INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 1, p. 370-376, 2015

RESEARCH PAPER OPEN ACCESS

Ability to reduce the risk of free radicals and fenton reaction by ethanol extract of *Catharanthus roseus* L

Shahin Mardani-Nejad[1], Ramazan Ali Khavari-Nejad[1*], Sara Saadatmand[1], Farzaneh Najafi[2], Parviz Aberoomand Azar[3]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Faculty of Biology Sciences, Kharazmi University, Tehran, Iran*

[3]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran*

Key words: *Catharanthus roseus*, nitric oxide radical inhibition, fenton reaction, ferric reducing power activity, cupric chelating activity.

http://dx.doi.org/10.12692/ijb/6.1.370-376 Article published on January 10, 2015

Abstract

Oxidative stresses caused by free radicals are the reasons of many diseases such as inflammation and cancer; therefore, the plant or plant products that can act as inhibitors of these radicals can have an important role in controlling the related diseases. For the purpose of investigating the anti-oxidation ability of *Catharanthus roseus* L., ethanol extract of the root, stem, leaf, flower, seed pod, and seed of the plant was prepared, and this ability was measured based on nitric oxide scavenging radical and based on ferric reducing power activity (FRAP), and cupric chelating activity (CCA) to reduce the risk of fenton reaction against synthetic antioxidants (ascorbic acid, butylated hydroxyanisole, and butylated hydroxytoluene). The results showed that extract of root, seed, and leaf (228.75 ± 1.47, 209.31 ± 2.23, and 228.75 ± 1.47 µg mL^{-1}, respectively) had the best performance in inhibiting half of nitric oxide. The extract of root had the best reducing power activity between other extracts and the ethanol extract os seed had the best chelating activity. Pearson's coefficient of determination between flavonoid content and antioxidant capacity of extracts were high. *C. roseus*, particularly the root, leaf, and seed can be a useful source of natural antioxidants, its strong antioxidants.

* **Corresponding Author:** Ramazan Ali Khavari-Nejad ✉ ra.khavarinejad@gmail.com

Introduction

In recent years, in seeking to prove a relationship between oxidative tension and human diseases, a growing attention has been paid to food supplements compounds containing anti-oxidative properties. Reactive oxygen species (ROS) and also all reactive nitrogen species (RNS), in addition to lipid peroxidation, may cause damage to DNA which can lead to mutation (Ferreres *et al.*, 2008). In many countries, in order to prevent oxidative damage to food crops, synthetic antioxidants like BHA, BHT, and TBHQ have a wide range of applications as food additives. Synthetic antioxidants are cheap and available and are taken into consideration because of their stability and high performance. In recent years, the use of synthetic antioxidants, like other chemical additives, has been limited due to their probable toxicity and carcinogenic (Ahmadi *et al.*, 2007).

Phenolic compounds are found in fruits, leaves, root, seed and other parts of the plants (Stoilova *et al.*, 2007). Studies have found that reception of antioxidants through food supplement has an effective role in maintaining and promoting health. As an example, coronary artery diseases and some types of cancer have a reversed relationship with the consumption of food rich in polyphenols. These studies have led to a special attention to natural resources for the purpose of finding an antioxidant molecules (Kaviarasan *et al.*, 2007). These compounds act as regenerator, hydrogen donor, and metal chelating. Iron ion has an important role in hydroxyl radical formation and creation of oxidative stress conditions in live creatures. In biologic systems, the majority of hydroxyl radicals or oxidants which react with the similar activity of ferric ion (fenton reaction) will eventually lead to the formation of hydroxyl radical from Ferrous ion (Thomas *et al.*, 2009). Therefore the use of antioxidant to slow down the rate of oxidation in food seems necessary.

