SUPPLEMENTARY MATERIAL

Phytochemical screening, antioxidant activity, total phenolic and total flavonoid contents of seven local varieties of *Rosa indica* L.

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Abstract: *Rosa indica* symbol of godness and beauty known for various healing power, has astringent, sedative, anti-inflammatory and antidepressant qualities. Standard methods were used for Qualitative detection of phyto-compounds and quantitative detection of antioxidants was done using DPPH radical scavenging assay, total phenolics and total flavonoids content were expressed in mg GAE/g dry weight and mg QE/g dry weight. Results revealed phyto-compounds presence in all varieties under study however maximum % inhibition was observed by *Rosa indica* var pink perfume (94±0.6) with IC50 value 0.3376 ± 0.01 mg/ml. Highest phenolic and flavonoid content was observed in the leaves extracts of *Rosa indica* var cardinal red i.e. **3.3553±0.11** (ethanol) mg of Gallic acid equivalents (GAE)/g dry weight and **3.736± 0.001**(ethanol) mg of quercetin equivalents (QE)/g dry weight respectively at conc. 0.125mg/ml. Our finding provides evidence that all varieties of rose contain medicinally important bioactive compounds and justifies their use for treatment of different diseases.

Keywords: Phytochemicals; Antioxidants; Quercetin; Gallic acid.

1. Experimental

1.1 Materials

Leaves of all the selected seven local varieties were collected from Jinnah Garden, Lahore in the months of Feb -May (2016) authenticated and deposited in the Prem Madan Herbarium of Lahore College for Women University, Lahore (*Rosa indica* L. Voucher No: LCWU-15-122, *Rosa indica* var gulab bajaso Voucher No: LCWU-15-107, *Rosa indica* var marandi Voucher No: LCWU-15-115, *Rosa indica* var *pink perfume* Voucher No: LCWU-15-110, *Rosa indica* var indian chief Voucher No: LCWU-15-112, *Rosa indica* var cardinal red Voucher No:LCWU-15-106, *Rosa indica* var yellow album Voucher No: LCWU-15-120, *Rosa indica* var double delight Voucher No:LCWU-15-108).

The leaves extract were dried at room temperature, i.e., 21°C, and were crushed to coarse powder. About 150g of dried powder was extracted successively by double maceration for 3-4 days each methanol and ethanol (Inmaculade et al. **2005**).

1.2 Qualitative Assay for the Detection of Alkaloids, Phenolics, Flavonoids, Terpenoids and Tannins:

Phytochemical screening was performed for alkaloids (Salehi-Surmaghi et al. 1992), phenolic acids (Harbor **1973**), Flavonoids (Harbor **1973**), terpenoids (Indumathi et al. 2014) and tannins (Harbor **1973**). The color intensity or the precipitate formation was used as analytical responses to these tests.

1.2.1 DPPH free radical scavenging activity:

Antioxidant assay was performed using methanol as a solvent of selected plant material at four different concentrations (0.125, 0.25, 0.5 and 1mg/mg/mL). All the fractions showed significant antioxidant activity at all the concentrations. 50µL of plant extracts were added in methanol solution of DPPH (5ml of 0.05mM) at six different concentrations (0.125, 0.25, 0.5 and 1mg/mL). All the fractions were incubated for 30

min at room temperature and then absorbance was measured at 517nm using blank (containing all reagents except the test compound). The color of all the tested fractions became changed from deep-violet to light yellow that indicated the presence of antioxidants in all the tested fractions. Ascorbic acid was used as positive control and its values were compared with the decrease in absorbance in the tested fractions. The IC50 represents the concentration of fractions that means 50% inhibition was determined for each fraction (Lee et al. **1998**).

1.2.3 Determination of total phenolic content

The total phenolic content of the extracts was determined using Folin-Ciocalteu (FC) reagent. The blue complex (phosphotungstate-phosphomolybdate) was formed by the reduction of reagent by phenolics of extract in alkaline conditions. The test sample (20 µL) was mixed with 158 µL of de-ionized water and 100 µL of FC reagent. The mixture was left for 10 minutes at room temperature. After 10 min, 300 µL of 25% (w/v) sodium carbonate solution was added to the mixture, and then the mixture was incubated at 40^{o}C. After cooling for half an hour, absorbance was measured at 765 nm against methanol and ethanol used as blank. Gallic acid was used as a standard to construct calibration curve, and TPC was determined from this curve (Cliffe et al. **1994**).

