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Short communication

Characterization of phenolic compounds in petal extracts of rose

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ABSTRACT

The experiment was carried out during 2015-16 to evaluate the phenolic content and its chemical composition in rose petal extracts. Among 13 varieties tested, Ashwini recorded the highest phenolic content (427.59 mg GAE/100g FW) followed by Dr. S.S. Bhatnagar (379.24 mg GAE/100g FW) and Nehru Centenary (342.67 mg GAE/100g FW), whereas Pusa Ajay showed the lowest phenolic content (101.03 mg GAE/100g FW). High performance liquid chromatography (HPLC) analysis identified the presence of five major phenolic compounds in rose petal extract, *viz*. quercetin, catechin, epicatechin, rutin and 3-hydroxy cinnamic acid. From the present study it can be concluded that rose petals are rich in phenols and can be further utilised as natural source of antioxidants. Phenol rich varieties can be selected for processing extracts with health promoting properties or can be incorporated into functional foods with bioactive properties related to oxidative stress.

Key words: *Rosa hybrida*, phenolics, antioxidants, HPLC.

Rosa hybrida L. belonging to family Rosaceae, is commercially important flower crop. Its flowers are also edible 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. Rose petals have been consumed for many years in teas, cakes, and flavour extracts, as well as folk medicine to treat blood circulation disorders, and to control cancer growth (Shafei *et al*., 6). Many investigations have also revealed that roses contain a wide diversity of phenolic compounds such as gallic acid, catechin, epicatechin, kaempferol, rutin, myricetin, and quercetin that not only possess antioxidant activities but also exert antiallergic, anti inflammatory, antiatopic, antibacterial, antiviral, antifungal, antidepressant, and antistress effects (Boskabady *et al*., 1 and Ulusoy *et al*., 9). Phenolic compounds are a class of low molecular weight secondary plant metabolites. These compounds scavenge free radicals which are produced during cell metabolism [reactive oxygen species (ROS) or free radicals such as hydrogen peroxide, hydroxyl radical and singlet oxygen] that can lead to oxidative stress. Oxidative stress is associated with major chronic health problems like cancer, inflammation, neurodegeneration diseases, heart diseases, aging and also food deterioration. The antioxidant activity of phenolics is mainly attributed to their redox properties. Special attention has been paid to plants because they are very rich sources of phenolic compounds. However, there are only a few studies concerning the comparison of the phenolic composition of Indian rose varieties. This study is aimed to evaluate the total phenolic content and its profiling in Indian rose varieties.

In the present investigation, 13 rose varieties, *viz*., Pusa Arun (dark red), Bhim (red), Nehru Centenary (dark red), Raktima (red), Pusa Bahadur (red), Ashwini (dark red), Dr S.S. Bhatnagar (dark red), Raktagandha (red), Pusa Ajay (pink), Pusa Virangana (red), Suryakiran (orange), Surkhab (red+white) and Rose Sherbet (deep pink) were used for the estimation of total phenolic content and its profiling. Fresh rose petals were collected in the morning hours from the Research Farm of the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi.

The total phenols were estimated according to the procedure given by Singleton and Rossi (7). A 0.5 g sample of rose petal was extracted with 20 ml methanol (80%). The aliquot (1 ml) was taken in the test tubes and added with 2.9 ml of Folin and Ciocalteau's Phenol Reagent (1N) and 0.5 ml of distilled water. The test tubes were then shaken well; then, 2 ml of sodium carbonate (20%) solution was added and kept for incubation at room temperature for 30 minutes. The colour developed was measured in spectrophotometer at 750 nm wavelength. Standard curve was drawn using gallic acid as standard. Different concentrations of gallic acid were prepared and O.D was read at 700 nm wavelength. The concentration of samples was calculated based on the standard curve.

Identification and characterization of phenolic compounds from Indian rose varieties was done using HPLC. Five standard stock solutions, catechin (500 µg/

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ml), rutin (500 µg/ml), quercetin (500 µg/ml), epicatechin and 3-hydroxy cinnamic acid (500 µg/ml) were prepared in HPLC grade solvent mixture of acetonitrile/formic acid (1: 1; v/v) and filtered using membrane disc filter (0.45 µm). Solution (5 mg/ml) of all samples was prepared in HPLC grade solvent mixture of acetonitrile/ formic acid (1: 1; v/v) and filtered using membrane disc filter (0.45 µm). HPLC instrument (Alliance, Waters Corp., Milford, Mass., U.S.A.) equipped with e2695 quaternary pump, auto injector (20µL loop), a 2998 photodiode array detector and an "Empower 2" software programme was used for characterization of phenolic compounds. A C-18 column (Thermo, USA) 25 cm × 4.6 mm × 5µ was used for separation of phenolic compounds using a mobile phase comprising of gradient elution of solvent A: water (0.1 % formic acid) and solvent B: acetonitrile (0.1% formic acid) at a flow rate of 0.5 ml/min. The elution profile was 0 min 100 % A, then the solvent B was increased first to 20 % in 20 min, thereafter to 30% in 10 min followed by 50% in 10 more min and finally to 100% for 10 min. The total run time was 50 min. The injected volume was 20 µl. For detection and quantification of phenolics, the photodiode array detector was set at 280 nm.

