by Shalini and Srivastava [20]. The peak area was calculated with reference to Gallic acid standard. A column, MetaChem Polaris TM Amide C18, 5μm, 4.6 x 250 mm at 30°C was used in this analysis. HPLC running conditions include: injection volume, 10μl; mobile phase, methanol: 0.4% acetic acid (80:20 v/v); flow rate, 1 ml/min; and detection at 280 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 μm; E-Merck, Darmstadt, Germany) prior to injection in the sample port. Gallic acid was used as internal and external standards. Phenolic acid present in each sample was determined by comparing chromatographic peaks with the retention time ($R_t$) of standard and co-injection of prepared samples. The amount of phenolic acid is expressed as milligram per gram (mg /g GAE) of fresh weight unless otherwise stated.

**Genomic DNA Extraction:** Genomic DNA was extracted from fresh leaves of accessions using a method described by Sambrook *et al.* [21] with some minor modifications. Young leaves were washed with plenty of tap water and then with 70% ethanol for 5 min and with autoclaved deionized water for 2 min to avoid surface contamination. The samples were ground with LN and 2 g of leaves powder were added to 15 ml extraction buffer [0.1 M Tris HCl, 0.05 M EDTA, 0.5 M NaCl, 1% PVP, 1.4% SDS and 10 mM 2-mercaptoethanol]. The suspension was incubated in a water bath at 37°C for 1 h. DNA pellets were dissolved in TES. RNase (10 mg/ml) was added and incubated for 1 h at 37°C. The mixture was extracted by phenol/chloroform/isoamyl-alcohol twice, followed by precipitation with isopropanol and sodium acetate. The pellet was washed with 70 % ethanol twice. Then, the vacuum dried DNA pellet was dissolved in 1X TE buffer.

**RAPD Analysis:** RAPD-PCR was performed in a total volume of 25 μl mix containing 25 ng DNA, 1X PCR buffer, 4 mM $MgCl_2$, 0.4 mM dNTP mix, 2.5 U of *Taq* polymerase (Promega) and 0.6 μM primer (Proligo) [15, 16]. The DNA amplification was carried out in a thermal cycler (MJ Research). PCR cycles were as follows; 40 cycles of 95°C

for 30 sec, 38°C for 1 min and 74°C for 1 min. A final step of extension was carried out at 72°C for 10 min. Amplified products were separated on 1 % agarose-gel in 1X TAE buffer. The gels were stained with ethidium bromide and photographed under UV using Gel Documentation System. DNA ladder with 1kb (Promega) was used.

**Data Analysis:** Genetic diversity was estimated by the Shannon index [22]. To calculate it following formula was used.

$$H = \sum_{i=1}^{k} -(p_i * \ln p_i)$$

**Where:**

$k$ is the number of bands produced with the respective primer and $P_i$ is the frequency of the $i$th fragment.

The RAPD profile generated by RDP35 primer was used. The presence of a band was scored as one (1) and absence of respective DNA band as zero (0) in a band-pairwise distance (similarity matrices). Data was computed based on Jaccard's coefficient of similarity, using 'Numerical Taxonomy System of Multivariate Program' (NTSYS-pc). Dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using NTYSYS-pc V 2.0 [23].

## RESULTS AND DISCUSSION

Table 2: Total phenolic contents (phenolic acids) in methanol extracts of *I. balsamina* accessions

| | Total Phenolic Content (mg/g GAE) | | |
|---|---|---|---|
| Accession | Flower | Leaves | Stem |
| PNK1 | 4.19±0.12 | 0.89±0.08 | 0.16±0.04 |
| PW1 | 8.29±0.05 | 1.71±0.18 | 0.40±0.05 |
| PPL1 | 9.58±0.12 | 1.17±0.04 | 0.29±0.04 |
| PPL2 | 6.41±0.09 | 2.75±0.05 | 0.23±0.13 |
| DR1 | 4.80±0.31 | 4.58±0.14 | 0.70±0.01 |
| LR2 | 6.04±0.05 | 0.89±0.24 | 2.75±0.02 |
| RR3 | 6.84±0.11 | 1.26±0.02 | 12.20±0.03 |
| UK1 | 4.95±0.06 | 6.79±0.02 | 0.65±0.12 |
| WP1 | 4.20±0.02 | 1.12±0.13 | 4.76±0.03 |
| WP2 | 4.23±0.03 | 1.08±0.02 | 4.86±0.08 |

