Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 1065-1068
Received: 04-03-2019
Accepted: 06-04-2019

Malavika Bhattacharya
Department of Biotechnology, Techno India University, EM-4, Sector-V, Salt Lake, Kolkata, West Bengal, India

Chandrima Das
Department of Biotechnology, Techno India University, EM-4, Sector-V, Salt Lake, Kolkata, West Bengal, India

Sirshendu Chatterjee
Department of Biotechnology, Techno India University, EM-4, Sector-V, Salt Lake, Kolkata, West Bengal, India

Correspondence
Malavika Bhattacharya
Department of Biotechnology, Techno India University, EM-4, Sector-V, Salt Lake, Kolkata, West Bengal, India

Comparative analysis of anti-oxidative properties of leaf and stem extracts of the medicinal plant *Vinca rosea* cultivars, *Alba* versus *rosea*

Malavika Bhattacharya, Chandrima Das and Sirshendu Chatterjee

Abstract
The current study aimed at evaluating the anti-oxidative properties of extracts from leaves and stems of two commonly available cultivars, *alba* and *rosea*, of the well known medicinal plant *Vinca rosea*. Comparative analysis revealed presence of significantly higher levels of antioxidants in the leaves of *alba* cultivar in contrast to the levels in the respective parts of the *rosea* cultivar. A similar trend was also observed on the comparison between extracts from stems of both cultivars. These findings pave the way for identifying and characterizing the components which are selectively abundant in the *alba* variety, thereby focussing towards developing suitable herbal medicines in the future.

Keywords: Plant extract, anti-oxidant, *Vinca rosea*, phytochemicals, herbal medicine

Introduction
Traditional practice of using herbal medicines, especially in rural populations, has been known for ages. Their usage has been manifested right from ancient civilizations to modern day treatment methodologies (such as Ayurveda) (Sarker and Nahar, 2007) [18]. India is home of over 45,000 plant species, out of which at least 7,000 species are of medicinal importance (Shiva, 1996) [19]. According to a recent report by World Health Organization (WHO), primary healthcare needs of around 4.3 billion people are catered by such traditional plant based therapies (Attisso, 1983) [2]. Even in Allopathic practices, medicinal plants contribute tremendously as they rely on products either derived or synthesized from plant sources (Srivastava *et al.*, 1995) [22].

Vinca rosea is a well known medicinal plant belonging to the family Apocynaceae. It is also known by several additional names, including *catharanthus roseus*, *Lochnera rosea* and *Rose periwinkle* (Gayatri and chakravarty, 2013) [8]. Since it originated in island of Madagascar, it is commonly referred to as Madagascar periwinkle. Over 100 cultivars of this plant are known worldwide (Ku *et al.*, 2013) [11]. In India, two commonly found cultivars are *rosea* and *alba*. They are distinguished on the basis of the colour of their flowers (rosea- pink, alba-white) (Tolambiya and Mathur, 2016) [24].

It is a perenial sub shrub which grows throughout the year under most climatic conditions. The plant can grow upto a height between 20 cm and 1 m. Single solitary flowers, ranging in colour from white to pink-purple, grow at the tip of their leaf axil (Coroner and Litz, 1978; Swanberg and Dai, 2008) [4, 23]. The corolla of each flower has five petal lobes. Its fruits are arranged as follicle pairs having length of 2-4 cm and breadth of 3 mm. Each fruit contains several small, black, oblong or cylindrical seeds (Swanberg and Dai, 2008; Devis, 1981) [23, 7]. The leaves are elliptical, oval or oblong in shape, and are arranged in opposite pairs, their length ranging between 1-3 inches (Rajora *et al.*, 2013) [16]. It has been reported that they can grow under various types of sunlight exposure conditions as well as in different soil types (for example, slightly acidic or having higher moisture content) (Sain and Sharma, 2013) [17].