Catharanthus roseus is a family of Apocinacea and native of island of Madagascar, but nowadays it is widely distributed in the tropics. The main reason of attention to this plant is due to its ability in synthesizing a wide range of terpenoid indole alkaloids that have a medical value in treating leukemia, lymphoma, and high blood pressure. However, other features of natural plant compounds, except for alkaloids, have been studied extremely low. Few studies have been done on phenolic compounds, especially in cell culture and there is very little information regarding the antioxidant of this important plant, and few experiment have been done on the aqueous extract of this plant (Ferreres *et al.*, 2008). No studies have ever been carried out on other antioxidant potentials of the plant as well as its antioxidant reducing power and chelating effect, thus the results are reported for the first time. Every year a lot of waste resulted from the extraction of alkaloids in this plant produced which is usually disposed. Therefore, the present study that was conducted on the antioxidant capacity of various plant organs compared to synthetic antioxidants can be of other promising applications of this valuable plant.

Materials and methods

Plant materials and preparation of ethanol extract

The seeds were planted under the greenhouse conditions in May 2013 and the vegetative organs and reproductive organs of the plant were collected in September 2013, dried in an oven at the temperature of 40 °C for 72 h and then ground and powdered by the mill (IKA universal, Germany). The extract was taken from the powdered samples in each extraction with 1:1 ratio by ethanol 85% while stirring at the temperature of 40 °C for 2 h. After this period, the mixture was filtered and the residue was remixed with the same solvent mixture ratio and the operation was repeated for 2 h. The filtered solutions of both stages were mixed together, condensed by the help of a rotary evaporator (Heidolph 4001, Germany) at the temperature of 40 °C and dried by a freeze dryer (Cherist 1-4 LD, UK). The extracts were kept at the temperature of -50°C until testing.

Nitric oxide-scavenging activity

Investigation of nitric oxide radical inhibition was done by the help of griess reagent (Govindarajan *et al.*, 2003). In this experiment, the reaction mixture (3

mL) containing 2 mL sodium nitroprusside, (5 mM) and 0.5 mL phosphate buffer with 0.5 mL extract or standard (25-400 mg mL^{-1}) was incubated at room temperature for 150 min. Then 0.5 mL of reaction was mixed with 1 mL of sulfinic acid (0.33%) in 20% glacial acetic acid. After 5 min, 1 mL naphthyl ethylenediamine dihydrochloride 0.1% was added to and mixed with it, then placed at room temperature for 30 min. A pink color was formed in the solution. The absorption of this colored solution was measured at 540 nm against blank. Ascorbic acid was used as standard. The inhibition percentage was calculated according to the following formula. The IC_{50} concentration of sample is capable of inhibiting 50% of nitric oxide radical. Inhibition% = $[1- A_s/A_0] \times 100$. Where A_s is the absorbance in the presence of ethanol extract or positive controls, while A_0 is the absorbance in the absence of ethanol extract and positive controls.

Ferric reducing power activity (FRAP)

Measuring the reducing power ability of ethanol extract from changing Fe^{3+} into Fe^{2+} was done according to the method of Ferreira *et al.* (Ferreira *et al.*, 2007). In this method, the concentration 6.25-400 µg mL^{-1} of each extract or standard was prepared, and 1mL of each sample, 2.5 mL phosphate buffer (pH = 6.6), and 2.5 mL potassium ferrocyanide solution were as incubated at the temperature of 50°C for 20 min. Then 2.5 mL TCA 10% was added to the mixture and was centrifuged for 10 min at 3000 revolutions per minute (RPM). 2.5 mL of the upper part of the mixture was removed and diluted with 2.5 mL water. After that, 0.5 mL ferric chloride was added to the mixture and the complex absorption was read at 700 nm against blank. Ascorbic acid, BHT, and BHA were used as positive controls for comparison. Reducing capacity of the samples was calculated by the following formula: Relative reducing effect % = $[A-A_{min}/A_{max}-A_{min}] \times 100$. Here, A_{max} is the maximum absorbance and A_{min} is the minimum absorbance in the test. A is the absorbance of sample.

