

Research Article

Rose leaves, a Potential Nutraceutical: An Assessment of the Total Anthocyanin Content and Total Phenolic Content

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Abstract

Roses are known as edible flowers and have been used for centuries as food components, either in the fresh form or in processed products, as well as medicinal remedies of various illnesses. Apart from petals, rose leaves are also rich source of anthocyanins and phenolic compounds. These compounds act as powerful antioxidants. However, there are only a few studies concerning the comparison of the phenolic content and anthocyanin content in leaves of Indian rose varieties. In the present study thirteen rose varieties were analysed for their total phenolic content, total anthocyanin content and chlorophyll content. Significant variations were observed in all the parameters among all the varieties. Variety Pusa Ajay recorded highest anthocyanin content (170.36±2.62 mg CGE/100g fresh weight) and total phenolic content (73.38±0.52 mg GAE/100g fresh weight). Lowest anthocyanin content was recorded in leaves of variety Raktagandha (27.22±2.81 mg CGE/100g fresh weight). A strong positive correlation ($r=0.974$) was detected between total anthocyanin content and total phenolic content of rose leaves. The present investigation suggests that rose leaf extracts can be utilised as natural sources for nutraceuticals.

Keywords: Rose, Anthocyanins, Phenolics, Antioxidants, Nutraceuticals

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Introduction

Rose is one of the most important commercial flower crops belongs to Rosaceae family and have been used for centuries as food components, either in the fresh form or in processed products, as well as medicinal remedies of various illnesses. Apart from petals, rose leaves are also rich source of anthocyanins and phenolic compounds. Phenolic compounds have antioxidant, anticarcinogenic, antimutagenic, antibacterial and antiallergic properties. The antioxidant activity of phenolics is mainly attributed to their redox properties. Leaf colour of the rose is attributed to the presence of anthocyanins and chlorophylls. Anthocyanins, a major constituent of natural food colorants, are a group of water-soluble pigments that are comprised of an aglycone anthocyanidin and sugar moieties [1]. They belong to flavonoid family and have gained a growing interest due to their extensive range of colours and beneficial health effects. Anthocyanins have high free radical scavenging capacity and play an essential role in the prevention of cardiovascular disease, obesity, cancer, diabetes and other diseases [2]. The high radical scavenging ability of anthocyanins is mainly due to the phenolic hydroxyl groups in molecule [3]. Recently roses have gained growing attention due to their antioxidant and other beneficial properties [4]. The antioxidant properties of plant extracts connected with the content of anthocyanins and phenols [5]. Plants are potential sources of natural antioxidants, which have attracted more attention. Numerous investigations have been carried out in attempts to find natural antioxidant compounds that may take the place of synthetic ones. Synthetic antioxidants commonly used in the food industry have been reported to cause cancer, as well as toxic effects to the human body [6]. For this reason, there is a need for new natural antioxidants to be obtained from natural sources, which are more effective and less toxic. However, there are only a few studies concerning the estimation of anthocyanin content and phenolic content in rose leaves. Hence, the present study was carried out to evaluate anthocyanin content, phenolic content and chlorophyll content of leaves of different rose varieties.

Materials and Methods

In the present investigation, thirteen rose varieties *viz.* Lalima, Bhim, Nehru Centenary, Pusa Arun, Raktima, Pusa Bahadur, Ashwini, Dr. S.S. Bhatnagar, Raktagandha, Pusa Ajay, Pusa Virangana, Suryakiran and Rose Sherbet were used for the estimation of total anthocyanin content, total phenolic content and total chlorophyll content. Fresh rose leaves were collected from the Research Farm of the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi.

Total anthocyanin content

Total anthocyanin content was determined by the pH-differential method given by Guisti and wrolsted [7]. The extract was diluted both in buffer pH 1.0 (0.025 potassium chloride) and buffer pH 4.5 (0.4 M sodium acetate). After 30 min of incubation at room temperature, absorption was measured at 520 and 700 nm. Results were calculated using molar absorptivity of 26900 L/mol/cm and molecular weight of 449.2 (Da) and expressed as cyanidin 3-glucoside equivalents (CGE) in milligrams per 100 g fresh weight.

Total phenolic content

Total phenols were estimated according to the Folin-Ciocalteu's reagent procedure described by Singleton and Rossi [8]. Diluted extract (0.1 mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted with distilled water 1:4, v/v) and 1.5 mL of sodium carbonate solution (7.5%, w/v) and the final volume was adjusted to 10 mL with distilled water. The mixture was incubated for 30 min at room temperature before absorption was measured at 750 nm. Total phenolic content was expressed as gallic acid equivalents (GAE) in grams per 100 g on a fresh weight.