The two main classes of active compounds in *Vinca* are alkaloids and tannins. So far, at least 86 alkaloids have been extracted from plants in the *Vinca* genus. It is mainly cultivated for its alkaloids having anticancer activities (Jaleel *et al.*, 2006) [9]. The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine (Kulkarni *et al.*, 1999) [12]. Vinblastine and vincristine have already been characterized and have been found to have significant anti-cancer effects (Meenakshi *et al.* 2013; De Luca and Laflamme, 2001) [13, 6]. Water extracts of different parts of the plant are also known for their anti-diabetic property. The leaves are used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes (Cowley and Bennett, 1928) [4].

The leaves have been known to contain 150 useful alkaloids among other pharmacologically active compounds. Significant antihyperglycemic and hypotensive activity of the leaf extracts (hydroalcoholic or dichloromethane-methanol) have been reported in laboratory animals (Pillay *et al.,* 1959) [15]. Fresh leaf juice of *Vinca rosea* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits (Nammi *et al.,* 2003) [14]. In addition, its leaves and twigs have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats (Singh *et al.,* 2001) [20]. A recent study was carried out to evaluate the antidiabetic activity of *Vinca rosea* methanolic whole plant extracts in alloxan induced diabetic rats for 14 days. The methanolic whole plant extract at high dose (500 mg/kg) exhibited significant anti-hyperglycemic activity than whole plant extract at low dose (300 mg/kg) in diabetic rats. The methanolic extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of -cells of pancreas in diabetic rats. Histopathological studies reinforce the healing of pancreas, by methanolic *Vinca rosea* extracts, as a possible mechanism of their antidiabetic activity (Ahmed *et al.,* 2010) [1].

In this study, attempt was made to indentify whether the levels of active components show any difference with respect to variation in cultivars. Hence, phytochemical characterization of various parts of the plants from the two most prominent cultivars found in India was initiated.

Materials and Methods

Instruments and Apparatus

The instruments that were used for the experiments included Analytical digital weighing balance (Wensor), UV- Visible Spectrophotometer (Systronics117), Centrifuge (Remi PR 23).

Chemicals and Reagents

The reagents and chemicals that were used for quantitative estimation of polyphenols content, flavonoids content and their anti-oxidants activity in *Vinca rosea* samples inlcuded: Gallic Acid standard (Sigma), Ascorbic acid (Sigma), Sodium carbonate (Merck), Sodium Hydroxide(Merck), Anhydrous Sodium Nitrite (Merck), Anhydrous Aluminium Chloride(Merck), methanol (Merck), catechins (Sigma), Follin-ciocalteu reagent (Merck) and 2,2 diphenyl-1-picrylhydrazyl DPPH (Merck). All chemicals were of analytical graded. HPLC grade water (Merck) was used throughout the experiment.

Sample collection

Both varieties of *Vinca rosea* plants were collected from local regions of Kolkata, West Bengal.

Preparation of plant extracts

Leaves and stems were separated from the plants and washed under tap water. Extracts were prepared from either fresh or dried samples of leaves and stems. For extraction, fresh or dried samples were grinded using mortar pestle and passed through a sieve for obtaining a homogeneous fine powder. Equal quantity of the grinded sample of both parts of the two cultivars was extracted with 10 ml of solvent (methanol). The assays were carried out with 15 times diluted samples.

Determination of moisture content

The various parts of the plants were weighed before and after drying and moisture content was calculated to ensure complete drying of the samples.

Phytochemical analysis

Phytochemical analysis of all extracts was carried out for estimation of their quantity as well as for getting an idea about the variation in relative abundance both in context of plant part and variety.

Quantitative analysis of Total Phenolic contents (TPc) (Follin- Ciocalteau Assay)

Estimation of total phenolic contents of the samples was done by Folin-ciocalteau method, with slight modification (Singleton and Rossi, 1965) [21]. Breifly, 300 µl of 15 times diluted sample was mixed with 1500 µl of 1:10 dilued Floin-ciocalteau reagent. This mixture was incubated with 1200 µl of 7% sodium bicarbonate solution for 2 hours. Subsequently, absorbance was measured at 765 nm in spectrophotometer. The calibration curve was prepared by using a solution of Gallic acid (in methanol) as standard (Singleton and Rossi, 1965; Chatterjee *et al.,* 2014) [21, 3].