Cupric chelation activities (CCA)

Metal ion binding properties of the extracts was performed by using complexometry method of Blazovics *et al.* (Blazovics *et al.*, 2003). In this experiment 60 µL of copper sulfate (20 mM L^{-1}) was added to the hexamine hydrochloric acid buffer (pH = 5.0, 30 mM L^{-1}) containing 30 mM L^{-1} potassium chloride and 0.3 µM murexide. After incubation at room temperature for 1 min, 1.5 mL of the extract or standard with concentration of 50-500 µg mL^{-1} was added to the complex and it was intensely stirred and placed at room temperature for 10 min. Absorption of the solution was measured at 485 and 520 nm. Absorption ratio of A_{485}/A_{520} refers to the released Cu^{2+}. Chelating percent of copper was obtained from the following equation: Cupric chelating effect% = $[(A_{485} / A_{520})_{max} - (A_{485} / A_{520})] / (A_{485} / A_{520})_{max} \times 100$. where (A_{485} / A_{520}) is the absorbance ratio in the presence of the sample, while (A_{485}/A_{520}) max is the maximum absorbance ratio without any samples.

Determinaition of total flavonoid content (TFC)

Total flavonoid compounds of extracts were measured by colorimetrically aluminium chloride method of Zhishen *et al.*, 1999. In this method, 500 µL of ethanol extract (10 mg mL^{-1}) of different plant organs was added ti 2 mL twice water. Then 150 µL sodium nitrite 5% was added. After 6 min, 1 mL sodium hydroxide 1 M was added. In the end the contents of the tube was brought to a volume of 5 mL. After 15 min, the absorption of samples was read at a wavelength of 510 nm. As standard curve was drawn based on 5-100 µgmL catechin and the amount of flavonoid compounds in the plant was measured as catechin equivalents (µg CAE mg^{-1} extract) according to the following equation: $T = (C \times V)/M$. In this equation, C is the catechin concentration in µg mL^{-1} according to the standard diagram; V is the volume of extract in mL and M is the weight of extract in mg.

Statistical analysis

The experimental results are expressed as mean ± S.E.M. All measurements were taken in triplicate. The data were analyzed by an analysis of variance ($P \leq 0.05$) and the means were separated by Duncan's multiple range tests. The IC_{50} values were calculated from a linear regression analysis. The statistical

analysis was performed by SPSS.

Results and discussion

Nitric oxide-scavenging activity

Fig. 1 shows that nitric oxide radical trapping activity has increased significantly with increasing concentration of ethanol extracts of various organs and positive control. 50% inhibition of free radicals (IC_{50}) by extracts and positive control were as follow, respectively: ascorbic acid > seed, root, and leaf > flower > seed pod > stem (Table 1).

Table 1. The half maximal inhibitory concentration (IC_{50}) of nitric Oxide, Fe^{3+} reducing and Cu^{2+} chelaiting of ethanol extracts of *C. roseus* and standards. Data show means of three replicates with standard error.

Sample	Nitric oxide radical inhibition (IC_{50})	Reducing power (IC_{50})	Metal chelaiting (IC_{50})
Roots	218.63±0.99 bc	407.70±4.75 c	767.75±21.42 b
Stems	538.75±8.55 f	1205.73±10.25 g	2522.92±46.49 f
Leaves	228.75±1.47 c	745.68±6.46 e	1216.02±36.23 d
Flowers	267.83±5.47 d	917.36±17.85 d	959.05±21.85 c
Seed Pods	338.68±5.95 e	1061.06±21.31 f	1685.04±96.13 e
Seeds	209.31±2.23 b	630.53±3.96 e	501.75±3.87 a
BHT	-	114.33±1.18 a	-
BHA	-	162.89±2.45 b	-
Ascorbic acid	142.98±1.01 a	147.90±2.52 b	521.67±3.79 a