Chlorophyll content

Chlorophyll content was estimated as per the method described by Arnon [9]. The absorbance of the known volume of solution containing known quantity of leaf tissue at two respective wavelengths (663 and 645) was determined. Fresh leaf samples were added to the test tubes containing 4 ml DMSO. Tubes were kept in dark for 4 h at 65 °C. Then the samples were taken out cooled at room temperature and the absorbance was recorded at 663 and 645 nm using DMSO as blank and was expressed as mg g⁻¹ fresh weight.

Statistical analysis

Results were expressed as means with standard error (SE) from triplicate measurements. Statistical comparisons were performed with Tukey's tests. Differences were considered significant at P≤0.05.

Results and Discussion

Among the rose varieties studied, the total anthocyanin content ranged from 27.22±2.81 to 170.36±2.62 mg/100g fresh weight of leaves. Variety Pusa Ajay showed highest total anthocyanin content (170.36±2.62 mg/100g). However, lowest total anthocyanin content was obtained by Raktagandha, (27.22±2.81 mg/100g) fresh weight of leaves. Notably high amount of anthocyanin pigment was found in rose leaves. The exact role of anthocyanins in leaves is unclear: both photoprotective [10, 11] functions have been attributed to these pigments. It is possible that anthocyanins, which are often located in epidermal vacuoles, serve a similar photoprotective function by absorbing visible light when leaves have not fully developed photosynthetic competence. Beside these roles anthocyanin pigment also act as a source of antioxidants. Stone [12] noted that anthocyanins in young developing leaves of *Iguanura geonomaeformis* and *Pinganga* species appear brown due to the masking effect of chlorophyll. The total phenolic content varied from 7.75±0.99 mg GAE/100g fresh weight to 73.38±0.52 mg GAE/100g fresh weight of leaves among various varieties of rose taken into the study. As results indicated in **Figure 1** showed that variety Pusa Ajay (73.38±0.52mg GAE/100g fresh weight) recorded highest phenolic content followed by Lalima (55.97±1.86 mg GAE/100g fresh weight). Rose Sherbet exhibited lowest phenolic content (7.75±0.99 mg GAE/100g fresh weight). Total phenolic contents of rose leaves were in the same range that has been previously reported by Nowak and Gawlik-Dzik *et al.* [13] for seventeen rose species. Our results are in confirmation with the finding of Bitis *et al.* [14] who reported total phenolic (203.8 mg gallic acid equivalents/g) content of *Rosa sempervirens* leaves. In the present study it was also found that the chlorophylls content depends upon genotypes. Among the rose genotypes studied, the

total chlorophyll content ranged from 0.34 ± 0.01 mg/g to 1.13 ± 0.01 mg/g fresh weight of leaves (**Table 1**). Rose Sherbet showed highest total chlorophyll content (1.13 ± 0.01 mg/g fresh weight). However, lowest total chlorophyll content was obtained in variety Pusa Ajay (0.34 ± 0.01 mg/g fresh weight). Results obtained are in agreement with the findings of Dolatkahi *et al.* [15]. A strong statistically significant positive correlation ($r = 0.974$) between total anthocyanin content and phenolic content of rose leaves was observed (**Table 2**). Chlorophyll a, chlorophyll b and total chlorophyll content in leaves showed a statistically significant negative correlation with total anthocyanin content. It is evident from the present investigation that, as the anthocyanin content of leaves increases, chlorophyll content decreases. Total phenolic content also showed a statistically significant negative correlation with chlorophyll a, chlorophyll b and total chlorophyll content in leaves.