Quantitative analysis of Total Flavanoid contents (TFc) (Aluminium chloride Assay)

For determination of flavonoids, aluminium chloride colorimetric method (Jia and Tang, 1995) [10] was used with slight modification. For estimation of total flavanoids, 300 µl of 15 times diluted sample was incubated with 90 µl of 5% $NaNO_2$ for 5 minutes. This was followed by incubation of the mixture with 90 µl of 10% $AlCl_3$ for 6 minutes. Finally, the mixture was incubated with 600 µl of 1M NaOH and the absorbance was measured at 510 nm in spectrophotometer. The calibration curve was prepared by using a solution of Catechin as standard (Jia and Tang, 1995; Chatterjee *et al.,* 2014) [10, 3].

Statistical Analysis

For statistical analysis of the data, Graph Pad Prism7 was used. In addition, Paired t test was performed to study the significant changes among the data <0.005.

Results and Discussion

Moisture content

In this study, the moisture content was observed to be in the range of 70-80%. Table 1 shows the moisture content values for different samples.

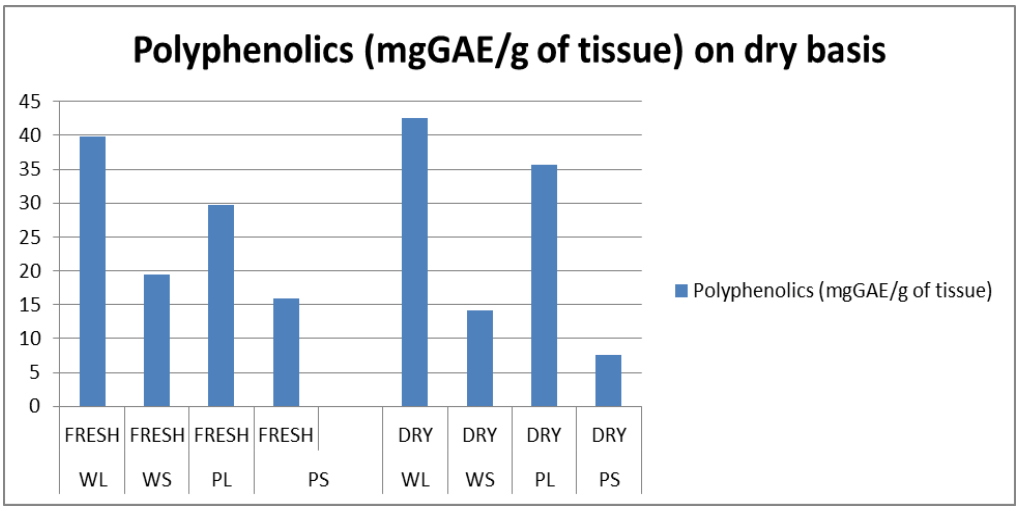
Table 1: Moisture content of various samples.

Sample Code	Dry/ Fresh	% Moisture Content (M)
WL	FRESH	78.89
WS	FRESH	80.158
PL	FRESH	73.336
PS	FRESH	76.835

WL – cultivar *alba*, leaves; WS – cultivar *alba*, stems;
PL – cultivar *rosea*, leaves; PS – cultivar *rosea*, stem

Total Phenolic contents (TPc)

Data analysis from the current study indicated that there is significant and consistent difference between TPc of the various samples. TPc was found to be higher in the leaf extracts of the *alba* cultivar in comparison to those of the *rosea* cultivar. This observation was consistent in both fresh and dried samples. A similar trend was observed on comparative analysis of the TPc of the stem extracts of both cultivars. Moreover, TPc levels were observed to be higher in the leaf extracts in comparison to the stem extracts in the respective cultivars (Fig. 1).