Table 3: DPPH scavenging effect activity in methanol extracts of *I. balsamina* accessions

| | DPPH Activity (%) | | |
|---|---|---|---|
| Accession | Flower | Leaves | Stem |
| PNK1 | 85.81±0.22 | 73.87±0.02 | 18.10±0.01 |
| PW1 | 16.17±0.01 | 74.31±0.01 | 28.19±0.01 |
| PPL1 | 44.97±0.04 | 60.26±0.04 | 21.45±0.82 |
| PPL2 | 77.47±0.03 | 25.85±0.44 | 11.96±0.01 |
| DR1 | 91.28±0.01 | 81.98±0.01 | 69.07±0.01 |
| LR2 | 32.41±0.04 | 76.33±0.04 | 8.54±0.01 |
| RR3 | 13.87±0.01 | 63.00±0.04 | 10.91±0.14 |
| UK1 | 91.66±0.01 | 80.61±0.02 | 69.07±0.01 |
| WP1 | 8.53±0.06 | 43.48±0.02 | 79.00±0.01 |
| WP2 | 8.53±0.04 | 43.49±0.02 | 79.00±0.01 |

**Total Phenolic Content and DPPH Scavenging Activity:** Results showed that in average *I. balsamina* flowers tissue contains more phenolic acids in comparison to its steam and leaf tissues (Table 2). The average total phenolic content in flowers, leaves and stem tissues was 5.95, 2.22 and 2.70 mg/g GAE respectively. However, among the four forms of *I. balsamina*, purple color flower tissues contains maximum (8 mg/g GAE) total phenolic content in comparison to red, pink and white color flowers. The DPPH activity % range was in between 8.53 to 91.66 %. The results (DPPH activity %) are shown in Table 3. Based on the DPPH activity in methanol extracts of flower, leaf and stem tissues of ten accessions the DPPH activity was maximum (62.32 %) in average in leaf tissues. The DPPH activity % in average in flower and stem tissue was 47.07 and 39.53 % respectively.

The fluctuation in total phenolic content and DPPH activity % can be attributed to the physiological state of the plant, respective tissues and or the variety (forms). The antioxidant activity of phenolic compound is due to the reactivity of phenol moiety (hydroxyl group on aromatic ring) [24]. They have the ability to scavenge free radicals via hydrogen donation or electron donation. Quantification of polyphenols was done using Folin-Ciocalteu (FC) reagent. In this method, phenols form the blue colored phosphomolybdic–phosphotungstic–phenol complex in alkaline solution [25, 26].

Different plant materials contain different phenolic compounds in different forms (24, 27]. Phenolic compounds occur in plants mainly as aglycones, glycosides or esters, or are bound to the cell wall. In this study, methanol was used for extraction for the best separation of Gallic acid [28, 29]. The scavenging activity on DPPH radicals has been widely used to determine the free radical-scavenging activity of different matrices [30]. DPPH is a stable free radical that is dissolved in methanol and its purple color shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the color from the DPPH assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation [18]. The *I. balsamina* leaves tissue extracts shows high DPPH activity % in average and it reflects the potential

