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Comparative Studies on Antioxidant Properties of Catharanthus Rosea and Catharanthus Alba

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Abstract: Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism and play a dual role in our body as both deleterious and beneficial species. In low/moderate concentrations, free radicals are involved in normal physiological functions but excess production of these species or decrease in antioxidant level leads to oxidative stress. The adverse effects of oxidative stress on human health have become a serious issue. Majority of the diseases / disorders are mainly linked to oxidative stress generated due to free radicals. The plant sources are rich of antioxidants, phyto-constituents which are capable to terminate free radical reactions and prevent our body from oxidative damage. Current research is therefore directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine Catharanthus roseus is used in Ayurvedic medicines for number of ailments. The present study deals with comparative evaluation of antioxidant potential of ethanolic extracts of the roots of the two varieties of Catharanthus roseus L. namely 'rosea'(pink flowers) and 'alba'(white flowers) using different systems of assay, e.g. Hydroxyl radical-scavenging activity, superoxide radical-scavenging activity, DPPH radical- scavenging activity and nitric oxide radical inhibition method. The results revealed that the ethanolic extracts of the roots of Periwinkle varieties extracts exhibited satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner; however Catharanthus rosea had more antioxidant activity than Catharanthus alba.
Key words: Antioxidant, Catharanthus rosea, Catharanthus alba.

INTRODUCTION

Free radicals are electrically charged molecules that are produced as by-products of our own metabolism. They are continuously produced by our body's use of oxygen such as in respiration and some cell-mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air pollution, pesticides, etc.[1]Free radicals and reactive oxygen species (ROS) are inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases. Normally there exists a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/quench these free radicals preventing them from causing deleterious effects in the body.[2] The antioxidant defense systems in the body can only protect the body when the amount of the free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, increasing their burden in the body either due to environmental condition or produced within the body, it leads to oxidative stress which may result in tissue injury and subsequent diseases.[3] It has been proved that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity[4]. Therefore, it is of great significance and necessity that research focuses on discovering potential natural effective antioxidants to replace synthetic ones. As plants produce significant amount of antioxidants to prevent oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Plant and

plant products are being used as a source of medicine since long. Traditional herbal medicines form an important part of healthcare system in India. The medicinal plant selected for the present study was Catharanthus roseus L., which belongs to family Apocynaceae which is commonly known as 'periwinkle' and is an important source of indole alkaloids, which are present in all plant parts. Vinca is used for the treatment of diabetes, fever, malaria, throat infections, and chest complaints. It is also used for the regulation of menstrual cycles, and as a euphoriant[5]. The physiologically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine are reported to be present in the roots [6]. Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia[7,8]. These Catharanthus alkaloids are also used for the treatment of both malignant and non-malignant diseases and in platelet and platelet associated disorders. In the present work, an attempt has been made to study the in vitro antioxidant activities of root extracts of two varieties of Catharanthus roseus, which are distinguishable on the basis of their flower colors Catharanthus 'rosea' (pink colored flowers) and Catharanthus 'alba' (white colored flowers).

MATERIALS AND METHODS

REAGENTS

All the chemicals used were of analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma chemical company, USA and Loba chemicals, Mumbai.

PREPARATION OF THE PLANT EXTRACTS

The plants of C. rosea and C. alba were collected from the campus of Govt. College of Pharmacy, Karad and further authenticated by the Department of Botany, Science College, Karad. The root samples were separated, weighed, washed, shredded and dried. The dried and powdered roots of C. rosea and C. alba (100g) each were separately Soxhlet extracted exhaustively with (95%) ethanol. The extracts were concentrated to dryness under reduced pressure in a rotary evaporator to yield dried ethanolic extracts which were stored in a dessicator and used for further studies.

HYDROXYL RADICAL SCAVENGING ACTIVITY

This assay was based upon the benzoic acid hydroxylation method.[9] Hydroxyl radicals were generated by direct addition of iron (II) salts to the reaction mixture containing phosphate buffer. In a screw –capped tube, 0.2ml of sodium benzoate (10mM) and 0.2ml of FeSO4.7 H2O (10mM) and EDTA (10mM) were added. Then the sample solution and a phosphate buffer (pH 7.4, 0.1M) were added to give a total volume of 1.8ml. Finally, 0.2ml of an H2O2 solution (10mM) was added. The reaction mixture was then incubated at 37^0 C for 2h. there after, the fluorescence was measured at 407nm emission (Em) and excitation (Ex) at 305nm. The measurement of spectrofluorometric changes has been used to detect the damage by the hydroxyl radical.

