Int. J. Chem. Sci.: 12(4), 2014, 1328-1336
ISSN 0972-768X
www.sadgurupublications.com

PHYTOCHEMICAL AND ELEMENTAL SCREENING ON LEAVES AND FLOWERS OF *CATHARANTHUS ROSEUS* : AN IMPORTANT MEDICINAL PLANT OF BANGLADESH

SHAHIN AZIZ[*], KOUSHIK SAHA[a], NASIM SULTANA[b], SHAMIM AHMED[b] and ABDULLAH AL-MANSUR[b]

Chemical Research Division, BCSIR, DHAKA, BANGLADESH
[a]Jahangirnagar University, SAVAR, BANGLADESH
[b]Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, DHAKA, BANGLADESH

ABSTRACT

Qualitative analysis of *Catharanthus roseus* plant confirms the presence of various phytochemicals like alkaloids, flavonoids, terpenoids, saponins, steroids, carbohydrates, anthaquinone glycosides etc in different extracts of its leaves and flowers. Some minerals have also been identified in the leaves and flowers of the plant by ICP-MS techniques.

Key words: Phytochemical screening, *Catharanthus roseus*, Plant species.

INTRODUCTION

Bangladesh is a rich storehouse of medicinal plants. All secondary metabolites in natural products can be termed bioactive molecules, as every diverse molecule possesses one or multiple kinds of pharmacological activities. The beauty of natural products is the uniqueness in their chemical and structural diversity that in relation to their various biological actions. Herbal medicine refers to use different parts of plants for medicinal purpose. Herbal drugs played an important role in drug discovery and were the basis of most early medicines. *Catharanthus roseus* commonly called Madagascar periwinkle is an evergreen shrub or herbaceous plant, which exhibits the anticancer activity due to the presence of vincristine and vinblastine[1]. The different parts of the medicinal plant, *Catharanthus roseus* is used for medicinal purposes for thousands of years in India, or subcontinetal. It is one of the chief herbs for treating dermatitis, abscesses, eczema, psoriasis,

[*]Author for correspondence; E-mail: shaziz2408@yahoo.com; Tel: 088-01713005011;
Fax: 88-02-8613022

sores, corns, ringworm, scabies, epilapsy, malaria, heart tonics and tumor. Mainly leaves and flowering tops of the plant are used for the extraction of oil. This oil has been found to have anti-bacterial and anti-yeast action. Researchers have been found that it can kill some intestinal parasites and have mild antibiotic effects. It was also observed that the its leaves are used extensively in folk medicine for decreasing sugar level of blood and showed significant anti-hyperglycemic effect[1-10]. The literature review reveals that scientists are much more interested to find out elements and organic compounds present in different parts of the plants. Some phytochemical works have been done previously on this medicinal plant in some other countries like India, Pakistan, Thailand etc. But in Bangladesh, no systematic phytochemical screening was done on extracts from different parts of this plant so far. After all, Bangladesh is not only geographically but zeologically different from other countries. It is well known that physiological changes do occur in plants due to changes in geographical sites, climatic and environmental conditions, which result in the production of nonidentical plant metabolites in this plant grown in different geographical regions. By considering theses facts and the medicinal values of the plant, phytochemical screening on different extracts from leaves and flowers solvent extract of *Catharanthus roseus* was carried out along with the identification of metals in dried leaves and flowers using ICP-MS techniques.

EXPERIMENTAL

Materials and methods

Collection of plant material

Fresh leaves and flowers of *C. roseus* were collected from the gardens of Botany Department of Dhaka University, Bangladesh in June, 2013 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. = 39512) has been deposited.

Preparation of the solvent extracts (Cold extraction)

Freshly collected leaves and flowers of *C. roseus* were dried in an oven at 38^{o}C and powdered by using a grinding machine. The powder of leaves (510 g) was extracted with methanol at room temperature for 5 days. The filtrate was dried into a gummy mass using rotary evaporator under reduced pressure. The methanol extract (40.0 g) was then triturated by n-hexane (100 mL x 3), then by ethyl acetate (100 mL x 3) and finally by n-butanol (100 mL x 3). Then these extracts were dried by using a rotary evaporator to get n-hexane extract (11.0 g), ethyl acetate extract (9.0 g) and n-butanol extract (8.5 g). The residual methanol soluble part (11.5 g) was finally denoted as methanol extract. Powder of the flower (200 g) was extracted successively with different solvents at room temperature. At first, it was

extracted with n-hexane for 5 days and the extract was dried to get a gummy mass (7.15 g) using rotary evaporator. Then the residual part of the flower was extracted with dichloromethane for 5 days and the extract was dried to gummy mass (5.80 g). Again the residual part was extracted with methanol and the filtrate was dried under reduced pressure to gummy mass (22.69 g). All solvents were of analytical grade and obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA).

