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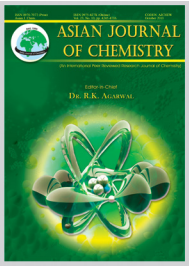

Comparative Evaluation of Reducing Potential of Flowers from Two *Catharanthus roseus* Subspecies

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Catharanthus roseus L. (*Vinca rosea*) flowers have wound healing, antiasthmatic properties and flower extract had also been used for eyewash in infants. The aim of the present study is to demonstrate antioxidant potential of the flower extracts of *Catharanthus roseus* subspecies. The flowers of two *Catharanthus roseus* subspecies (pink and white) were extracted, by soaking the fresh chopped material in *n*-hexane, chloroform, methanol and ethanol. All the extracts were evaluated for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, ferric reducing ability of plasma assays. Total phenolics and flavonoids were also estimated for all the extracts. The reducing potential of the extracts ranged from 91.96 ± 1.26 to 20.53 ± 1.04 % in DPPH assay while 0.307 ± 0.05 to 0.572 ± 0.01 mmoles in ferric reducing ability of plasma assay phenolics and flavonoids estimation show that methanol and ethanol extracts contain large amount of phenolics and flavonoids. A linear relationship was found in between antioxidant potential and phenolic content of the extracts of white and pink flowers (R^2 = 0.902) and (R^2 = 0.819) respectively.

Key Words: *Catharanthus roseus* L., Antioxidant activities, Ferric reducing ability, 1,1-Diphenyl-2-picrylhydrazyl.

INTRODUCTION

Natural products are the ultimate source of synthetic and traditional herbal medicines and are still the primary health care system in this modern age[1]. Plants produce a diverse array of secondary metabolites many of which have potential to cure various diseases. The presence of these life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain acute as well as management of chronic diseases. Antioxidant agents are closely associated to the prevention of degenerative diseases, such as cardiovascular and neurological illnesses, oxidative stress malfunctions and cancer[2].

Catharanthus roseus L. (Family: Apocynaceae), is distributed mainly in tropical regions. So the plant is easily growing and commonly available in the sub-continent with other common names like periwinkle, madagascar periwinkle, sadabahar, and baranmassa. It is also cultivated as an ornamental plant due to its attractive flowers. For the treatment of various ailments like cancer[3], diabetic mellitus, Hodgkin's and non- Hodgkin's diseases, hypertension, nose bleeding, sore throat and mouth ulcers, extracts of *C. roseus* had been used for many decades[4]. Antiviral, antibacterial and antifungal activities of this plant are also reported[5]. Mainly, the flowers have wound healing, antiasthmatic properties and flower extract had also been used for eyewash in infants. The alkaloids like vincristine and vinblastine from *C. roseus* are famous for their anticancer activity[6]. Furthermore wound healing action of the extracts of this plant has also been reported in rats[7]. It is also known to possess antidiabetic action *via* peroxisome proliferator-activated receptors[8]. Previously, there is no study reported regarding comparison of antioxidant properties and phenolic contents of the white and pink flower extract of *C. roseus*. To our best of knowledge, this is the first report on the comparative antiradical activity, reducing properties, total phenols and flavonoids of solvent extracts of pink and white flowers of *Cantharanthus roseus*.

EXPERIMENTAL

Folin-ciocalteu reagent, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxy toluene (BHT), Gallic acid, Quercetin, were purchased from Sigma (St. Louis, MO, USA).

Plant material: The flowers of *Catharanthus roseus* pink and white subspecies were collected in the month of October 2010 from Botanical Garden of Government College University, Lahore.

TABLE-1
% INHIBITION OF DPPH, FRAP, TOTAL PHENOLICS AND FLAVONOIDS OF THE EXTRACTS OF WHITE AND PINK FLOWER EXTRACTS

Flower	Extract	DPPH		FRAP	PHENOLICS	FLAVONOIDS
		Inhibition (%)	IC_{50}	µM equivalent to $FeSO_4.7H_2O$/g of sample	mg GAE/g of sample	mg equivalent of quercetin/g of sample
White	CrWM	91.96 ± 1.26	153.53	$0.572 \times 10^6 \pm 0.01$	178 ± 0.02	22 ± 0.12
	CrWE	88.85 ± 1.45	109.60	$0.521 \times 10^6 \pm 0.02$	142 ± 0.50	10 ± 0.35
	CrWC	26.45 ± 1.05	-	$0.38 \times 10^6 \pm 0.013$	68 ± 0.013	19 ± 0.013
	CrWH	20.53 ± 1.04	-	$0.319 \times 10^6 \pm 0.02$	83 ± 0.65	13 ± 0.55
Pink	CrPM	90.098 ± 1.05	107.37	$0.450 \times 10^6 \pm 0.02$	98 ± 0.15	20 ± 1.15
	CrPE	81.76 ± 1.17	126.93	$0.430 \times 10^6 \pm 0.012$	110 ± 0.016	15 ± 0.16
	CrPC	24.13 ± 1.01	-	$0.347 \times 10^6 \pm 0.015$	62 ± 0.02	18 ± 0.20
	CrPH	19.65 ± 1.72	-	$0.307 \times 10^6 \pm 0.05$	75 ± 0.056	14 ± 0.56
Standard	BHT	95.12 ± 1.15	-	-	-	-

