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Original Research Article

Antioxidant and Cytotoxic Activities of Stem and Root Extracts of *Catharanthus roseus* Cultivated in Bangladesh

Md Farid Uddin[1], Ananta K. Das[2], Koushik Saha[1]*

[1]*Department of Chemistry, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
[2]*Department of Pharmacy, Gono Bishwabidyalay, Savar, Dhaka-1344, Bangladesh*

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ABSTRACT

Catharanthus roseus apocynaceae is an important medicinal plant for its traditional uses and a valuable source of new drugs. This research was aimed at comparing the antioxidant and cytotoxic activities of different extracts from the stems and roots of *C. roseus* cultivated in Bangladesh. Chloroform, n-hexane, ethyl acetate, and methanol were used to extract the stems and roots of *C. roseus*. The total antioxidant capacity (TAC), total phenolic content (TPC), and total flavonoid content (TFC) of the various stem and root extracts were evaluated. The brine shrimp lethality bioassay was performed to determine the cytotoxicity of the various extracts. The results revealed that the chloroform extract of the roots had a moderate TAC with an IC_{50} value of 93.79 µg/mL when compared to the standard ascorbic acid, while the others had a weak activity. Methanol, chloroform, and ethyl acetate root extracts had higher TPC with values of 84.87±4.24, 62.20±2.36, and 57.87±3.77 mg/g, respectively, as GAE (gallic acid equivalent). However, the n-hexane extract of the stems had higher TFC with a value of 209.50±1.33 mg/g, equivalent to quercetin. On the other hand, a higher value of TAC (203.38±5.05 mg/g) was obtained for the chloroform extract of the *C. roseus* roots. There was a significant cytotoxic effect of the n-hexane extracts of stems and roots with LC_{50} values of 4.51 and 9.54 µg/mL, respectively, compared to the standard vincristine sulfate. According to the findings of this study, *Catharanthus roseus* roots and stems growing in Bangladesh may possess strong natural antioxidant and anticancer activities.

Keywords: Antioxidant activity, *Catharanthus roseus*, Cytotoxicity, Flavonoid content, Phenolic content.

Introduction

Plants were the primary source of drugs when primitive man realized that he could relieve disease symptoms and soothe aches and pains by employing plant parts or their products.[1] Although advances in medicine and pharmacology have led to the discovery of some therapeutics, medicinal plants in the form of decoctions, teas, powders, poultices, and several other formulations are still in high demand and use today.[2] From prehistoric times, medicinal plants have been used for various diseases as a source of life-saving drugs for most of the world's population due to their bioactive constituents.[3,4] As a result, global interest in medicinal plants for extending life and improving health has grown significantly.[5] Cancer is one of the leading causes of morbidity worldwide. The unregulated reproduction and survival of abnormal cells are characteristics of cancer cells. According to the International Agency for Research on Cancer (IARC), there were 8.2 million cancer deaths globally in 2012, with this figure expected to climb to 13 million by 2030. Because chemotherapy may harm healthy cells while killing cancer cells, resulting in unfavourable side effects, there is a constant effort to find novel anticancer medicines.[6] Cancer is linked to specific body areas where cells proliferate out of control. Such malignant tumours are treated and managed using products with plant origins.
Plants are abundant in phytochemicals, which have biological effects and can be used to treat cancer.[7] There is an increasing demand for herbal products as alternative medicine. About 80% of the world population uses herbal medicines for primary healthcare, particularly in developing countries. They have stood the test of time for their safety, efficacy, and cultural acceptability.[8] *Catharanthus roseus*, locally known as Nayantara, is found in different regions all over Bangladesh; and belongs to the Apocynaceae family. It is an evergreen subshrub that can grow up to 1 meter tall. The leaves are oval to oblong, 2.6-9 cm long and 1-3.6 cm broad, glossy green, hairless, with a pale midrib and 1-1.9 cm long petioles, which are arranged in opposite pairs. The flowers are white to dark pink with a darker red center, a 2.6-3 cm long basal tube, and a 2.1-5 cm corolla with five petal-like lobes. The fruit is a pair of follicles 3-4 cm long and 3 mm broad.[9] *Catharanthus roseus* is cultivated in gardens all over Bangladesh as an ornamental and medicinal plant. It has long been used in various medical systems to treat cancer,[10,11] diabetes,[12,13] high blood pressure, inflammation,[12] angiogenesis effects,[12] malaria,[14] and is also potent for anti-bacterial activities.[15,16] *Catharanthus roseus* is an important source of indole alkaloids, which are found in all parts of the plant. Antineoplastic alkaloids, such as vincristine and vinblastine, are used to treat cancer,[17] while antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine have been found in the roots.[18] Previous phytochemical screenings also resulted in the isolation of flavonoids and flavonoids glycosides like kaempferol,[19] kaempferol trisaccharides,[20] quercetin,[21] hirsutidin,[22] quercetin trisaccharides,[19] syringetin glycosides,[23] malvidin,[20] malvidin-3-O-glycosides,[23] malvidin-3-O-(6-O-p-coumaroyl),[23] petunidin,[21] petunidin-3-O-glycosides,[23] petunidin-3-O-(6-O-p-coumaroyl),[23] hirsutidin-3-O-glycosides,[23] and hirsutidin-3-O-(6-O-p-coumaroyl).[23] The present study was conducted to compare the antioxidant and cytotoxic activities of various extracts from the stems and roots of *C. roseus* grown in Bangladesh.