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Superoxide radical scavenging activity of the plant extract was measured according to the method of Mc Cord and Fridovich, [10] which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560nm. The percentage inhibition was calculated from the formula. [11]

DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity was measured according to the method of Braca et al.[12] An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37˚C for 30 min and absorbance of the test mixture was read at 517nm.The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula;

Percentage inhibition = Ao - A1/ Ao X 100
Where Ao = Absorbance of the control;
A1 = Absorbance of the plant extract/ standard.

NITRIC OXIDE RADICAL INHIBITION METHOD

Sodium nitroprusside, 0.2998 gm, was weighed accurately and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask[13] (10 mM). Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions, which were measured by using Griess Ilosvog Reaction[14-17]. The reaction mixture (6ml) containing sodium nitroprusside (10 mM, 4ml), phosphate buffer saline (1ml) and extracts (1ml) was incubated at 25 ^{0}C for 150 minutes. After incubation, 0.5ml of the reaction mixture containing nitrite was removed, 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed well and allowed to stand for 5 minutes for completing

diazotization, and then 1ml of 1- Naphthylamine (5%) was added, mixed and allowed to stand for 30 minutes. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. IC50 value is the concentration of sample required to inhibit 50% of nitric oxide radical.

RESULTS AND DISCUSSION

The antioxidant activities of ethanolic extracts of the roots of C. rosea and C. alba were measured in different systems of assay, e.g. Hydroxyl radical scavenging assay, superoxide radical scavenging assay, DPPH assay and nitric oxide radical inhibition method. Taking 0% inhibition in the mixture without plant extract, regression equations were prepared from the concentrations of the extracts and percentage inhibitions of free radical formation. IC50 values were calculated from these regression equations. IC50 value is inversely related to the activity. Figure 1 demonstrates the hydroxyl radical scavenging activities of the ethanolic extracts of the roots of C. rosea and C. alba. Hydroxyl radical is the major active oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions causing lipid peroxidation and biological damage. The scavenging of hydroxyl radical increased with increasing concentrations of the extracts of roots of Periwinkle with maximum scavenging effect of 91.56 % for C. rosea and 82.88 % for C. alba at the concentration of 200 µg/ml respectively. The IC50 values of the root extracts were found to be 98.5µg/ml and 112.4 µg/ml respectively. The results of the superoxide radical scavenging activities of the selected plant extracts are depicted in Figure 2.

