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Mechanisms of non-enzymatic antioxidant defense system of different organs of *Catharanthus roseus* for protective of cell membrane

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;*

[*]**Email:** *ra.khavarinejad@gmail.com*

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ABSTRACT

Background & Aim: When the level of free radicals is increased and also when both the enzymatic systems and low molecular antioxidants are not sufficient to protect the organism, it seems necessary to get antioxidants from external sources. This study aimed to evaluate the antioxidant potential of different parts of *Catharantus roseus*

Experimental: The antioxidant potential of ethanol extracts of roots, stems, leaves, flowers, seed pods and seeds of *C. roseus* plant were measured based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, inhibition of linoleic acid peroxidation by ferric thiocyanate (FTC), and malondialdehyde inhibition by thiobarbituric acid method (TBA) against the standards: Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) and ascorbic acid.

Results & Discussion: The phenolic content in the root (61.61 ± 2.58 µg of gallic acid per mg of dried weight extract) according to Folin–Ciocalteu method were more than any other organs. In scavenging a half of DPPH$^{\bullet}$ free radicals (IC_{50}), the extract of root, seed, and leaf (238.9 ± 2.02, 253.78 ± 1.42, 277.95 ± 2.56 µg mL^{-1}), respectively, had the best performance. In the inhibition of linoleic acid peroxidation, the root extract had the best inhibitory power after ascorbic acid and BHT, and the leaf and seed extract had the performance similar to BHA. The root (78.68 ± 0.54%), and seed (77.44 ± 0.66%) had the best performance in the inhibition of MDA compared with other extracts. Pearson correlation coefficients between the phenolic content and antioxidant capacity of extracts were high and equal to -0.838, 0.895, and 0.740, respectively, according to DPPH, FTC and TBA methods.

Industrial and practical recommendations: The results of this study can be promising in the potential of other applications of the plant organ especially in the inhibition of free radicals and lipids' peroxidation.

1. Introduction

The oxidative stress is obtained as the result of an imbalance between the internal production of free radicals and the antioxidant defense mechanisms. In living organisms, the peroxidation of lipids in the wall of living cells is among the most important objectives of free radicals. In these circumstances, not only the structure of wall and its function are affected, but also some of the products resulting from the oxidation such as malondialdehyde can make a chemical reaction with biomolecules and show the cytotoxic and genotoxic effects. Therefore, the presence of free radicals especially the peroxides play the key roles in the pathogenesis of a number of diseases such as cancer, diabetes, heart-cardiovascular disease, and other diseases (Wardle, 2005). In most of the countries, the synthetic antioxidants such as BHA, BHT and TBHQ are widely used as the food additives in order to prevent the oxidative degradation of these products (Schillaci *et al*., 2014). The synthetic antioxidants are cost-effective and available and taken into account because of their high stability and performance. In recent years, the use of synthetic antioxidants has been limited as other chemical additives due to their possible toxicity and carcinogenesis (Panicker *et al*., 2014). Among the plant compounds with the antioxidant, the phenolic compounds are widely distributed in most of the plants. Phenolics are widespread constituents of plant foods and beverages, these compounds are generally existed in the fruits, leaves, roots, seeds and other plant parts (Stoilova *et al*., 2007; Dai & Mumper, 2010). The antioxidant features of phenolic compounds are mainly due to their regenerative power and chemical structures which enable them to neutralize the free radicals, make the complex with metal ions, and quench the single and triplet oxygen molecules. The phenolic compounds inhibit the lipid oxidation reactions by giving the electrons to free radicals and play the important roles in food storage and providing the human health (Ahmadi *et al*., 2007).