*Corresponding author. E mail: ksaha@juniv.edu
Tel: *+880715258598*

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Materials and Methods

Collection and identification of plant materials

Stems and roots of *C. roseus* were collected from the gardens at Jahangirnagar University, Savar, Bangladesh, from April 2020 to September 2020. The plant materials were identified by a taxonomist at the Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. 39512) was assigned.

Extraction of Catharanthus roseus stems and roots

The stems and roots of *C. roseus* were cut into little pieces and allowed to dry thoroughly under the shade. After that, the dried stems and roots were then ground into powder using a grinding machine. The dried powder of the stems and roots was then extracted at room temperature with n-hexane, chloroform ($CHCl_3$), ethyl acetate, and methanol (MeOH). The extracts were then collected and dried on a rotary evaporator to eliminate the solvents. The extracts were denoted as CR-1 (n-hexane extract of the stem), CR-2 ($CHCl_3$ extract of the stem), CR-3 (ethyl acetate extract of the stem), CR-4 (MeOH extract of the stem), CR-5 (n-hexane extract of the root), CR-6 ($CHCl_3$ extract of the root), CR-7 (ethyl acetate extract of the root), CR-8 (MeOH extract of the root).

Determination of the antioxidant capacity of Catharanthus roseus extracts

The free radical scavenging activity of the *C. roseus* extracts was analyzed spectrophotometrically by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.[24] DPPH is a reactive free radical that functions as an electron acceptor and causes the oxidation of other substances. Antioxidants, on the other hand, serve as electron donors. They neutralize DPPH by being oxidized themselves. DPPH is found as a dark-coloured crystalline powder composed of stable free-radical molecules and forms a deep violet colour in solution. Different concentrations (6.25, 12.5, 25, 50, 100, 200, 400, or 800 mg/ml in methanol) of ascorbic acid solution (1 ml) as well as *C. roseus* stem or root extract solution (1 ml) were mixed separately with 3 ml of 0.4 mM DPPH solution. After 30 minutes in the dark, the mixture was measured for absorbance at 517 nm with a UV-Visible spectrophotometer, with ascorbic acid serving as a positive control. The entire procedure was performed three times for each test solution. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH free radical scavenging is indicated by the deep violet colour changing to pale yellow or colourless, and it was calculated using the formula:

$$\text{Percentage inhibition} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100$$

The IC_{50} is the concentration at which 50% of the total DPPH free radical is scavenged or neutralized and can be determined by the linear regression method by plotting the percentage inhibition against the corresponding concentration.