CrWM = Crude extract of white flower in methanol; CrWE = Crude extract of white flower in ethanol; CrWC = Crude extract of white flower in chloroform; CrWH = Crude extract of white flower in *n*-hexane; CrPM = Crude extract of pink flower in methanol; CrPE = Crude extract of pink flower in ethanol; CrPC = Crude extract of pink flower in chloroform; CrPH = Crude extract of pink flower in *n*-hexane; BHT= Butylated hydroxyl toluene

Preparation of extracts: The solvent extraction of the flowers of two *Catharanthus roseus* subspecies (pink and white) was carried out by soaking the fresh and chopped material of both varieties in *n*-hexane, chloroform, methanol and ethanol for 24 h with continuous shaking. The four extracts of *C. roseus* white flowers in *n*-hexane chloroform, methanol and ethanol were CrWH, CrWC CrWM and CrWE, while in the same sequence, extracts of *C. roseus* pink flowers were labeled as CrPH, CrPC CrPM and CrPE.

DPPH Free radical scavenging assay: Antiradical activity of *n*-hexane, chloroform, methanol and ethanol extracts was determined by mixing 250 µL of the extracts with 2 mL of methanolic solution of DPPH (10 mg/L)[9]. The mixture was shaken vigorously and incubated at room temperature for 0.5 h in dark. The absorbance was recorded at 517 nm against methanol and DPPH as blank. The total decrease in absorbance indicated the antioxidant activity. The antioxidant activity was calculated as inhibition of DPPH and determined by the formula.

$$\% \text{ age inhibition of DPPH} = \frac{(A - B)}{A} \times 100$$

where, A is the absorbance of blank and B is the absorbance of sample.

Butylated hydroxyl toluene (BHT), a standard antioxidant was tested against DPPH and used as a reference. To calculate the IC_{50} value, percentage inhibition of DPPH was plotted against concentration of the extracts in the range of 50-250 µL.

Ferric reducing ability of plasma assay: Ferric reducing ability of plasma working solution (2800 µL) was incubated at 37 ºC for 5 min, then mixed with 200 µL (1 mg/mL) concentration of the plant extract and further incubated at 37 ºC for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of $FeSO_4$, $7H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured for the sample solutions. The antioxidant potential of all extracts of each variety was determined against a standard curve in µM equivalent to $FeSO_4$, $7H_2O$/g of sample.

Determination of total phenols: Total phenols in the extracts were determined using Folin-Ciocalteu reagent. The stock solutions of methanol, ethanol, chloroform and *n*-hexane extracts were prepared in concentration of 1 mg/mL. 80 µL of each sample (1 mg/mL) was mixed with 0.25 mL of Folin-Ciocalteu reagent and 0.8 mL of 10 % sodium carbonate solution. 2 mL of methanol was added to the reaction mixture. The mixture was allowed to stand for 0.5 h and the absorbance was measured at 765 nm. Gallic acid was used in the range of 0.00625 mg/mL - 0.2 mg/mL to produce standard calibration curve. The total phenolic contents were expressed as mg equivalent of gallic acid (GAE) per gram dry weight of the extract through standard calibration curve.

Total flavonoids assay: To evaluate the total flavonoids in the plant extracts Ordon *et al.*[10] method was used. To 100 µL (1 mg/mL) of sample and 100 µL of 2 % $AlCl_3$ ethanol solution was added. After incubation of 1 h at room temperature, absorbance was measured at 420 nm. Total flavonoid content was calculated as mg quercetin equivalent per gram of sample extract using the following equation based on the calibration curve: $y = 0.0128x + 0.0455$, $R^2 = 0.9965$.

Statistical analysis: The statistics was applied by Microsoft Excel 2010. All the experiments were run as triplicate and presented here as average ± confidence level.

RESULTS AND DISCUSSION

Natural products, which possess antioxidant activity are able to inhibit reactive oxygen species and trap free radicals generated in the metabolic system. Therefore, they can reduce the risk of many chronic diseases induced by over activity of such reactive intermediates. Colorimetric measurement of $DPPH^{\bullet}$ free radical provides a cheap, facile and reproducible method for the determination of antioxidant activity in the plant extracts. Diphenyl picrylhydrazyl (DPPH) free radical scavenging activity of the extract was measured as percentage scavenging of $DPPH^{\bullet}$. Generally the polar extracts of both varieties were found stronger free radical scavenger than the nonpolar ones. The polar extracts exhibited % inhibition in the range of 81-91 %, while IC_{50} values 107-153 µg/mL. CrPM was the most effective extract against DPPH with IC_{50} = 107.37 µg/mL (Table-1). CrWH, CrWC, CrPH and CrPC showed insignificant results in the assay. The polar solvents more effectively extracted antioxidants from flowers *C. roseus* than the non-polar solvents. The comparison of DPPH radical

scavenging % inhibition of pink and white flower subspecies indicated that the white and pink flower methanol extracts (CrWM, CrPM) had nearly the same antioxidant activity, while white flower ethanol extract (CrWE) had greater % inhibition than the pink flower ethanol extract (CrPE). The % inhibition exhibited by the chloroform and *n*-hexane extracts of both varieties were not enough to consider as shown in Fig. 1.