Catharanthus roseous (L.) G. Don (formerly *Vinca rosea* L., Apocynaceae) is commonly known as the Madagascar perwinkle. It is a perennial, every green herb, 30-100 cm height that was originally native the island of Madagascar but it is now widely dispersed in the tropic (Aslam *et al*., 2010). The significant importance of this plant is its ability to synthesize a wide range of terpenoid indol alkaloids which have the medicinal values. However, other features of natural compounds in the plant except for alkaloids have been a little investigated. A few studies have been conducted on phenolic compounds especially in cell culture and there is a little information about the antioxidant potential of this important plant. The limited tests are performed on the aqueous extract of this plant (Ferreres *et al*., 2008; Pereira *et al*., 2010) and no research is conducted on some of the antioxidant potentials of this plant particularly the ethanol extract of this plant. Every year, lots of residues remain from the extraction of alkaloids in this plant and they are usually destroyed. Thus this study, which is conducted on the antioxidant potential of plant vegetative and reproductive organs compared to the synthetic antioxidants, can be promising for other applications of this valuable plant.

2. Materials and Methods

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3. Results and discussion

3.1. Total phenol content

The results show the significant difference between the vegetative and reproductive organs of plant in terms of phenolic content. The phenolic compounds in the root 61.61 ± 2.58 (highest), in the leaf 48.32 ± 0.39, in the flower 39.49 ± 0.49, in the seed pod 28.84 ± 0.69, seed 47.51 ± 0.92 and the lowest amount in stem 18.37 ± 1.16 were measured equal to µg of gallic acid per mg of dried weight of extract according to the standard curve ($Y= 0.000532X + 0.0079$, $r^2= 0.997$). The result showed that most of the organs of this plant had the high phenolic content. The plant root had higher phenolic content than other vegetative organs and the amounts of these compounds were notable in the leaves and seeds. The phenolic compounds have shown wide significant antioxidant activity in food products derived from plant sources (Msagati, 2013). The studies have shown that the increased levels of phenolic compounds in the food diet can reduce the diseases significantly (Petti & Scully, 2009).

3.2. DPPH• radical-scavenging activity

Fig. 1 shows that the free radical trapping activity was significantly increased by enhancing the concentration of extracts. The inhibitory potential of standards was limited and the application of higher concentrations was not able to higher free radical scavenging at the significant level. Ascorbic acid with the concentration 5.43 ± 0.40, BHA with the concentration 13.35 ± 0.80, BHT with the concentration 22.29 ± 0.40, the ethanol extract of root with the concentration of 238.9 ± 2.02 µg ml^{-1}, seed with concentration of 253.78 ± 2.46 µg ml^{-1}, leaf with concentration of 277.95 ± 4.443 µg ml^{-1}, flower with concentration of 489.22 ± 8.29 µg ml^{-1}, stem with concentration of 809.24 ± 12.67 µg ml^{-1}, seed pod extract with concentration of 1077.86 ± 44.24 µg ml^{-1} scavenged a half of free radicals. DPPH radical scavenging model is one of the mostly used methods for investigation of inhibitory potential of samples (Elmastas *et al*., 2007). This radical is a stabilized nitrogen center and the dark purple color is created by dissolving in the alcohol and it is changed to yellow during the process of hydrogenation or electronation. The compounds which are able to do this reaction can be considered as the antioxidants and the radical scavenger (Brand-Williams *et al*., 1995). DPPH radicals make a chemical reaction with available antioxidants in extracts and become as the stable DPPHH, thus its color is changed from dark purple to bright yellow and its rate of absorption is reduced.

According to the study on the aqueous root extract, the concentration of 153 µg ml^{-1} (Periera *et al*., 2010) and in another research the concentrations of 265 and 447 µg ml^{-1} were reported for scavenging a half of free radicals in the aqueous extract of seed and leaf (Fereres *et al*., 2008). The performance of aqueous extract of root and seed had no significant difference with the output of the alcoholic extract exhibitory potential in this test, but the performance of alcoholic extract in the leaf in this test was better than the aqueous extract in the test by

(Fereres *et al*., 2008). The inhibition impact of 50% free radicals by extracts and positive controls was reduced for ascorbic acid, BHA and BHT > root, seed and leaf > flower > stem > seed pod, respectively.