Fig. 1 depicts the total phenolic content of the extracts that were analysed. Among the 13 rose varieties tested, the total phenolic content varied from 101.03 mg GAE/100g FW to 427.59 mg GAE/100g FW of petals. The variety Ashwini showed highest phenolic content(427.59 mg GAE/100g FW) followed by Dr S.S. Bhatnagar (379.24 mg GAE/100g FW), Nehru Centenary (342.67 mg GAE/100g FW), Bhim (333.20mg GAE/100g FW) and Pusa Arun (306.78 mg GAE/100g FW). Variety Pusa Ajay recorded lowest phenolic content (101.03 mg GAE/100g FW). It was evident from the results that light colour variety showed lesser phenolic content whereas the bright red colour variety showed higher phenolic content in the petals. Our results are in accordance with the findings of Vinokur *et al*. (10) and Qin and Xiaojun (4) in rose. Roman *et al*. (5) also reported that the total phenolic content in petal of *Rosa canina*, varied from 326 mg/100g to 575 mg/100 g. The differences among the rose varieties regarding the phenolics compounds could be due to genetic derivation.

HPLC chromatogram was generated for phenols in all varieties indicating several peaks. Characteristic peaks showed the typical absorbance of phenols. Phenolic identification was conducted by comparing RT (retention time) and elution order of sample with those of standards under the same HPLC conditions. Several peaks were detected in all varieties corresponding to different kinds of phenolic fractions. A total of five different types of phenolic compounds were identified in crude extract of rose petals (Table 1). It was observed that quercetin was present in all the varieties and its content ranged from 23.83 to 6038.78 µg/g. The presence of catechin was observed in variety Nehru Centenary (27.50 µg/g) and Suryakiran (38.73 µg/g). Epicatechin was found among most of the varieties except Nehru Centenary and Pusa Arun and its content ranged from 20.43 to 1980.66 µg/g Rutin was observed in four varieties, *viz*. Raktagandha, Bhim, Raktima and Nehru Centenary and its content ranged from 11.68 µg/g to 94.37 µg/g. 3-hydroxy cinnamic acid was observed only in one variety Pusa Ajay (9.76 µg/g). The identified compounds in this study are in agreement with the findings of Hvattum (2) and Stanila *et al*. (8) in rose. The present results are also in confirmation with the findings of Kumar *et*

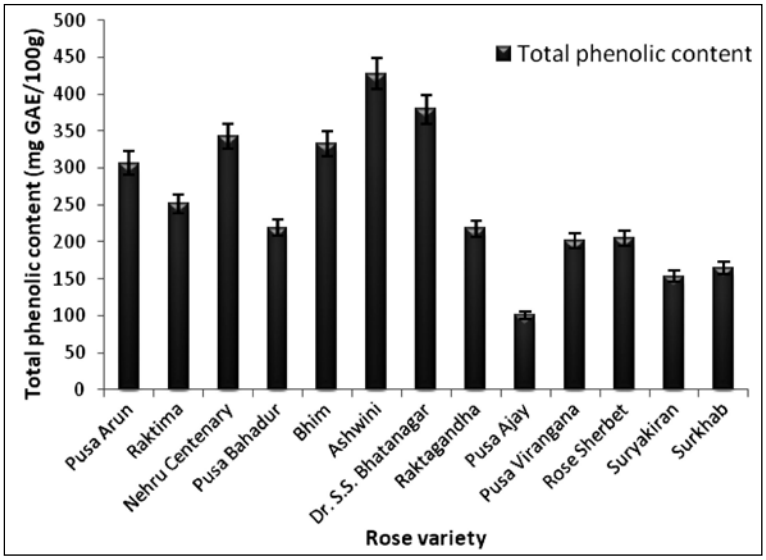

Fig. 1. Total phenolic content of Indian rose varieties.

Table 1. Characterization of phenolic compounds of rose varieties using HPLC.

Variety	RT (min.)	Peak area (%)	Identified compound	Content (µg/g)
Nehru Centenary	24.270	0.39	Catechin	27.50
	30.608	0.94	Rutin	20.30
	41.223	98.67	Quercetin	6038.78
Dr. S. S. Bhatnagar	29.629	27.42	Epicatechin	135.09
	41.268	72.58	Quercetin	327.74
Suryakiran	24.097	24.37	Catechin	38.73
	29.819	58.49	Epicatechin	88.70
	41.420	17.15	Quercetin	23.83
Pusa Bahadur	29.784	21.26	Epicatechin	256.06
	41.398	78.74	Quercetin	869.05
Bhim	29.758	4.95	Epicatechin	20.43
	33.476	10.27	Rutin	13.70
	41.365	84.78	Quercetin	321.02
Surkhab	29.698	16.10	Epicatechin	291.02
	41.312	83.90	Quercetin	1390.03
Pusa Ajay	29.686	77.46	Epicatechin	1980.66
	34.842	4.04	3-hydroxy cinnamic acid	9.76
	41.277	18.50	Quercetin	433.50
Pusa Virangana	29.774	62.31	Epicatechin	370.93
	41.348	37.69	Quercetin	205.69
Rose Sherbet	29.734	17.76	Epicatechin	184.73
	41.352	82.24	Quercetin	784.06
Pusa Arun	41.305	100.00	Quercetin	82.44
Raktagandha	29.673	16.95	Epicatechin	298.83
	30.761	2.05	Rutin	11.68
	41.272	81.00	Quercetin	1308.94
Raktima	30.026	63.85	Epicatechin	791.28
	30.848	23.58	Rutin	94.37
	41.367	12.58	Quercetin	142.87
Ashwini	30.026	86.47	Epicatechin	275.47
	41.405	13.53	Quercetin	39.50

al. (3), who reported gallic acid, catechin, epicatechin, rutin, m-coumaric acid, quercitrin, myricetin, quercetin, apigenin, and kaempferol in fresh flowers of *Rosa bourboniana* and *Rosabrunonii*.

From the present investigation it can be concluded that rose petals are rich in phenols and can be further utilised as natural source of antioxidants. Phenol rich varieties can be selected for processing extracts with health promoting properties or to be incorporated into functional foods with bioactive properties related to oxidative stress.

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