Sodium nitroprusside was used in the radical inhibition method of nitric oxide. This material in an aqueous solution and physiological pH produce nitric oxide, which is measured by griess reagent. Inhibitors of nitric oxide compete with oxygen to reduce the production of nitric oxide, so the substance that decreases the production of nitric oxide can be considered as an antioxidant scavenging these radicals that is accompanied by a decrease in the intensity of the pink color follow by reduction in absorption. Ethanol extract of root with concentration of 218.63 ± 1.711, stem extract with 538.75 ± 14.816 and leaf with 227.95 ± 4.443 µg mL^{-1} inhibited half of nitric oxide radicals. A study on aqueous root extract (Pereira *et al.*, 2010) has proved concentration of 189 mg mL^{-1} inhibits 25% of the radicals. In another study, concentrations of 546 and 505 mg mL^{-1}, respectively to inhibit 25% of the radicals (Ferreres *et al.*, 2008). The results showed that ethanol extracts, compared to aqueous extract, had significantly a better performance in inhibition of nitric oxide radical. In addition to active oxygen, nitric oxide also plays a role in other pathological conditions such as inflammation and cancer (Lee *et al.*, 2003). Plant or plant products that can prevent formation of nitric oxide can be considered as an important place in disease inhibition.

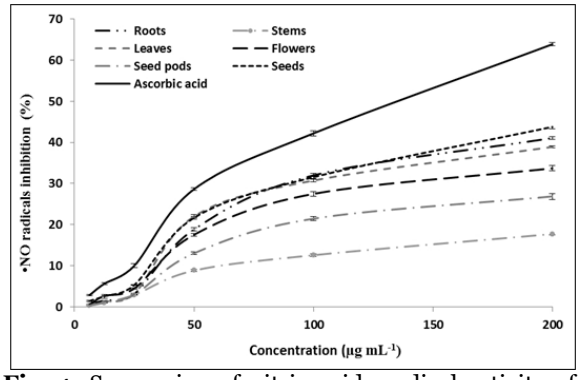

Fig. 1. Scavenging of nitric oxide radical activity of ethanol extract of various organs of *C. roseus* and standard (ascorbic acid). Data show means of three replicates with standard error.

Ferric reducing power activity (FRAP)

Fig. 2 shows that ferric reducing power of extracts have significantly increased with increasing concentration. There was a significant difference between extracts of different organs. Strength of the electron donation and the end of 50% of a chain reaction of extracts and positive controls contained BHT > ascorbic acid, BHA > root > flower > seed and leaf > seed pod > stem, respectively (Table 1).

The reducing power (antioxidant) was measured in the sample of reducing Fe^{3+} to Fe^{2+} with donating electron in the presence of ethanol extracts of plant and positive controls. Reducing power of compounds can be an indicator of its potential antioxidant activity (Akbar Dar *et al.*, 2014). Fe^{2+} complex rate can be measured by measuring prussian blue formation rate at 700 nm. The material with more ability to donate electron will cause an increase in absorption in this wavelength; in other words, it has the higher antioxidant ability. Among the ethanol extract of the plant, root had higher reducing power than other organs. Meanwhile, transition elements such as iron and copper have the ability to form free radicals based on fenton reactions that can be the cause of cardiovascular diseases in human (Uttara *et al.*, 2009). Since the Cu^{2+} can induce the production of oxy radicals and lipid peroxidation, reduction in its concentration in the Fenton reactions would somehow create a shield against oxidative damage.

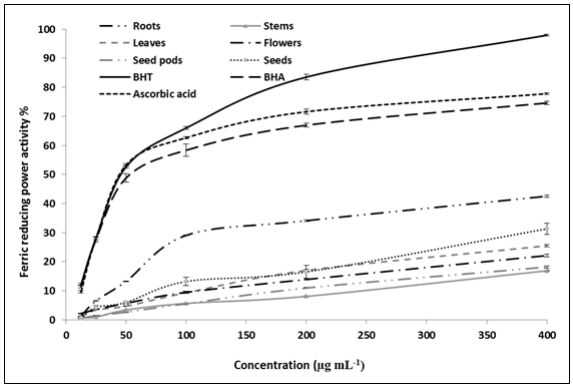

Fig. 2. Reducing power activity of various organs of *C. roseus* and standards (ascorbic acid, BHA and BHT). Data show means of three replicates with standard error.