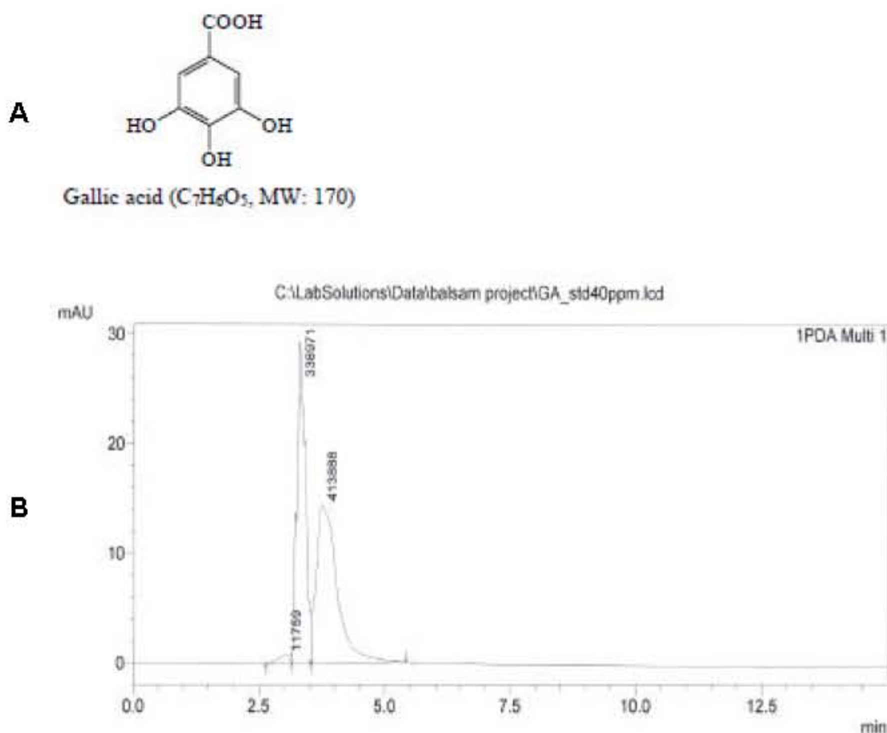


Fig. 1: A), Gallic acids structure. B), HPLC chromatogram of the Gallic acid standard at 280nm. Peaks area 338971 is of Gallic acid

Table 4: The concentration of Gallic acid in flower, leaf and stem extract samples of *I. balsamina* accessions analyzed by HPLC

| | Gallic Acid Concentration(mg/l) | | |
|---|---|---|---|
| Accession | Flower | Leaves | Stem |
| PNK1 | 15.23±0.05 | 11.65±0.07 | 13.04±0.04 |
| PW1 | 13.10±0.31 | 15.02±0.34 | 13.78±0.36 |
| PPL1 | 11.54±0.66 | 13.06±0.76 | 12.68±0.47 |
| PPL2 | 13.13±0.52 | 12.57±0.05 | 12.83±0.51 |
| DR1 | 11.65±0.52 | 15.87±0.35 | 20.81±0.77 |
| LR2 | 12.08±0.73 | 18.42±0.45 | 13.83±0.24 |
| RR3 | 12.40±0.51 | 16.09±0.21 | 11.90±0.39 |
| UK1 | 34.63±0.42 | 13.78±0.73 | 12.44±0.41 |
| WP1 | 13.46±0.53 | 13.56±0.28 | 13.10±0.32 |
| WP2 | 13.61±0.42 | 13.62±0.97 | 13.20±0.40 |

use of *I. balsamina* leaves in herbal preparations, herbal tea, in food industry and some other applications in biotech industry.

**HPLC Analysis:** A column MetaChem PolarisTM Amide C18, 5µm, 4.6 x 250 mm and mobile phase methanol-acetic acid-water was chosen for Gallic acid analysis by using Shidmazu HPLC. The separation of Gallic acid standard compound in HPLC is depicted in Figure 1. A good separation can be achieved in short separation time of 15 min. The concentration of Gallic acid in flower, leaf and stem tissue extracts of *I. balsamina* accessions is shown in Table 4. The UK1 *I. balsamina* accession showed the maximum (34.63 mg/l) Gallic acid content in its flower tissue. However, in average for ten accessions, the Gallic acid content in flower, leaf and stem was 15.08, 14.36 and 13.76 mg/l respectively. In RR3 *I. balsamina* accession, the leaves, stem and flower tissue contains about 16.09, 11.90 and 12.40 mg/l of Gallic acid respectively (Table 4). The HPLC chromatogram of the leaves, stem and flower tissue extracts for RR3 accession are shown in Figure 2. The column we used to determine the contents of Gallic acid is commonly used for Gallic acid content determination. The similar column was used by other researchers to determine the contents of Gallic acid in green tea products by HPLC Analysis [31].

**RAPD Analysis:** Out of 20 RAPD arbitrary primers, only 3 primers, RDP11, RDP35 and RDP119 were selected based on generated RAPD polymorphism profile. The RAPD-PCR amplification profiles for total genomic DNA from ten accessions using three arbitrary decamer

