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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 315-317

www.thepharmajournal.com
Received: 11-02-2019
Accepted: 15-03-2019

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Assessment of variation in biochemical characteristics and radical scavenging properties of two cultivars of the medical plant *Catharanthus roseus*

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Abstract
The present study was carried out to get an understanding of the basis of differential medicinal effects of various components of the plants of two commonly available cultivars of *Catharanthus roseus*, *alba* and *rosea*. Fresh leaves and stems of both cultivars were processed simultaneously and their equivalent concentrations were used for performing the analyses. Although most of the biochemical parameters appeared to be consistent across different parts of the plant as well as different cultivars, levels of anti-oxidants appeared to be significantly higher in the corresponding parts of the *alba* cultivar as compared to the *rosea* cultivar. Further studies aimed towards identifying the active components which are more abundant in the *alba* cultivar can be useful in determining the basis of differential medicinal properties of the various cultivars.

Keywords: *Catharanthus roseus*; plant extracts; biochemical attributes; anti-oxidant properties

Introduction
Plant derived compounds have been used for treating diseases since inception of mankind. Numerous such examples of usage of local plants can still be found in various treatment forms, including Ayurveda, Homeopathy and Allopathy [1-3]. India is a country which is rich in biodiversity. Due to the vast geographical variation available in this country, around 45,000 plant species are present here. Out of them, at least 7,000 species are estimated to be of medicinal importance [4].
Catharanthus roseus is an important plant belonging to the Apocynaceae family [5]. All over the world, at least 100 cultivars of this plant species are known [6]. The two most common cultivars found in India are *rosea* and *alba*. They are distinguished by the colour of their flowers. While *rosea* cultivar has purple flowers, those of the *alba* cultivar are white in colour [7, 8].
The traditional system of medicine practiced in India involves herbal treatment which focuses on the medical potential of plants. *Catharanthus roseus* is one such plant recognized well in herbal treatment [9]. Numerous studies have highlighted their protective role in various diseases. *C. roseus* leaves and stem have been used for treatment of various types of non-communicable diseases (NCDs). It is known for its antitumour, anti-diabetic, anti-microbial, anti-oxidant and anti-mutagenic effects [10-14].
This study is carried out to evaluate the biochemical characteristics and antioxidant potential of the fresh leaves and stem extracts of the two commonly available cultivars of *C. roseus*.

Materials and Methods
Sample collection and preparation of plant extracts
The plants of both cultivars of *Catharanthus roseus* were collected from local places in Kolkata, West Bengal.
For preparation of plant extracts, fresh leaves and stems were separated from the plants, washed under tap water and grinded with the help of mortar pestle, and passed through a sieve. The homogenous fine powder thus obtained was weighed and equal quantities of all samples were dissolved in solvent. In the next step, these samples were centrifuged at 10,000 rpm for 5 minutes and the tests were carried out with the supernatants collected after centrifugation.

Qualitative analysis of the samples

1. Determination of presence of Sugar

The samples were subjected to Benedict's test for checking presence of simple carbohydrates. For performing this test, approximately 1 ml of sample was placed into a clean test tube. 2 ml (10 drops) of Benedict's reagent ($CuSO_4$) was added to it. Subsequently, the solution was heated in a boiling water bath for 5 minutes and color change was observed in the solution of test tubes. The observed data were collected and tabulated.

2. Determination of presence of Starch

Iodine solution was used to test for the presence of starch which turns the solution intense blue-black in color. Absence of starch is indicated by unchanged brown color of the solution. For performing this test, the test tubes containing samples were placed in a water filled beaker on a hot plate. The mixture was heated for 5 minutes while continuously stirring the water in beaker with a glass rod. The solutions were filtered and the filtrates were poured into clean test tubes. A few drops of iodine solution were added to the filtrates and observations on color change were noted.

3. Determination of presence of Lipid

For performing this test, 2 ml of ethanol was added to each sample, the mixtures were shaken well and allowed to settle for 2 minutes. Any clear liquid was emptied into a test tube containing 2 ml of distilled H_2O. Presence of lipid was indicated by appearance of a milky-white emulsion. A clear mixture indicated absence of fats in the sample.

4. Determination of presence of Vitamin C

This test is based on the fact that a blue substance, known as 2,6-dichlorophenolindophenol (DCPIP) changes its colour from blue to red in presence of acids. However, this colour is lost in the presence of certain chemicals, including ascorbic acid (vitamin C). Thus, DCPIP solution can be used to test for the presence of vitamin C samples. For performing this test, a small amount of the sample was put into a test tube to a depth of about 2cm. An equivalent similar amount of distilled water was added to it and the mixture was stirred with a glass rod. Next, it was allowed to stand for a few minutes. Subsequently, a small amount of the clear liquid was transferred into to a test tube and DCPIP solution was added to it drop wise and the observations on color change/disappearance were noted.

Quantitative analysis of the samples

1. Determination of presence of Protein

Presence of proteins was estimated with the help of Bradford Reagent. For this assay, equal volumes of sample and Bradford reagent were mixed and the mixtures were incubated at room temperature for 30 minutes in dark. O.D. was measured at 595 nm to test the absorbance of the standards and unknowns using Spectrophotometer. The exact concentration of proteins was calculated in each sample using standard curve prepared with Bovine serum albumin (BSA).