Reagent preparation for the detection of different class of compounds

(a) 1% Picric acid: 1 mL of picric acid dissolved in 99 mL distilled water.

(b) Dragendroff's reagent: It is used for the detection of alkaloids. 0.17 g Bismuth nitrate was dissolved in 2 mL acetic acid solution and 8 mL of distilled water was added. (Solution A). 4 g of potassium iodide was dissolved in 10 mL acetic acid solution and 20 mL of distilled water was added (Solution B). Solution A & B were mixed and made up to 100 mL with distilled water.

(c) Mayer's reagent: It is used for the detection of alkaloids. Solution (A) was made by dissolving 0.68 g mercuric chloride in 30 mL of distilled water. Solution (B) was made by dissolving 2.5 g of potassium iodide in 10 mL of distilled water. Solution A & B were mixed and the volume was adjusted to 100 mL with distilled water.

(d) Molisch's reagent: 10 g of α-naphthol was dissolved in 100 mL of 95% alcohol. It is used for the detection of carbohydrates.

(e) Fehling's solution: It is used for the detection of reducing sugar. 3.4650 g Copper sulphate was dissolved in distilled water and the volume was made up to 50 mL (Solution A). 17.30 g of potassium sodium tartarate and 5 g of sodium hydroxide was dissolved in distilled water and volume was made up to 50 mL (Solution B) with water. Two solutions were mixed in equal volume prior to use.

Test for qualitative estimation of bioactive compounds from different solvent extracts of leaf and flower of *Catharanthus roseus*

The extracts of leaves and flowers of *Catharanthus roseus* were subjected to qualitative analysis to detect the presence of different classes of chemical constituents in the plant.

(i) Test for alkaloids

n-Hexane, ethyl acetate, n-butanol and methnol extracts of leaf & n-hexane, dichloromethane and methanol extracts of flower of *Catharanthus roseus* were warmed

separately with 2% H_2SO_4 for two min. It was filtered and few drops of following reagents were added, which indicated the presence of alkaloids.

(a) Dragendroff's reagent: A red precipitation indicated the positive test.

(b) Mayer's reagent: A creamy white colored indicated the positive test.

(c) Picric acid (1%): A yellow precipitation indicated the positive test.

(ii) Test for flavonoids

A small quantity of the extract was heated with 10 mL of ethyl acetate in boiling water for 3 min. The mixture was filtered and the filtrates were used for the following test.

(a) The filtrate was shaken with 1 mL of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed in ammonia layer indicating the presence of flavonoid.

(b) The filtrate was shaken with 1 mL of 1% ammonium chloride solution, where light yellow color was observed. It indicated the presence of flavonoid.

(iii) Test for carbohydrates

The extracts were shaken vigorously with water and then filtered. A few drops of Molisch's reagent was added to the aqueous filtrate, followed by vigorous shaking again. Concentrated H_2SO_4 (1 mL) was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive test.

(iv) Test for saponins

A small quantity of different extracts was diluted with 4 mL of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake, which indicated the positive test.

(v) Test for steroids

2 mL of acetic anhydride and 2 mL H_2SO_4 were added to the extracts. The color changed from violet to blue or green, which indicated the presence of steroids.

(vi) Test for anthraquinone glycosides (Borntragers's test)

Dil. H_2SO_4 was added to the extracts and boiled. Then it was filtered and cooled. To the cold filtrate, 3 mL of benzene was added and mixed. The benzene layer was separated

and ammonia (2 mL) solution was added to it. A rose pink to red color in ammonical layer was observed, which indicated positive test.

(vii) Test for cardiac glycosides (Legal's test)

To each extract, 1 mL of pyridine and 1 mL of sodium nitroprusside solution were added and observed. A deep red color was observed indicating the positive test.