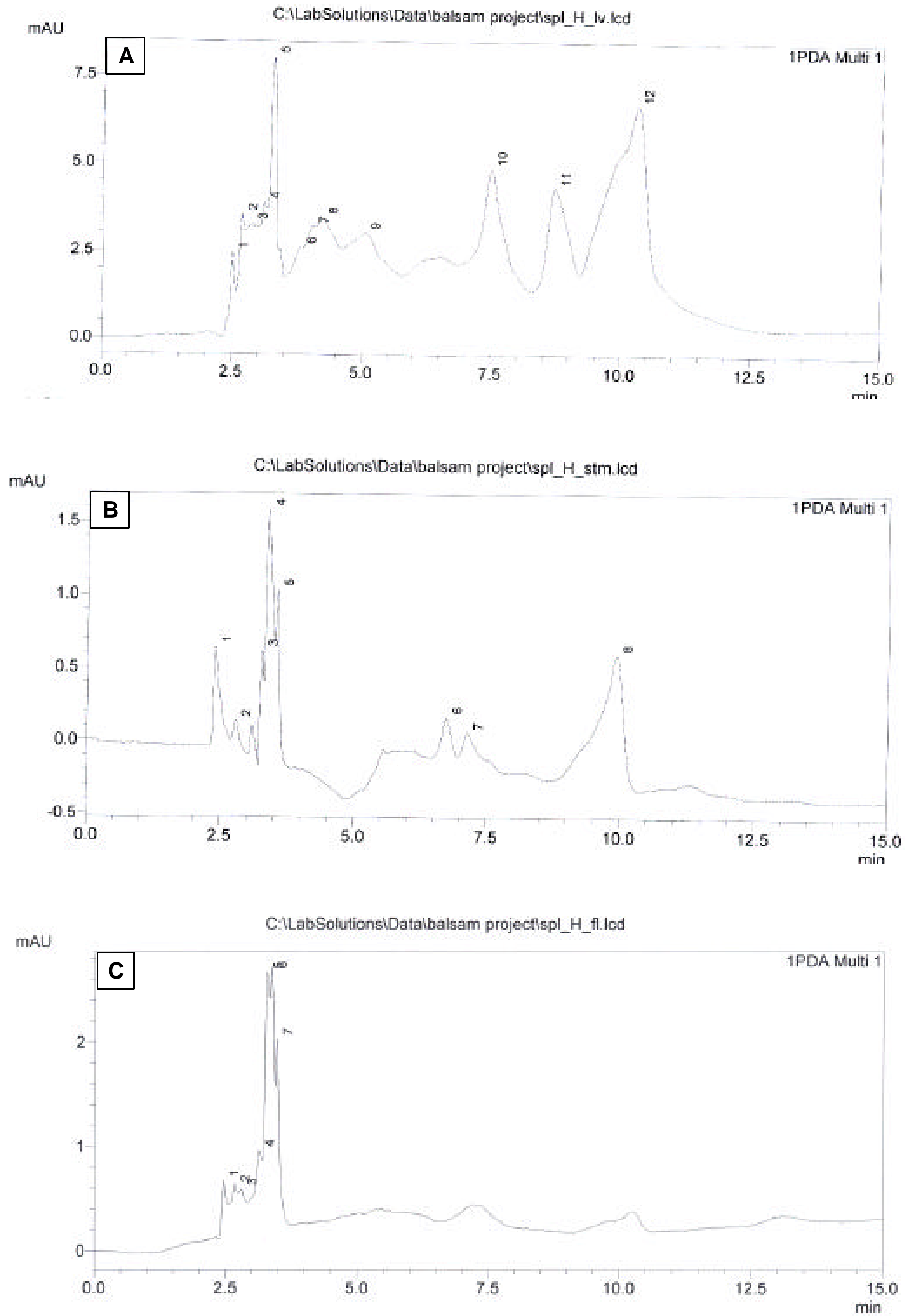


Fig. 2: HPLC chromatogram of extracts from different plant parts of balsam accessions RR3 detected at 280 nm. Peak 5 is of Gallic Acid. A), HPLC chromatogram of leaf extract; B), HPLC chromatogram of stems extract; C), HPLC chromatogram of flower extract