1.2.4 *Determination of total flavonoid content (TFC)*

The total flavonoid content was estimated by the modified protocol of (Dewanto et al. **2002**).

2. Statistical Analysis

All the *in vitro* experimental data were presented as mean ± Standard error of three parallel measurements and data was evaluated by SPSS software, Microsoft Excel and Graphpad prism Software 5.04 (Levesque, **2007**).

Table S1 Phytochemical screening of seven local varieties of *Rosa indica* L.

Plant species	Results				
	Terpenoids	**Alkaloids**	**Flavonoids**	**Phenolics**	**Tannins**
Rosa indica	+	+	+	+	+
Rosa indica var pink perfume	+	+	+	+	+
Rosa indica var marandi	+	+	++	++	+
Rosa indica var indian chief	+	+	+	+	+
Rosa indica var bajaso	+	+	+	+	+
Rosa indica var cardinal red	+	+	+	+	+
Rosa indica var double delight	+	+	+	+	+
Rosa indica var yellow album	+	+	+	+	+

+ Presence, ++ Maximum Presence, - Absence

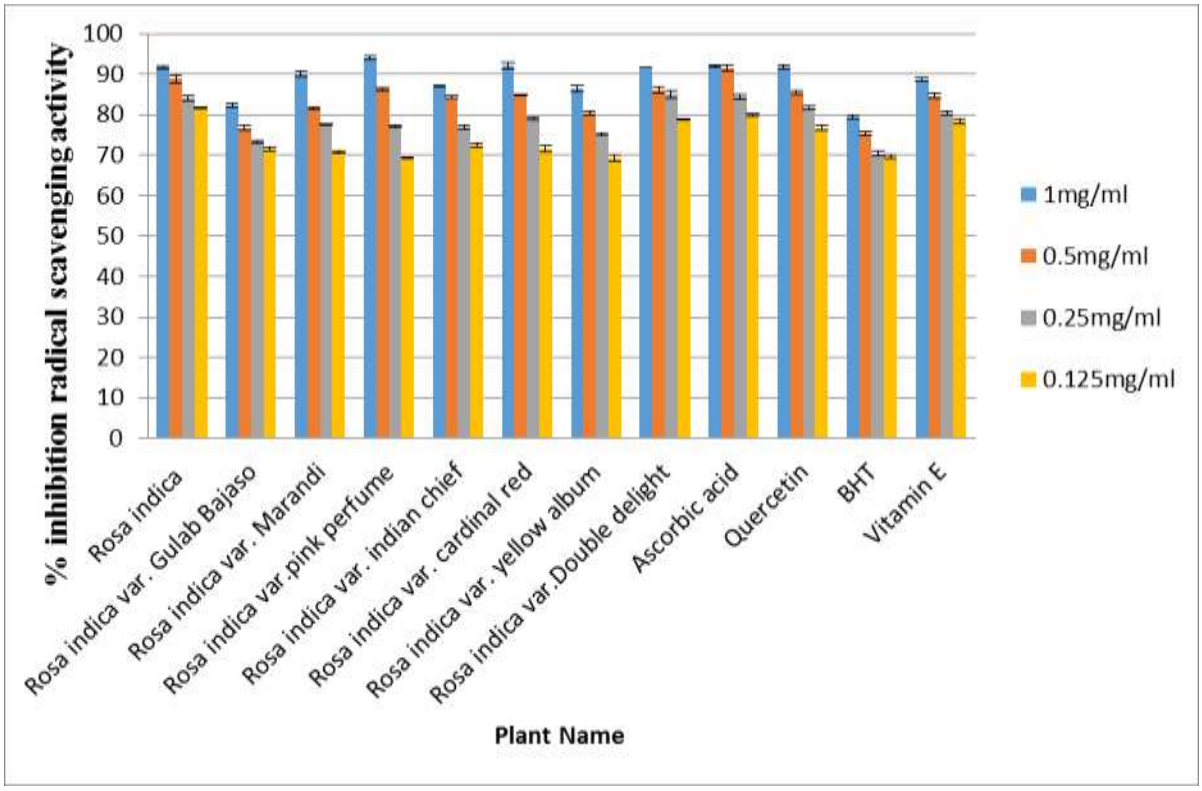


Figure S1 Anti-radical scavenging activity of 7 local varieties of *Rosa indica* L.