(viii) Test for terpenoids (Salkowski test)

Each extract was mixed with 2 mL of chloroform and then concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration at the interface indicated positive result for the presence of terpeniods.

(ix) Test for gum and mucilages

Each extract was dissolved in 10 mL of distilled water and 25 mL of absolute alcohol was added to it with constant stirring. White or cloudy precipitate indicated the presence of gum and mucilages.

(x) Test for proteins and amino acids

Each extract was dissolved in 10 mL of distilled water and the filtrate was subjected to test the presence of proteins and amino acids.

(a) **Biuret test:** 2 mL filtrate was treated with one drop of 2% copper sulphate solution and then 1 mL of ethanol (95%) was added to it followed by excess potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

(b) **Ninhydrin test:** Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

Determination of elements

Major minerals/elements serve as structural components of tissues and function in cellular and basal metabolism, water and acid-base balance, clotting of blood and formation of bones and teeth etc.[11-15] Inductively Coupled Plasma-mass Spectrometry (ICP-MS) (Franklin, USA) was used for the determination of elements in the dry powder of leaves and flowers of *Catharanthus roseus.* Definite amount (10 g) of leaf and flower samples were taken and kept in furnace at 400^{o}C for 8 hrs. Then there was a formation of ash. The ash

samples were transferred into a mixture of HNO_3 and $HClO_4$ (2:1), which was prepared earlier in a Kjeldahl flask and left overnight. Before starting digestion, ice bath was used for cooling Kjeldahl flask. Then it was placed on heating mantle set at low temperature. Once boiling was initiated, red orange fumes of NO_2 were driven off. Gentle heating was continued until HNO_3 and H_2O was driven off. At this point, effervescent reaction occurred between organic material and $HClO_4$. Then the flask was put on heating mantle, which was at room temperature and digestion was allowed to proceed with occasional heating from mantle. It is important that the reaction between organic material and $HClO_4$ should not to go too fast, because then charring would occur. If charring occurred, immediately the flask is placed in ice bath to stop digestion. Then I mL of HNO_3 will added and resume gentle boiling. After reaction of test portion with $HClO_4$ was completed (identified by cessation of effervescent due to reaction between organic material and $HClO_4$). High heat was applied for Ca for 2 min. Then the flask was removed from heating mantle and left to cool. Each digest sample was transferred to 50 mL volumetric flask and diluted with H_2O. Then each digest sample from leaves and flowers of *Catharanthus roseus* was ready for elemental analysis in ICP-MS spectrometry.

RESULTS AND DISCUSSION

The results of the qualitative analysis of different extracts from leaves and flowers of *Catharanthus roseus* are presented in Table 1. The medicinal value of these plants lies in some chemical substances that have a definite physiological action on human body. The most important of these bioactive constituents of the plants are alkaloids, terpenoids, flavonoids, steroids, cardiac glycosides and protein compounds. Results showed that the polar compounds like alkaloids, carbohydrates, amino acids and glycosides are present in the polar fractions of extracts from leaves and flowers. But flavonoids, terpenoids and steroids are present in all the extracts; may be because of their polarity difference due to the availability of various substituents in their structure.

Table 1: Results of screening various extracts from leaves and flowers of *Catharanthus roseus*

Test for	LH	LE	LBn	LM	FH	FD	FM
Alkaloids							
(a) Dragendroff;s test	Negative	Positive	Positive	Positive	Negative	Positive	Positive
(b) Mayers test	Negative	Positive	Positive	Positive	Negative	Positive	Positive
(c) Picric acid test	Negative	Positive	Positive	Positive	Negative	Positive	Positive

Cont…

Test for	LH	LE	LBn	LM	FH	FD	FM
Flavonoids	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Carbohydrates	Negative	Positive	Positive	Positive	Negative	Negative	Positive
Saponins	Negative	Negative	Positive	Positive	Negative	Negative	Positive
Steroids	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Anthraquinone glycosides	Negative	Positive	Positive	Positive	Negative	Negative	Positive
Cardiac glycosides	Negative	Positive	Positive	Positive	Negative	Negative	Positive
Gum and mucilages	Positive	Positive	Negative	Negative	Positive	Positive	Positive
Terpenoids	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Proteins and amino acids	Negative	Positive	Positive	Positive	Negative	Negative	Positive