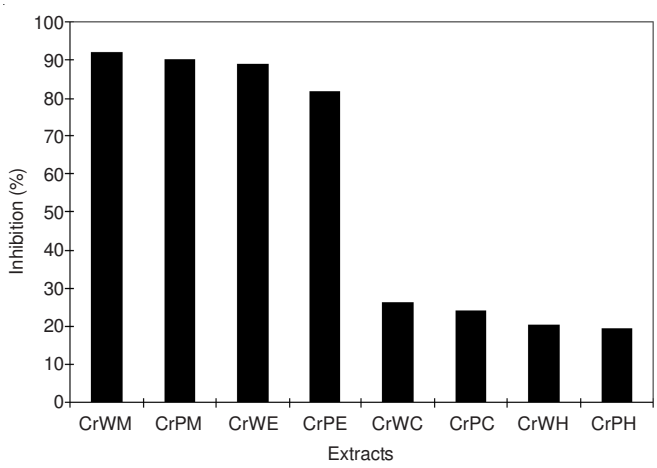

Fig. 1. DPPH antiradical scavenging activity of extracts of white and pink flowers

Ferric reducing ability of plasma assay depends upon the formation of ferrous tripyridyl triazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue colour and can be monitored at 593 nm[11]. The literature shows that ferric reducing ability is sensitive in the measurement of total antioxidant power of the fresh biological fluids, such as plant homogenates and pharmacological plant products[12]. All the extracts were subjected to the ferric reducing ability of plasma assay and the results are shown in Table-1. Comparatively the methanol and ethanol extracts of white flower extracts (CrWM, CrWE) expressed greater antioxidant activity than the pink flower methanol and ethanol extracts (CrPM, CrPE). No significant results were exhibited by the extracts of *n*-hexane and chloroform of both species as shown in Fig. 2.

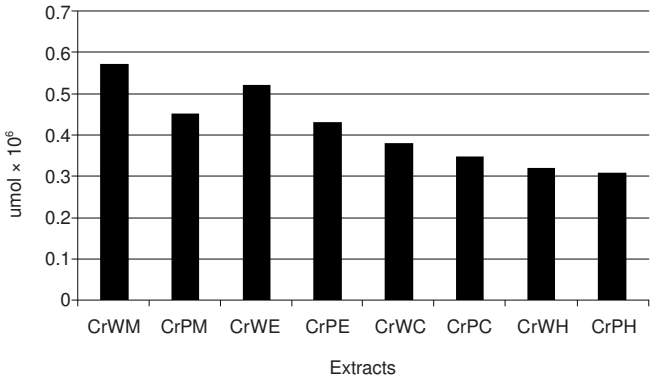

Fig. 2. Ferric reducing ability of plasma assay antioxidant activity of extracts of white and pink flowers

The antioxidant activity of plant extracts mainly due to the presence of polyphenolic compounds[13]. These polyphenolic compounds have the reducing ability by adsorbing and neutralizing free radicals, quenching singlets and triplet oxygen or decomposing peroxides[14]. These polyphenol type compounds possess significant antioxidant capacities and are considered to be the main source of lowering the risk of several diseases associated with the excessive generation of free radicals[15].

The total phenolic content of all the extracts was determined by Folin-Ciocalteu reagent as mg GAE/g dry weight of extract and the results are given in the Table-1. The amount of total phenolics varied in different extracts and ranged from 1.78×10^2-0.68×10^2 mg GAE/g of dry extract. The total phenols in all the extracts of both white and pink varieties ranged 0.13×10^2-0.22×10^2 mgGAE/g of sample. The results showed a strong correlation in between total phenols and % inhibition of DPPH for white flowers ($R^2 = 0.9029$) and pink flowers ($R^2 = 0.8197$). While with ferric reducing ability of plasma values and polyphenols showed the correlation for white flowers ($R^2 = 0.8826$) and pink flowers ($R^2 = 0.676$). A lower R^2 value for pink flowers suggested the involvement of compounds other than phenols in the reducing action of the extract. These correlations indicated that the polyphenols extracted excessively in the polar solvents, were the main constituents responsible for the antioxidant activity. The flavonoids estimation results suggested very less amount of this class of compounds in the flower extracts of *C. roseus* (Table-1).

Conclusion

This study showed that the *Catharanthus roseus* white flowers methanol and ethanol extracts have larger amounts of phenolics as compared to the pink flowers. Hence, the white flowers have shown greater antioxidant activity than pink flowers. Further studies about purification of the extracts is requried to identify the active principals.

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