Cupric chelation activities (CCA)

Fig. 3 shows that the Cu^{2+} chelating power increased significantly with increasing concentration of the extracts. Significant difference between ascorbic acid and seed in chelating power of half of the Cu^{2+} ions, was not observed, although their ability was significantly better in comparison with other organs. The results show that the chelating ability can be used as another mechanism of antioxidant effect of *Catharanthus roseus* plant. After positive controls, seed extract had the high chelating ability.

Total flavonoid content (TFC)

According to the equation (Y= 0.00289X-0.0076, r^2= 0.989) flavonoid content of various organs of the plant express as equivalent of catechin (µg CAE mg^{-1} extract) was measured to be, 42.9 ± 1.34, 38.56 ± 1.15, 32.02 ± 0.91, 18.82 ± 0.19, 17.27 ± 0.2, 9.64 ± 0.69 in the leaf, seed, root, flower, seed pod, and stem, respectively. Many phenolic compounds have antioxidant activity (Orcic *et al.*, 2011), from which flavonoids are considered as one of the most important groups of these compounds. Flavonoids have widely shown a significant antioxidant activity in food products derived from plant sources (Amic *et al.*, 2007). Studies have shown that an increase in levels of flavonoids in diet can reduce certain diseases. Thus, the flavonoid content and antioxidant effect in different organs of the plant *Catharanthus roseus* were examined. The results showed that different organs of the plant contain high flavonoid contents. The leaf, seed and root had higher flavonoid content than those of other organs, respectively.

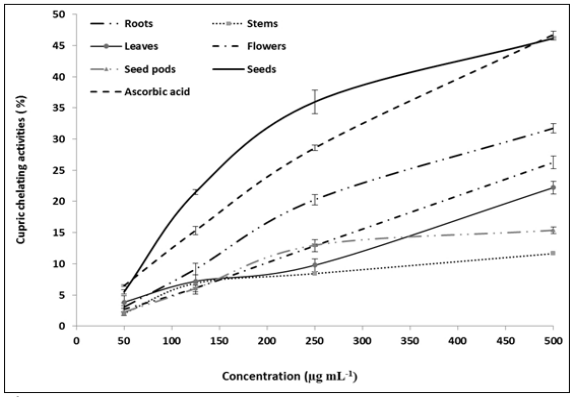

Fig. 3. Chelating activity of *C. roseus* and standard (ascorbic acid). Data show means of three replicates with standard error.

Pearson's coefficients of determining the relationship between flavonoid content and antioxidant capacity of the extracts based on nitric oxide inhibition method, reducing power of Fe^{3+} and Cu^{2+} chelating were -0.187, -0.728 and -0.787, respectively. A high and negative correlation between flavonoid contents and the plant antioxidant capacity at a significant level ($p < 0.01$) was evident in the applied methods in a way that the extract with higher flavonoid contents and lower concentration was capable of nitric oxide inhibition, and reducing Fe^{3+} or Cu^{2+} chelating.

Conclusions

The results presented in this study show that the majority of vegetative and reproductive organs of *Catharanthus roseus* plant, especially root, seed and leaf, contain high flavonoid content. High correlation between flavonoid contents with antioxidant ability of plant was observed between regenerative ability and extract chelating with inhibition of nitric oxide radicals. Since nitric oxide plays a role in pathologic conditions such as inflammation and cancer, plant or plant products which can act as inhibitors of free radicals can have an important role in controlling the diseases. Extraction of strong antioxidant from this plant can also be very useful in the food and pharmaceutical industries, and it may be used as a substitute for synthetic antioxidants from this plant.

Acknowledgments

This research was supported by a grant from the Science and Research branch, Islamic Azad University of Tehran.