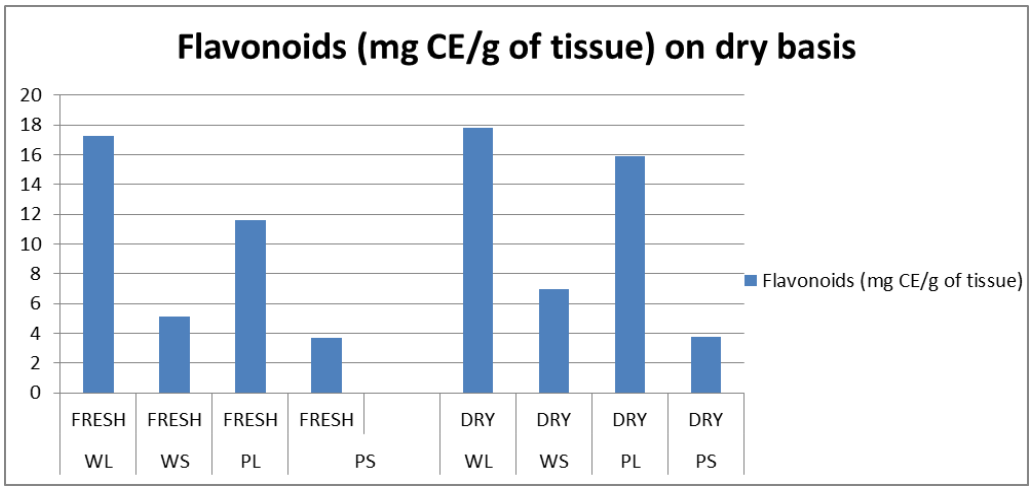


WL – cultivar *alba*, leaves; WS – cultivar *alba*, stems;
PL – cultivar *rosea*, leaves; PS – cultivar *rosea*, stem

Fig 1: Total Polyphenolics content (TPc) of various samples.

Total Flavanoid contents (TFc)

Analysis of Total Flavanoid contents (TFc) revealed a similar pattern as observed in TPc. Leaf extracts of the *alba* cultivar showed higher levels of TFc as compared to those obtained from the *rosea* cultivar. TFc of the stem extracts was also found to exhibit a similar trend. This pattern was found to be consistent in both fresh and dried samples. Further analysis also revealed that TFc was higher in the leaves as compared to the stems of each cultivar. Figure 2 summarises the results on TFcs.



WL – cultivar *alba*, leaves; WS – cultivar *alba*, stems;
PL – cultivar *rosea*, leaves; PS – cultivar *rosea*, stem

Fig 2: Total Flavanoids content (TFc) of various samples.

Concluding Remarks

Cantharanthus roseus has been known as a plant species of medicinal importance since ages. As part of folklore and other forms of medicinal practices, it is used to treat a wide variety of diseases, such as diabetes, leukemia, ulcers, wounds etc. Since around 100 different cultivars of this plant species are known across the world, understanding the variations in the levels of different active components in context of the various cultivars would be of importance. Since it has been observed that there is significant variation in the total phenolic contents and total flavanoid contents, both in terms of plant components and cultivars, these observations can be utilized for investigating how a particular cultivar may be selectively utilized for medicinal purposes in future.

Acknowledgements

The authors are thankful to chancellor, Techno India University, West Bengal for providing the necessary infrastructural and laboratory facilities.

References

1. Ahmed MF, Kazim SM, Ghori SS, Mehjabeen SS, Ahmed SR, Ali SM, *et al*. Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats. International Journal of Endocrinology. 2010; Volume 2010, Article ID 841090, 6 pages.
2. Attisso MA. Phytopharmacology and Phytotherapy. In: Bannerman RH, Burton J, (eds.), Traditional Medicine and Health Care Coverage. World Health Organization, Geneva, 1983.
3. Chatterjee S, Roy N, Saha A, Roy S, Chatterjee A, Hazra N, *et al*. Black Tea Consumption Enhance Antioxidant Status, Reduce Inflammatory Stress vis-à-vis Insulin resistance: Hint from a Small Clinical Cohort Study on Pre-diabetic Subjects. International Journal of Pharmaceutical Sciences Review and Research. 2014; 28(50):278-283.
4. Coroner RA, Litz Re. *In vitro* propagation of *catharanthus roseus*. Hort. Sci. 1978; 13:241-242.