Evaluation of the total phenolic content of Catharanthus roseus extracts

The total phenol content of the *C. roseus* extracts was evaluated using the Folin-Ciocalteu reagent (FCR) based on colorimetric reaction.[25] The FCR, a mixture of phosphomolybdate and phosphotungstate, is used for the colorimetric assay of phenolic and polyphenolic antioxidants.[26] An aliquot of 1 mL of plant extract (200 µg/mL) or standard of different concentrations ((6.25, 12.5, 25, 50, 100, or 200 µg/mL) was taken in test tubes. Then, 5 mL of FCR and 7.5 % Na_2CO_3 were added and thoroughly mixed. Furthermore, the standard test tubes were incubated for 30 minutes to complete the reaction, whereas the extract solution test tubes were incubated for 1 hour at room temperature. A UV-VIS spectrophotometer was used to measure absorbance at 765 nm. Gallic acid was used as a standard to produce a calibration curve. The phenol content in the extracts is expressed as mg of gallic acid equivalents (GAE)/g of extract. The total phenolic content in plant extracts in gallic acid equivalents (GAE) was calculated using the following equation:

$C = (c \times V)/m$,

Where C is the total phenolic content of the extract (mg/g plant extract, in GAE), c is the concentration of gallic acid determined from the calibration curve (mg/ml), V is the volume of extract (ml), and m is the weight of crude plant extract (g).

Estimation of the total flavonoid content of Catharanthus roseus extracts

The total flavonoid content in plant extracts was measured using the colorimetric technique of aluminum chloride.[27] In this procedure, 1 mL of plant extract (200 µg/mL) or standard (3.125, 6.25, 12.5, 25, 50, 100, or 200 g/mL) was placed in the test tube. The test tube was filled with 3 mL of methanol, 200 µl of 10% aluminum chloride solution, and 200 µl of 1M potassium acetate solution. Finally, 5.6 ml of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 minutes at room temperature, and the absorbance of the solution was measured at 415 nm using a spectrophotometer against a blank. Quercetin was used as a reference standard, and the flavonoid content of the extracts was calculated as mg of quercetin equivalents (QE)/ g of extract. The total flavonoid content in the plant extracts in quercetin equivalents was calculated using the following equation:

$C = (c \times V)/m$

Where C is the total flavonoid content of the extract (mg/g plant extract, in quercetin equivalents), c is the concentration of quercetin determined from the calibration curve (mg/ml), V is the volume of the extract (ml), m is the weight of crude plant extract (g).

Determination of the total antioxidant capacity of Catharanthus roseus extracts

The antioxidant activity of *C. roseus* extracts was determined by the phosphomolybdenum method as described by Prieto et al. [28] The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of a green PO_4/Mo (V) complex at acidic pH. It is assumed that the molybdenum in the complex is easier to reduce, and an electron-transfer reaction occurs between reactants and Mo (VI), resulting in the creation of a green phosphate/Mo (V) complex with a maximum absorbance of 695 nm. In this method, 300 µl of each plant extract or standard of different concentrations (3.125, 6.25, 12.5, 25, 50, 100, or 200 g/mL) solution was placed in separate test tubes. An aliquot of 3 ml of phosphomolybdate reagent was added to each test tube. The test tubes were incubated at 95°C for 90 minutes to complete the reaction. Then the absorbance was measured at 695 nm using a spectrophotometer. The total antioxidant activity in the plant extracts is expressed as ascorbic acid equivalents and calculated using the following equation:

$C = (c \times V)/m$

Where C is the total antioxidant content of a compound (mg/g plant extract, in ascorbic acid equivalents), c is the concentration of ascorbic acid determined from the calibration curve (mg/ml), V is the volume of extract (ml), and m is the weight of crude plant extract (g).

Evaluation of the cytotoxic activity of Catharanthus roseus extracts

The cytotoxic activity of *C. roseus* extracts was performed by the brine shrimp lethality bioassay.[29] In this method, 32 mg of each of the test samples were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO), and finally, the volume was made up to 20 ml with seawater. As a result, the stock solution had a concentration of 1600 µl/ml. The solution was then serially diluted to 6.25, 12.5, 25, 50, 100, 200, 400, or 800 µl/ml with seawater. The plant extract solution (5 mL) was then mixed with 5 mL of saltwater containing 10 nauplii and incubated at room temperature for 24 hours. The test tube was examined after 24 hours with a magnifying glass against a black background, and the number of survived nauplii in each tube was counted. The percentage of brine shrimp nauplii mortality at each concentration was calculated using these data. The mortality was calculated using the formula:

$$Percentage\ mortality = \frac{No.of\ nauplii\ taken - No.of\ nauplii\ alive}{No.of\ nauplii\ alive} \times 10$$

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The effectiveness of the concentration mortality relationship of plant products is usually expressed as a median lethal concentration (LC_{50}). This represents the chemical concentration that produces death in half of the test subjects after a certain exposure time and is determined by the linear regression method by plotting percentage mortality against the corresponding log of concentration. Vincristine sulfate was used as a positive control in this assay to compare the cytotoxicity of the test samples.