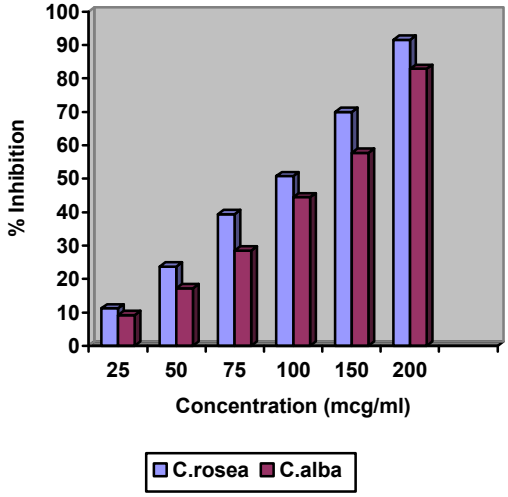


Figure 1: Hydroxyl radical scavenging potential of C. rosea and C. alba

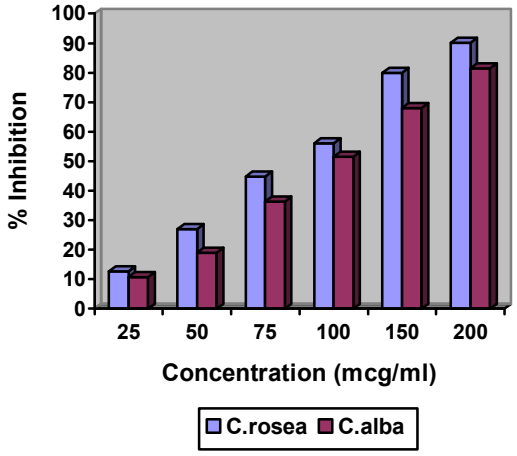


Figure 2: Superoxide radical scavenging potential of C. rosea and C. alba

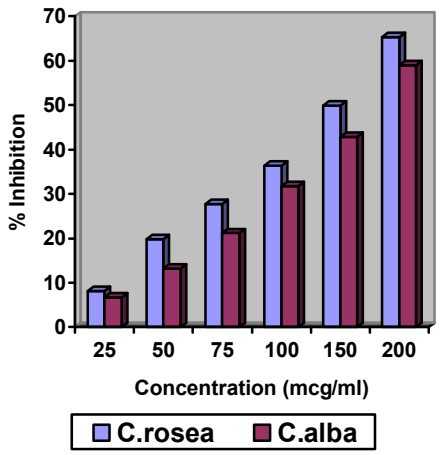


Figure 3: DPPH radical scavenging potential of C. rosea and C. alba

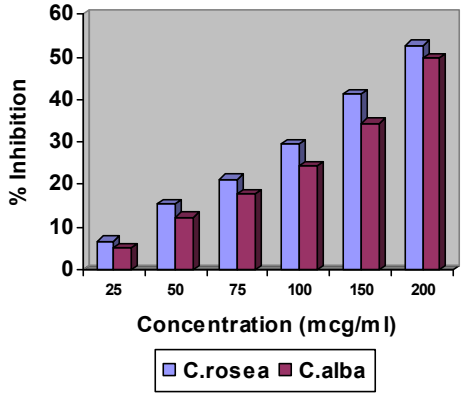
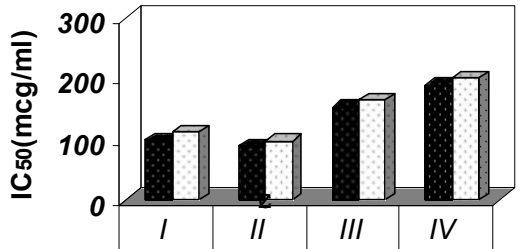


Figure 4: Nitric oxide radical scavenging potential of C. rosea and C. alba



	I	II	III	IV
C.rosea	98.5	89	153.2	189.5
C.alba	112.4	97.2	164.7	201.3

Figure 5: Comparison of IC_{50} values of C.rosea & C. alba extracts in (I) Hydroxyl radical scavenging assay, (II) Superoxide radical scavenging assay, (III) DPPH radical scavenging assay and (IV) Nitric oxide radical scavenging assay.

The maximum scavenging effect of 90.05 % and 81.47 % was observed for the ethanolic extracts of roots of C. rosea and C. alba respectively at a concentration of 200 µg/ml. The IC50 values for the extracts of roots of C. rosea and C. alba were found to be 89.0µg/ml and 97.20µg/ml respectively. DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. In the in-vitro antioxidant studies (Figure 3) the extent of DPPH radical scavenging at different concentrations of the ethanolic extracts of roots of Periwinkle varieties was measured. The radical scavenging effect was found to increase with increasing concentrations. The extracts of the roots of C. rosea and C. alba showed their maximum activity of 65.27 % and 59.03 % respectively at a concentration of 200 µg/ml .The extracts exhibited IC50 values of 153.2µg/ml and 164.7 µg/ml respectively. Figure 4 shows the nitric oxide radical scavenging activities of the two plant extracts. NO is a potent diffusible free radical generated by the endothelial cells and macrophages, which is a mediator of various physiological processes. The reduction of NO radical by the extracts of the roots of Periwinkle varieties was found to be concentration dependent and the maximum scavenging effect was found to be 52.77% for C. rosea and 49.67 % for C. alba extracts respectively with the IC50 values of 189.5µg/ml, and 201.3µg/ml respectively. Figure 5 highlights the comparative representation of the IC50 values (mcg/ml) exhibited by ethanolic extracts of the roots of C. rosea and C. alba in the various in-vitro antioxidant assay systems.

CONCLUSION

The present study revealed that the ethanolic extracts of the roots of C. rosea and C. alba exhibited satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner. However from this study it is concluded that the ethanolic extract of the roots of Catharanthus rosea had more antioxidant activity than Catharanthus alba, as evidenced from the various in vitro antioxidant assays.

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