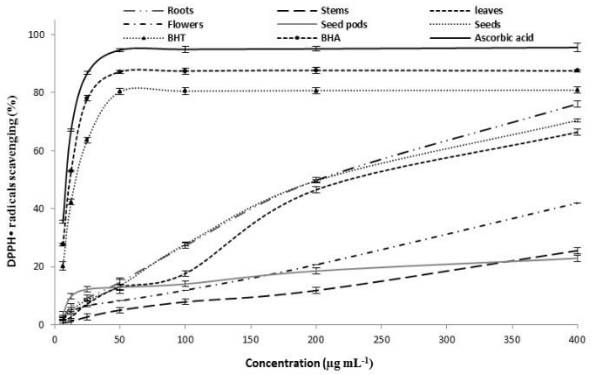

Fig 1. Scavenging of DPPH• activity by ethanol extracts different parts of *C. roseus* and standards (BHT, BHA and ascorbic acid). Data show means of three replicates with standard error.

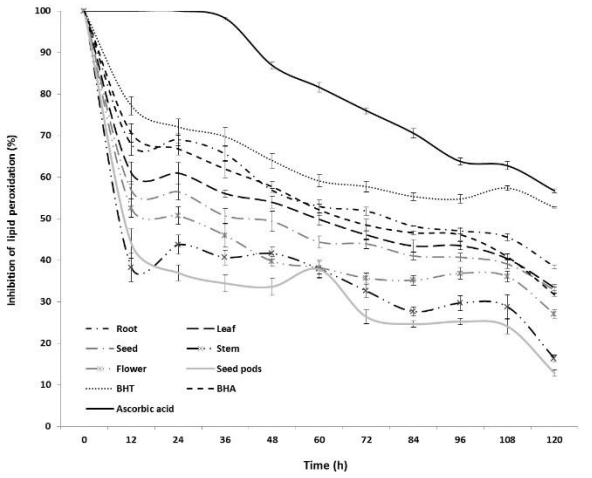

Fig 2. Inhibition of lipid peroxidation by ethanol extracts of different parts of *C. roseus* and standards (BHT, BHA and ascorbic acid) by FTC method. Data show means of three replicates absorbance values with standard error.

3.3. Ferric thiocyanate and thiobarbituric acid method

The results of lipid peroxidation inhibition in Fig. 2 showed that the increased time led to the decreased percentage of linoleic acid peroxidation inhibition. When the absorption control became maximal (after 108 h), the percentage of linoleic acid peroxidation inhibition was as follows: Ascorbic acid > BHT > root > BHA, leaf and seed > flower > stem > seed pod.

The malondialdehyde inhibition showed that the inhibition potential of malondialdehyde was as follows: BHT > root > seed > BHA > flower > ascorbic acid > leaf and seed pod > stem. Table. 1 shows absorbance and inhibition of malondialdehyde different parts of *C. roseus*.

Table 1. Absorbance and inhibition of malondialdehyde of ethanol extracts of various organs of *C. roseus* and standards by TBA method. Data show means ± S.E., n=3

Sample	Absorbance (nm)	Inhibition of malondialdehyde (%)	
Control	1.61 ± 0.005	0	
Roots	0.34 ± 0.007	78.68 ± 0.54	f
Stems	0.99 ± 0.01	38.31 ± 0.44	a
Leaves	0.90 ± 0.007	43.29 ± 0.59	b
Flowers	0.52 ± 0.02	67.53 ± 1.26	d
Seed pods	0.92 ± 0.02	42.50 ± 1.22	b
Seeds	0.35 ± 0.01	77.44±0.66	f
BHT	0.19 ± 0.008	87.47 ± 0.55	g
BHA	0.44 ± 0.003	72.14 ± 0.38	e
Ascorbic acid	0.66 ± 0.07	59.31 ± 0.52	c

Means values with different lowercase letters in the column are significantly different ($p < 0.05$).