LH : Leaf n-hexane extract, LE : Leaf ethyl acetate extract, LBu-leaf butanol extract,
LM : Leaf methanol extract FH : Flower nahexane extract, F : Flower dichloromethane extract,
FM : Flower methanol extract

There was a tremendous legacy of folklore uses of different parts of *Catharanthus roseus* in medicine. At present for the prevention of several diseases, there is an increasing interest for the importance of dietary minerals. The trace elements, together with other essential nutrients, are necessary for growth, normal physiological functioning and maintaining life. They must be supplied by food, since the body can not synthesize them. So it is necessary to find out, which elements are present in the selected plant. The results of elemental detection in the leaves and flowers of *Catharanthus roseus* are presented in Table 2. Results indicated the presence of Na, K. Fe, Ni, S and Cl_2 in both; the leaves and flowers.

Table 2: Minerals/elements detection (calculated on dry matter basis) by ICP-MS techniques for leaves and flowers of *Catharanthus roseus*

Component as element	Leaf	Flower
Magnesium (Mg)	Absent	Absent
Calcium (Ca)	Absent	Absent
Sulphur (S)	Present	Present
Iron (Fe)	Present	Present
Sodium (Na)	Present	Present

Cont…

Component as element	Leaf	Flower
Chorine (Cl)	Present	Present
Potassium (K)	Present	Present
Manganese (Mn)	Absent	Absent
Chromium (Cr)	Absent	Absent
Lead (Pb)	Absent	Absent
Nickel (Ni)	Present	Present
Cadmium (Cd)	Absent	Absent

CONCLUSIONS

The plant studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent. We therefore, suggest further the isolation, purification, and characterization of the bioactive compounds of the leaf and flower part of *Catharanthus roseus* with a view to obtain some useful chemotherapeutic agents.

ACKNOWLEDGEMENT

We are thankful to the former Director, BCSIR Laboratories, Dhaka, Mr. Abu Anis Jahangir for providing necessary facilities to carry out this research work.

REFERENCES

1. S. K. Jain, Medicinal Plant Lore of Tribal of Bastar: Text Book of Ethanobotany, **19**, (1965) pp. 236-250.
2. S. K. Jain, Observation on Ethano Botany of the Tribal's of Central India in In (Ed.) LC (1981) pp. 193-198.
3. S. K. Jain, Medicinal Plants, National Books Trust of India Publication, New Delhi (1968).
4. S. K. Jain, J. Adv. Plant Sci., **13(1)**, 329-339 (2000).
5. A. J. Pareek, Phytological Res., **8(2)**, 91-04 (1995)
6. R. J. Suryanarayan, Aurvidyan, **9(4)**, 251-255 (1996).
7. U. S. Patil, Ph.D. Thesis, Amravati University, Amravati (2002).

8. Y. Asakawa, Progress in the Organic Chemistry of Natural Products, 65(1) (1995) p. 618.

9. A. Korolkovas, Essentials of Medicinal Chemistry, 2nd Ed. Willey, New York (1998) p. 111.

10. A. Burger, Medicinal Chemistry, Inter Science, York (1960) pp. 27-37.

11. R. Macrae, R. R. Robinson and M. J. Sadler, (Eds.) Encyclopedia of Food Science, Food Technology and Nutrition, San Diego, CA Academy Press Inc., Vol. 5 (1993).

12. F. H. Nielsen, Ultratrace Elements in Nutrition, Ann. Rev. Nutrition, **4**, 21-41 (1984).

13. M. Ozcan, Mineral Contents of Some Plants used as Condiments in Turkey, Food Chem., **84**, 437-440 (2004).

14. K. Gupta and L. C. Gupta, A. Food and Nutrition Facts and Figures, 5th Edn., Joypee Brothers, Medical Publishers (P) Ltd. New Delhi (2000).

15. N. S. Rajurkar and M. M. Damame, Elemental Analysis of Some Herbal Plants used in the Treatment of Cardiovascular Diseases by NAA and AAS, J. Radioanal. Nucl. Chem., **219(1)**, 77-80 (1997).

Accepted : 23.07.2014