5. Cowley RC, Bennett FC. "*Vinca rosea*" Australian Journal of Pharmacy. 1928; 9:61.
6. De Luca V, Laflamme P. The expanding universe of alkaloid biosynthesis, Curr Opin Plant Biol, 2001; 4:225-233.
7. Devis dR. Cell and tissue culture potential for *Catharanthus roseus* plant breeding. Phil Tran R. Soc. (London), 1981; 292:547-556.
8. Gayatri CL, Chakravarty R. Micropropagation in *cantharanthus roseus*. Internat. J Innov. Tech exploring eng (IJITee), 2013; 2(5).
9. Jaleel CA, Gopi R, Lakshmanan GMA, Panneerselvam R. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don, Plant Science, 2006; 171(2):271-276.
10. Jia ZS, Tang MC. The determination of flavonoid contents in mulverry and their scavenging effects ion superoxide radicals, Food Chemistry. 1999; 64:555-559.
11. Ku C, Chung WC, Chen LL, Kuo CH. The Complete Plastid Genome Sequence of Madagascar Periwinkle *Catharanthus roseus* (L.) G. Don: Plastid Genome Evolution, Molecular Marker Identification and Phylogenetic Implications in Asterids, PLOS ONE, 2013; 8(6):1-11.
12. Kulkarni RN, Baskaran K, Chandrashekara RS and Kumar S. Inheritance of morphological traits of periwinkle mutants with modified contents and yields of leaf and root alkaloids, Plant Breeding, 1999; 118(1):71-74.
13. Meenakshi K, Neha SL, Ramesh C, Sheela C. *Catharanthus roseus* and Prospects of its Endophytes: A New Avenue for Production of Bioactive Metabolites, IJPSR. 2013; 4(7):2705-2716.
14. Nammi S, Boini KM, Lodagala SD, Behara RBS. The juice of fresh leaves of *Catharanthus roseus* Linn. Reduces blood glucose in normal and alloxan diabetic rabbits, BMC Complementary and Alternative Medicine, 2003; 3: article 4.
15. Pillay PP, Nair CPM, Santi Kumari TN. *Lochnera rosea* as a potential source of hypotensive and other remedies, Bulletin of Research Institute of the University of Kerala, 1959; 1:51-54.
16. Rajora RK, Sharma NK, Sharma V. Effect of plant growth regulators on micro propagation of *Catharanthus roseus*. Internat. J Adva. Biotechnol. Res. 2013; 4(1):123-130.
17. Sain M, Sharma V. *Catharanthus roseus* (An anti-cancerous drug yielding plant) – A Review of Potential Therapeutic Properties. Int. J Pure App. Biosci. 2013; 1(6):139-142.
18. Sarker SD, Nahar L. Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. England: John Wiley and Sons, 2007; 283-359.
19. Shiva MP. Assessment of NTFP Resources of India: A report for Formulation of the National Forestry Action Programme, Vol. 1. Ministry of Environment and Forests, New Delhi, 1996; 5(13):54-55.
20. Singh SN, Vats P, Suri S *et al*. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats". Journal of Ethnopharmacology. 2001; 76(3):269-277.
21. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965; 16:144-153.
22. Srivastava J, Lambert J, Vietmeyer N. Medicinal Plants: A growing Role In Development. World Bank. Washington D.C, 1995.
23. Swanberg A, Dai W. Plant Regeneration of Periwinkle (*Catharanthus roseus*) via Organogenesis Hort Science, 2008; 43(3):832-836.
24. Tolambiya P, Mathur S. A Study on Potential Phytopharmaceuticals Assets in *Catharanthus Roseus* L. (*Alba*) Internat. J. Life Sci. Biotech. Pharma Res. 2016; 5(1):1-6.