Statistical analysis

Microsoft Excel 2019 was used to analyze the data, plot a calibration curve of several standards, and prepare the result tables and figures. The data are presented as the mean ± SD (Standard Deviation) of three consecutive experiments.

Results and Discussion

DPPH free radical scavenging activity of Catharanthus roseus extracts

An antioxidant molecule donates an electron to DPPH. The DPPH is decolorized, which can be quantified using changes in absorbance. Table 1 and Figure 1 show the results of the antioxidant activity of *Catharanthus roseus* extracts. The DPPH radical scavenging is a widely used method to evaluate compounds' free radical scavenging activity or the antioxidant capacity of plant extracts. DPPH is a stable nitrogen-centered free radical that changes colour from violet to yellow when reduced by either hydrogen or electron donation.[30] The greater the decolorizing action, the greater the antioxidant activity, as indicated by a lower IC_{50} value. Substances that can perform this reaction can be considered antioxidants and, therefore, radical scavengers.

The extracts' DPPH radical scavenging assays revealed that chloroform extract of root (CR-6) had a moderate free radical scavenging capacity, with an IC_{50} value of 93.79 µg/mL when compared to normal ascorbic acid. Furthermore, the methanol extract of the roots (CR-8) and chloroform extract of the stem (CR-2) had weak free radical scavenging capacity with IC_{50} values of 201.85 and 224.32 µg/mL, respectively. Other extracts demonstrated non-significant free radical scavenging capacity compared to the standard.

Total phenolic and flavonoid contents of Catharanthus roseus extracts

The total phenolic content of *Catharanthus roseus* extracts was measured using the Folin-Ciocalteu reagent and expressed as Gallic acid equivalents (GAE) per gram of plant extract. It was determined using the standard curve of gallic acid ($y = 0.0075x + 0.0462$; $R^2 = 0.999$). The result of the total phenolic content is presented in Table 2 and Figure 2. Due to the high reactivity of phenolic compounds, antioxidative capabilities have emerged, allowing for the addition of hydrogen or electron donors, which can help to stabilize the unpaired electron.[31] Epidemiological research has linked low plasma ascorbate, tocopherol, and carotenoid concentrations to cardiovascular diseases. Oxidation reactions have been suggested to play a significant role in atherogenesis.[32] Inhibiting the cytochrome P450 superfamily of enzymes, which breaks down several pro-carcinogens into reactive chemicals before they interact with DNA and cause malignant transformation, has been demonstrated to reduce the development of reactive intermediates. The results of the present study showed that the methanol (CR-8), chloroform (CR-6), and ethyl acetate (CR-7) extracts of the root have higher phenolic content with 84.87±4.24, 62.20±2.36, and 57.87±3.77 mg/g, respectively, as equivalent to standard GAE. A higher phenolic content indicates good antioxidant properties.

The total flavonoid content of *Catharanthus roseus* extracts was determined using the aluminum chloride colorimetric technique. Total flavonoid content was determined using the quercetin standard curve ($y=0.008x + 0.0483$; $R^2 = 0.9989$) and expressed as quercetin equivalents (QE) per gram of plant extract. The results of the total flavonoid content are shown in Table 3 and Figure 3. Flavonoids are commonly known for their antioxidant properties, and the flavonoid content of the investigated plant is important for evaluating its antioxidant properties. There is a positive correlation between flavonoid content and the antioxidant capacity in plant extracts. The procedure involves the formation of a complex between flavonoid and $AlCl_3$, which results in a yellow-coloured solution.