The membrane lipids are rich of unsaturated fatty acids and make them susceptible to contribute in the oxidative processes. In this case and particularly, arashidonic acid and linoleic acid can be taken into account and the lipid oxidation is the aim (Yu, 2001). The peroxidation inhibition of fatty acid may be due to the free radical trapping activity. The superoxide ion indirectly starts the peroxidation of fatty acids and lipids since the superoxide anion acts as the radical precursor of oxygen and hydroxyl (Ordonez *et al*., 2006). Hydroxyl radicals reduce hydrogen atoms in the membrane. These atoms can lead to the lipid peroxidation. FTC method determines the amount of peroxide at the initial stage of lipid peroxidation (Saha *et al*., 2004). In this test, the divalent iron with the obtained lipid peroxide radicals is changed to the trivalent iron that make a chemical reaction with ammonium thiocyanate and thus ferothiocyanate, which is a red complex, is produced. The substances, which neutralize the peroxide radical, ultimately decrease the production of colored complexes which can be considered as the antioxidants. In this test, the utilized extracts shows lower absorption than the control group due to the presence of antioxidant compounds and the inhibitory properties of extracts were also reduced over time due to the accumulation of radicals. This test is first

reported for this plant, but no research is found in the available resources in this regard. The peroxidation inhibition model of linoleic acid by ethanol extracts of plant was consistent with the standards. TBA (MDA) method is one of the most widely used methods for determining the oxidation of lipids in foods. Malondialdehyde is produced during the oxidation of lipids (Grotto *et al*., 2009). This substance can make a chemical reaction with biomolecules and show the cytotoxic and genotoxic effects. Therefore, the further presence of free radicals particularly the peroxides play the key role in the pathogenesis of several diseases such as diabetes, heart-cardiovascular disease, cancer, aging and various other diseases (Wardle, 2005). In this method, malondialdehyde make the pink complex with thiobarbituric acid and it has the maximum absorption at the range of 530-535 nm. The substance, which are able to make chemical reaction with malondialdehyde do not let this substance make chemical reaction with thiobarbituric acid and they are considered as the antioxidants. Thus, the compounds in the extracts avoid from the pink complex formation by inhabitation of malondialdehyde. When the absorption control becomes maximal, malondialdehyde will be changed into acid and alcohol, thus it will not be followed by spectrophotometry (Rahmat *et al*., 2003). This test is first reported for this plant. The inhibition model of malondialdehyde in ethanol plant extracts was consistent with the standards. The high correlation was observed between the inhibition of linoleic acid peroxidation and malondialdehyde with the coefficient of 0.693.

In this test, the high correlation between the total phenolic content and the antioxidant capacity of ethanol extracts of plant organ was evident based on the applied methods. Pearson correlation coefficients (R^2) between the phenolic content and antioxidant capacity of extracts were equal to -0.838, 0.895, and 0.740, respectively, according to DPPH, FTC and TBA methods and the high correlation was evident between the phenolic content and antioxidant capacity of plant. On the other hand, the high and negative correlation was seen between DPPH radical scavenging at the level of $p < 0.01$ with FTC method with the coefficient (R^2) of -0.953. One of the most important roles of antioxidants is to inhibit the lipid peroxidation chain reaction by free radical scavenging (Jain *et al*., 2008). The high correlation between the between free radical scavenging and linoleic acid peroxidation by the organs of plant in this experiment represents the plant antioxidant potential.

4. Conclusion

The results presented in this study indicate that except for the stem and seed pod, most of the vegetative and reproductive organs of *C. roseus* especially the root have high phenolic contents. The regenerative ability of electrons, changing Fe III to Fe II, and the anti-lipid peroxidation activity were observed in the ethanol extracts of this organ. Therefore, this capability had high correlation with phenolic compounds. Therefore, in addition to the very important applications of pharmaceutical compounds in this plant, its potent antioxidant can be very useful in the food and pharmaceutical industries and inhibiting the fatty acid peroxidation and protective of cell membrane.

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6. References

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