Calculation

Regression curve was prepared by plotting optical density on the 'y' axis against standard protein, i.e. BSA. The protein in the sample was calculated from this standard graph.

2. Estimation of DPPH radical scavenging activity

DPPH radical scavenging assay (with some modifications) was used to estimate the antioxidant activity of the samples [15]. The absorbances were measured at 540 nm in spectrophotometer. Percentage scavenging activity was calculated using the formulae: % Scavenging = [(Control absorbance- sample absorbance)/Control absorbance] * 100

Results and Discussion

Results of qualitative tests

Benedict's test

After the observation, the results of Benedict's test showed that reducing sugar is not present in any of the samples. Table 1 summarizes the findings.

Table 1: Summary of qualitative analysis of the samples for detection of sugars

Sample	*Rosea*		*Alba*	
	Stem	Leaf	Stem	Leaf
Reducing sugar	×	×	×	×

Iodine test for Starch

Results showed that starch is present in all samples of *Catharanthus roseus*. Table 2 summarizes the findings.

Table 2: Summary of qualitative analysis of the samples for detection of starch.

Sample	*Rosea*		*Alba*	
	Stem	Leaf	Stem	Leaf
Starch	✓	✓	✓	✓

Test for lipid

Results showed that lipid is not present at any of the samples of *Catharanthus roseus*. Table 3 summarizes the findings.

Table 3: Summary of qualitative analysis of the samples for detection of lipids.

Sample	*Rosea*		*Alba*	
	Stem	Leaf	Stem	Leaf
Lipid	×	×	×	×

Test for Vitamin C

Results showed that none of the samples of *Catharanthus roseus* has vitamin C. Table 4 summarizes the findings.

Table 4: Summary of qualitative analysis of the samples for detection of vitamin C.

Sample	*Rosea*		*Alba*	
	Stem	Leaf	Stem	Leaf
Vitamin C	×	×	×	×

Results of quantitative tests

Estimation of protein concentrations

Protein contents were estimated for all the samples. Figure 1 summarizes the findings.

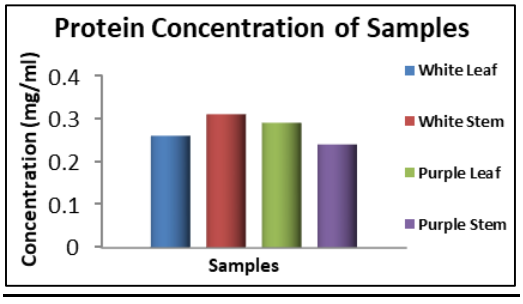

Fig 1: Protein concentrations of various samples of *Catharanthus roseus* (White: *alba*; Purple: *rosea*)

Determination of DPPH scavenging activity

To estimate the anti-oxidant capability, all the samples were analysed for their ability to scavenge DPPH. The findings of this assay are summarized in Figure 2.

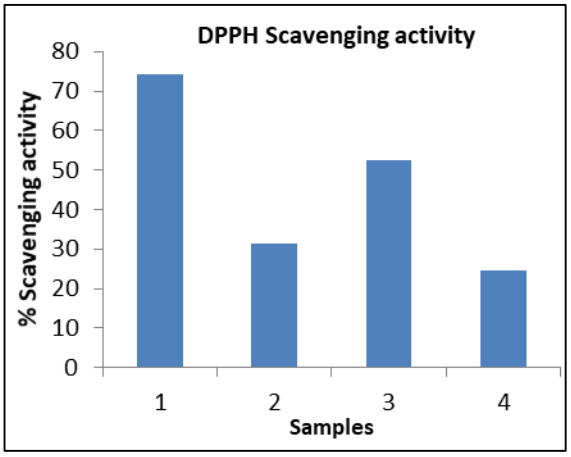

Fig 2: DPPH scavenging activity of various samples *Catharanthus roseus* (White: *alba*; Purple: *rosea*); 1-White Leaf, 2- White Stem, 3-Purple Leaf, 4- Purple Stem

From the present study we have observed that the biochemical parameters that have been studied show similar pattern across the two cultivars, *rosea* and *alba*. Moreover, the patterns do not show significant variation across various parts of the plants within a single cultivar. However, the ability to scavenge DPPH radical varies markedly, both in context of the cultivars as well as various parts of an individual plant. The maximum level of scavenging activity was observed in the extract prepared from leaves of the *alba* cultivar. In addition, extracts prepared from the leaves of both cultivars showed higher scavenging activity compared to the extracts prepared from their stems.

Conclusion

The observations of selective capability of DPPH scavenging activity despite having similar biochemical parameters can provide a clue to the selective medicinal effects that are obtained from a particular cultivar and not from the other. Further detailed study on this aspect can help towards better understanding of the underlying molecular mechanisms.

Acknowledgements

The authors are thankful to Chancellor, Techno India University, West Bengal for providing the necessary infrastructural facilities; and to Dr. Sirshendu Chatterjee for support and help. The authors also express gratitude to personnel of Acharya Jagadish Chandra Bose Indian Botanic Garden, Botanical Survey of India, Shibpur, Howrah, West Bengal for taxonomic authentication of the plants.

Conflicts of interest

Nil

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