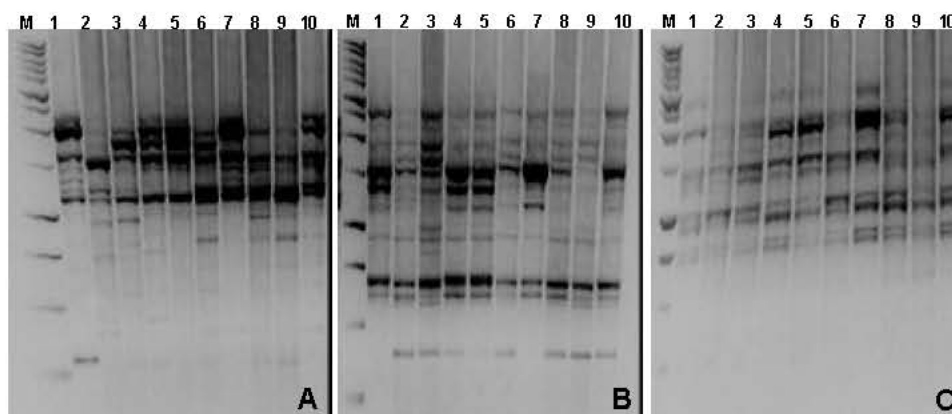


Fig. 3: RAPD profile of ten *Impatiens balsamina* accessions. Lane M, 1 kb DNA ladder; lane 1, 2, 3, 4, 5, 6, 7, 8, 9 and10 represents PNK1, PPL1, DR1, LR2, UK1, WP1, PW1, RR3, PPL2 and WP2 accession respectively. Accession details are depicted in Table 1. A), RAPD bands amplified by primer RDP11; B), RAPD bands amplified by primer RDP35; and C), RAPD bands amplified by primer RDP119

RAPD primers produced 164 discrete bands out of which 64 were polymorphic. The range of number of RAPD bands was from 5-11 per accession and the amplified products size range was from 0.25-3.0 kb (Fig. 3). To observe the genetic diversity and relatedness within and between four forms of *I. balsamina*, RAPD analysis was carried out for 10 accessions using RAPD profile generated by RDP35 primer (Fig. 3B). It was observed that RAPD primer, RDP35 generate robust RAPD banding pattern in comparison to other primers (data not shown for all RAPD primers) and hence considered suitable to study genetic variation in *I. balsamina* accessions.

Both polymorphic and non-polymorphic RAPD bands generated by RDP35 primer were analyzed using NTSYS-pc software version 2.0. It divided 10 accessions into 2 major big clusters (Figure 4 and Table 5). Major Cluster 1 comprised of 5 accessions. Under major cluster 1, there were 2 minor clusters. One minor cluster is composed of 3 accessions (PNK1, PPL2 and DR1) with the average *Jaccard Coefficient* (*J*) value $J = 1.0$. Whereas other minor cluster is composed of 2 accessions (UK1, WP1) with the same (one) average value $J = 1$. The difference in J values for two minor clusters in major cluster 1 is negligible. It indicates a very close genetic relatedness between 2 minor clusters, because the distance value is quite low. Almost identical banding patterns were observed in all accessions in both minor clusters in major cluster 1. It could be interpreted that these accessions share the nearly same genetic information and hence shows quite similar banding patterns.

Table 5: Major cluster groups of *I. balsamina* accessions based on similarity matrix generated by random primer, RDP35

| Major Cluster | Accessions |
|---|---|
| 1 | PNK1, PPL2, DR1, UK1, WP1 |
| 2 | PPL1, LR2, WP2, PW1, RR3 |

Major cluster 2 is comprised of 5 accessions. Under major cluster 2, there were 2 minor clusters. One minor cluster is composed of 3 accessions (PPL1, LR2 and WP2); while second minor cluster contains 2 accessions (PW1, RR3). WP2 accession shows close relation with PPL1 and LR2, but indicates difference at genome level. The similarity matrix obtained using *Jaccard coefficient* is shown in Table 6. The similarity coefficient range is from 0.56 to 1.0 in 10 accessions of *I. balsamina* tested in this study. However, the RAPD analysis based on RAPD band profile generated by RDP35 primer does not clearly differentiate four forms of *I. balsamina* based on Pink, Purple, Red and White flower color.

Based on Shannon Index (*H*), the genetic diversity shown by three primers (RDP11, RDP35 and RDP119) is depicted in Table 7. The high values of *'H'* would be representative of more diverse communities [22]. If the bands are evenly distributed then the *H* value would be high. The *H* value also indicates us to know not only the number of bands but how the abundance of the bands is distributed among all the accessions in the populations [32]. Though, based on total phenolic content in stem, leaf and flower tissues of ten accessions representing four forms of *I. balsamina* and their RAPD analysis does not show strong correlation;