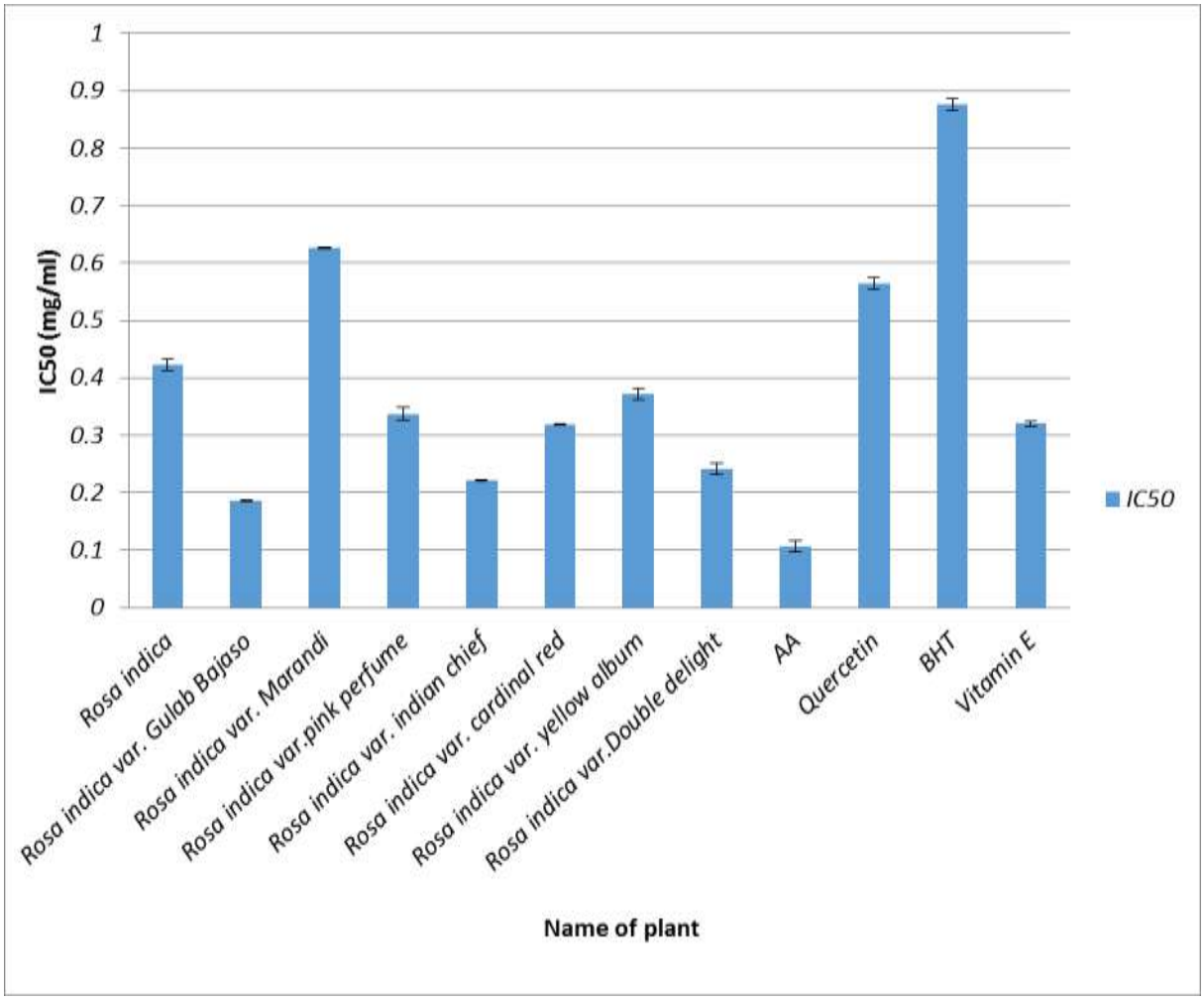


Figure S2 Half maximal inhibitory concentration (IC50) in 7 local varieties of *Rosa indica* L.