References

Ahmadi F, Kadivar M, Shahedi M. 2007. Antioxidant activity of *Kelussia odoratissima* Mozaff. in model and food systems. Food Chemistry **105,** 57-64.
http://dx.doi.org/10.1016/j.foodchem.2007.03056

Akbar Dar M, Masoodi MH, Wali AF, Mir MA, Shapoob NS. 2014. Antioxidant potential of methanol Root extract of *Mentha arvensis* L. from Kashmir Region. Journal of Applied Pharmaceutical Science **4,** 50-57.
http://dx.doi.org/10.7324/JAPS.2014.40311

Amic D, Davidovic-Amic D, Beslo D, Rastija V, Lucic B, Trinajstic N. 2007. SAR and QSAR of the Antioxidant Activity of Flavonoids. Current Medicinal Chemistry **14,** 827-845.
http://dx.doi.org/10.2174/092986707780090954

Blazovics A, Szentmihalyi K, Lugasi A, Balazs A, Hagymasi K, Banyai E, Then M, Rapavi E, Hethelyi E. 2003. In vitro analysis of the properties of beiqishen tea. Nutrition **19,** 869-875.
http://dx.doi.org/10.1016/S0899-9007(03)00157-6

Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. 2007. Free-radical cavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chemistry **100,** 1511–1516.
http://dx.doi.org/10.1016/j.foodchem.2005.11.043

Ferreres F, Pereira DM, Valentao P, Andrade PB, Seabra RM, Sottomayor M. 2008. New phenolic compounds and antioxidant potential of *Chataranthus roseus*. Journal of Agrcultural and Food Chemistry **56,** 9967-9974.
http://dx.doi.org/10.1021/jf8022723

Govindarajan R, Rastogi S, Vijaykumar M, Shirwaikar A, Rawat AK, Mehrotra S. 2003. Studies on antioxidant activities of *Desmodium gangeticum*. Journal Biological and Pharmaceutical Bulletin **26,** 1424-1427.
http://dx.doi.org/10.1248/bpb.26.1424

Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. 2003. Screening of medicinal plant extracts for antioxidant activity. Life Science **73,** 167-179.
http://dox.doi.org/10.1016/S0024-3205(03)00259-5

Kaviarasan S, Naik GH, Gangabhagirthi R, Anuradha CV, Priyadarsini KI. 2007. In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum* graecum) seeds. Food Chemistry **103,** 31-37.
http://dx. doi.org/10.1016/j.foodchem.2006.05.064

Orcic DZ, Mimica-Dukic NM, Franciskovic MM, Petrovic SS, Jovin ED. 2011. Antioxidant activity relationship of phenolic compounds in *Hypericum perforatum* L. Chemistry Central Journal **5,** 5-34.
http://dox.doi.org/10.1186/1752-153X-5-34

Pereira DM, Faria J, Gasparn L, Ferreres F, Valentao P, Sottomayor M, Andrade PB. 2010.

Exploiting *Catharanthus roseus* roots: source of antioxidants. Food chemistry **121,** 56-61. http://dox.doi.org/10.1016/j.foodchem.2009.12.002

Stoilova I, Krastano A, Stoyanova A, Denev P, Gargova S. 2007. Antioxidant activity of ginger extract *Zingiber officinale*. Food Chemistry **102,** 764-770. http://dox.doi.org/10.1016/j.foodchem.2006.06.023

Thomas C, Melissa M, Mackey MM, Diaz AA, Cox DP. 2009. Hydroxyl radical is produced via the Fenton reaction in submitochondrial particles under oxidative stress: implications for diseases associated with iron accumulation. Redox Report **14,** 102-108. http://dox.doi.org/10.1179/135100009X392566

Uttara B, Singh AV, Zamboni P, Mahajan RT. 2009. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Current Neuropharmacology **7,** 65–74. http://dox.doi.org/10.2174/157015909787602823

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chemistry **64,** 555–559. http://dox.doi.org/10.1016/S0308-8146(98)00102-2