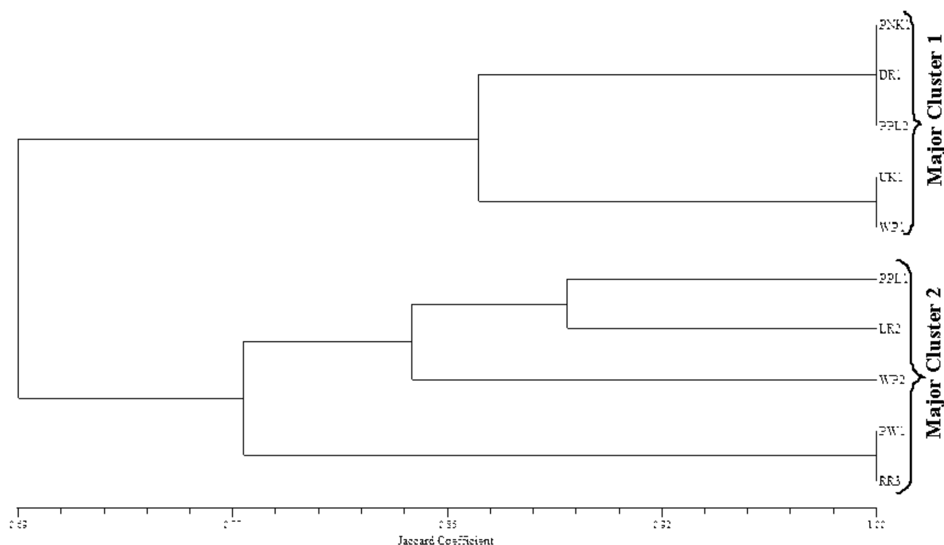


Fig. 4: Dendrogram showing the genetic relationships among 10 accessions of the *I. balsamina* based on RAPD analysis

**Table 6: RAPD-based genetic similarity matrix of ten *I. balsamina* accessions**

| | PNK1 | PPL1 | DR1 | LR2 | UK1 | WP1 | PW1 | RR3 | PPL2 | WP2 |
|---|---|---|---|---|---|---|---|---|---|---|
| PNK1 | 100 | | | | | | | | | |
| PPL1 | 67 | 100 | | | | | | | | |
| DR1 | 100 | 67 | 100 | | | | | | | |
| LR2 | 75 | 89 | 75 | 100 | | | | | | |
| UK1 | 86 | 78 | 86 | 87 | 100 | | | | | |
| WP1 | 86 | 78 | 86 | 87 | 100 | 100 | | | | |
| PW1 | 62 | 78 | 62 | 87 | 75 | 75 | 100 | | | |
| RR3 | 62 | 78 | 62 | 87 | 75 | 75 | 100 | 100 | | |
| PPL2 | 100 | 67 | 100 | 75 | 86 | 86 | 62 | 62 | 100 | |
| WP2 | 56 | 89 | 56 | 78 | 67 | 67 | 67 | 67 | 56 | 100 |

**Table 7: Comparison of genetic diversity and dissimilarity coefficients among 10 *I. balsamina* accessions studied using RAPD**

| Primer | Sequence (5'-3') | DNA bands# | Polymorphic bands# | Shannon Diversity Index (*H*) |
|---|---|---|---|---|
| RDP11 | CAG GCC CTT C | 53 | 19 | 2.38 |
| RDP35 | TGC CGA GCT G | 77 | 26 | 2.74 |
| RDP119 | AGT CAG CCA C | 34 | 19 | 3.31 |

#indicates total number

it does not preclude importance of our results. Use of isoenzyme analysis [33], AFLP [34] and other techniques of DNA fingerprinting remains to be tested to establish relationship between total phenolic content and four forms of Garden Balsam.

## CONCLUSION

The quantitative selective chemo-profiling analysis has showed that different accessions (genomes) of *I. balsamina* have an influence on production of total phenolic and Gallic acid contents in their stem, leaf and flower tissues. Results are indicating that the production of secondary metabolites is also genetic makeup dependant as the environmental conditions were same for all ten accessions. The combination of the selected chemotype, morphological traits and genetic data could be helpful in breeding strategy to develop new *I. balsamina* verities with more desirable health-promoting compounds. Our results could serve as the foundation for the further research work on *I. balsamina*.

## ACKNOWLEDGEMENTS

The authors are grateful to the Melaka State Government, Melaka, Malaysia for research funding [Grant Code: MIB(R&D) Pro (3001-9)]. Authors also do wish to acknowledge trainee (students) from Universiti Industri Selangor (UNISEL) for their assistance in accessions collection.

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