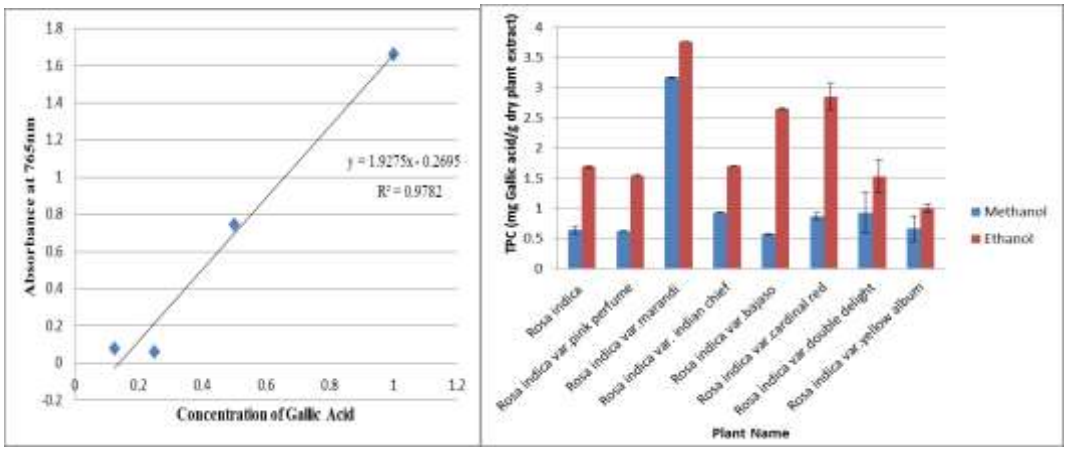
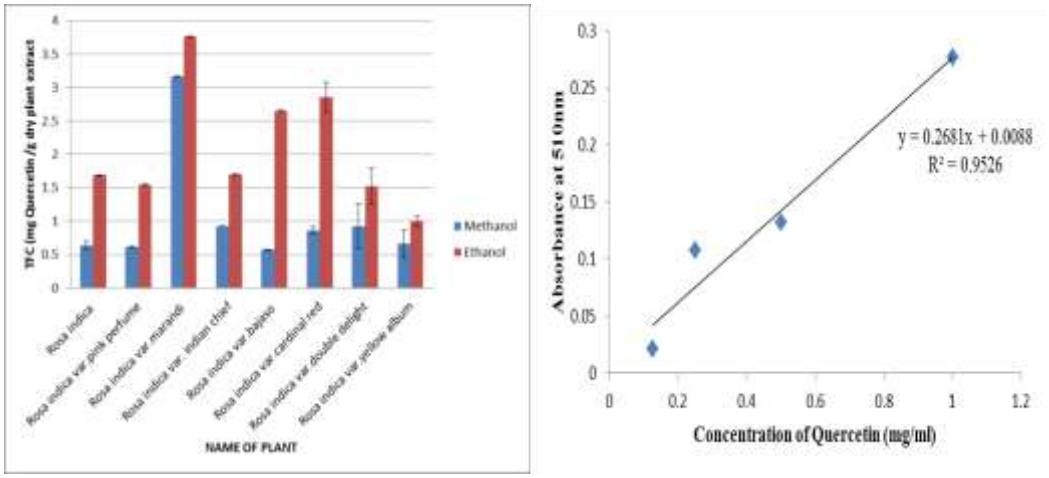


Figure S3 (a): Caliberation curve for Gallic Acid

Figure S3 (b): Total phenolic content (mg Gallic acid/g dry plant extract)



a) b)

Figure S4 (a) TFC (mg Quercetin/ g dry plant extract)

Figure S4 (b) Caliberation Curve of Quercetin (mg/ml)

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