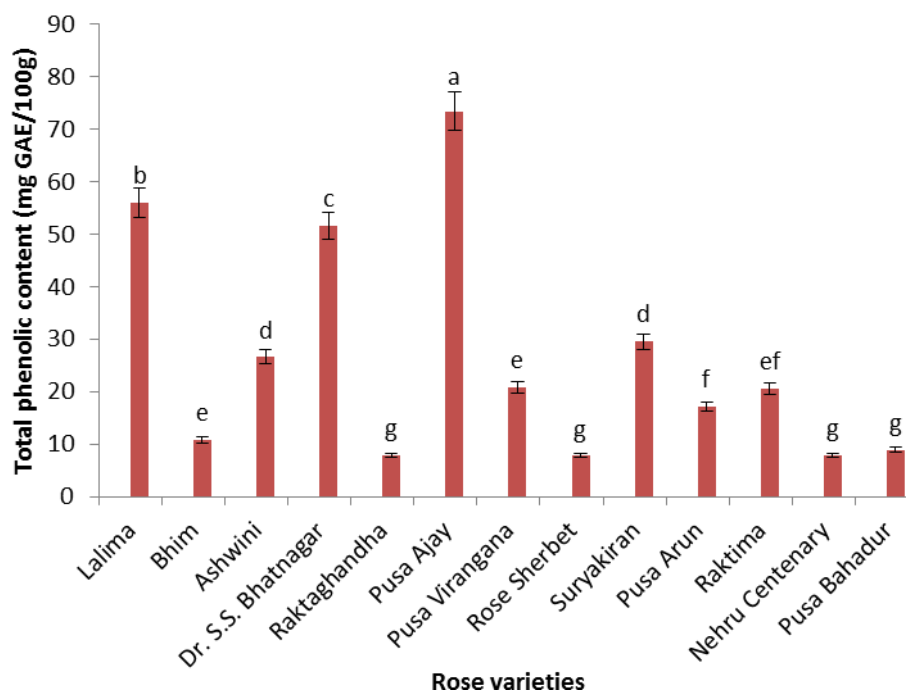


Figure 1 Estimation of total phenolic content (mg GAE/100 g fresh weight) of different varieties of rose leaves. Data represents the mean of three replicates and vertical bars on graph are \pm standard error. Same letters on the bar graph did not differ significantly at 5% level of significance when compared by Tukey's HSD test

Table 1 Anthocyanin content and chlorophyll content of leaves of different rose varieties

Varieties	Total anthocyanin content (mg CGE / 100g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Lalima	131.70 ± 6.07^{bb}	0.32 ± 0.01^g	0.11 ± 0.01^f	0.43 ± 0.01^g
Bhim	30.47 ± 4.33^f	0.85 ± 0.011^b	0.14 ± 0.02^{cde}	0.99 ± 0.01^c
Ashwini	88.68 ± 2.50^c	0.63 ± 0.01^d	0.13 ± 0.01^{ef}	0.75 ± 0.00^e
Dr. S.S. Bhatnagar	121.66 ± 2.08^b	0.32 ± 0.02^g	0.13 ± 0.01^{def}	0.45 ± 0.01^g
Raktagandha	27.22 ± 2.81^f	0.86 ± 0.01^b	0.19 ± 0.02^a	1.05 ± 0.01^b
Pusa Ajay	170.36 ± 2.62^a	0.23 ± 0.02^h	0.11 ± 0.01^f	0.34 ± 0.01^h
Pusa Virangana	72.25 ± 3.49^{de}	0.53 ± 0.03^f	0.16 ± 0.01^{abc}	0.69 ± 0.01^f
Rose Sherbet	29.39 ± 3.60^f	0.98 ± 0.01^a	0.15 ± 0.01^{cde}	1.13 ± 0.01^a
Suryakiran	89.66 ± 9.34^c	0.55 ± 0.02^{ef}	0.16 ± 0.06^{abc}	0.71 ± 0.01^f
Pusa Arun	69.01 ± 2.30^e	0.69 ± 0.03^c	0.18 ± 0.03^{ab}	0.87 ± 0.00^d
Raktima	81.73 ± 2.35^{cd}	0.57 ± 0.01^e	0.18 ± 0.03^{ab}	0.75 ± 0.00^e
Nehru Centenary	28.05 ± 2.14^f	0.86 ± 0.03^b	0.16 ± 0.03^{bcd}	1.02 ± 0.02^b
Pusa Bahadur	31.66 ± 0.46^f	0.86 ± 0.02^b	0.17 ± 0.02^{abc}	1.03 ± 0.01^b
SEm \pm	7.229	.0379	0.005	.041
CD (P \leq 0.05)	11.657	0.030	0.028	0.029
CV	9.289	2.835	11.209	2.206

Data represents the means \pm standard error. Means within a column that did not differ significantly at 5% level of significance when compared by Tukey's HSD test are followed by the same superscript.

Table 2 Linear correlation coefficients (r) between total anthocyanin content (TAC), total phenolic content (TPC), chlorophyll a content (CHL.a), total chlorophyll b content (CHL.b) and total chlorophyll content (T.CHL) in leaves of rose varieties obtained by Pearson's analysis

Parameters	TAC	TPC	CHL.a	CHL.b	T.CHL
TAC	1	0.974**	-0.966**	-0.690**	-0.974**
TPC		1	-0.937**	-0.773**	-0.954**
CHL.a			1	0.614*	0.997**
CHL.b				1	0.677*
T.CHL					1

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

Conclusion

From the present study, it can be concluded that among the thirteen rose varieties studied, leaves of var. Pusa Ajay exhibited higher anthocyanin content and total phenolic content. The present investigation suggests that extracts from rose leaves can be utilised in pharmaceutical and food industry.

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