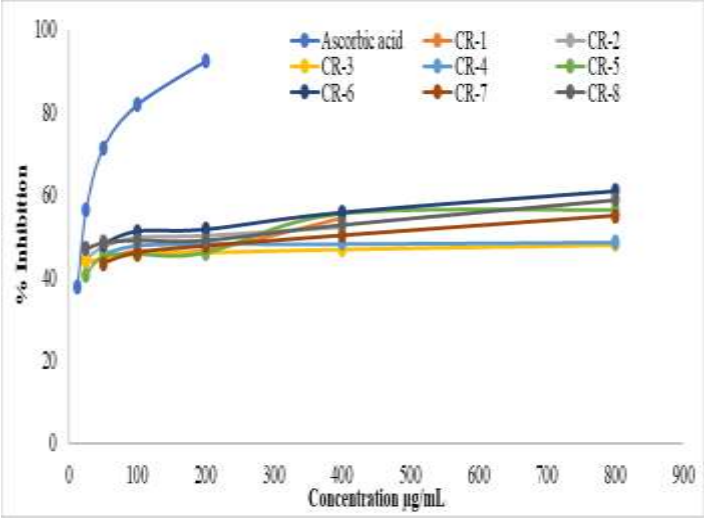

Figure 1: Comparative percentage inhibition of the various *Catharanthus roseus* extracts as determined by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.

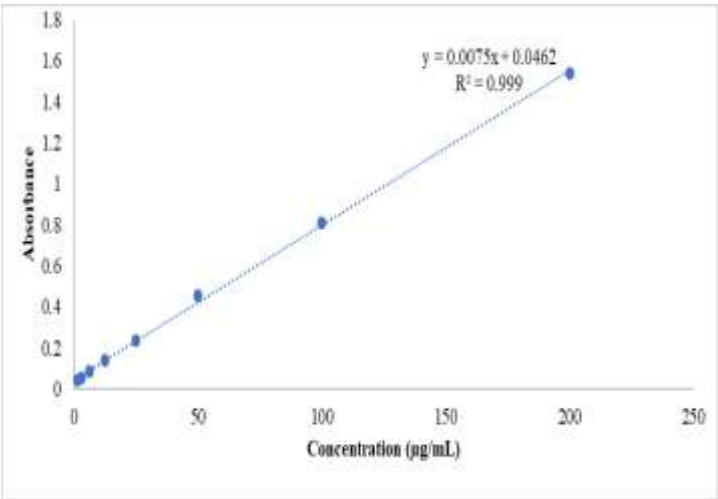

Figure 2: Calibration curve of gallic acid.

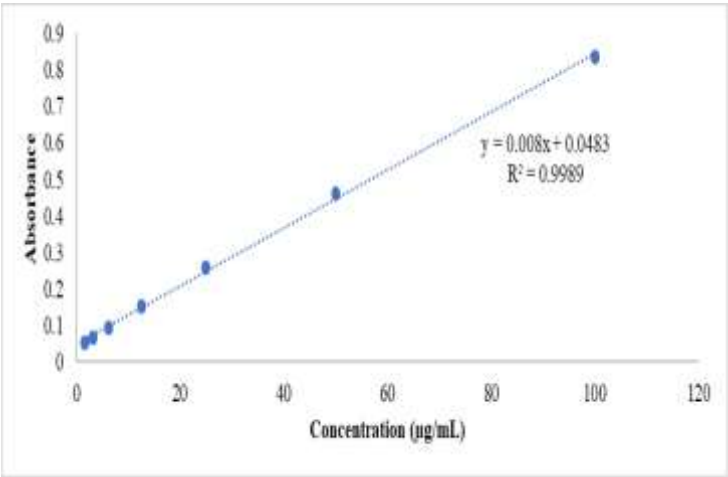

Figure 3: Calibration curve of quercetin.

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Table 1: IC_{50} values of the various *Catharanthus roseus* extracts as determined by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.

Sample	IC_{50} value (µg/mL)
Ascorbic acid	6.54
n-Hexane extract of the stem (CR-1)	258.04
Chloroform extract of the stem (CR-2)	224.32
Ethyl acetate extract of the stem (CR-3)	1223.48
Methanol extract of the stem (CR-4)	1236.4
n-Hexane extract of root (CR-5)	358.33
Chloroform extract of root (CR-6)	93.79
Ethyl acetate extract of root (CR-7)	415.18
Methanol extract of root (CR-8)	201.85

After that, the absorbance is then measured spectrophotometrically to determine the presence of flavonoid compounds. The analysis of the total flavonoid content in *Catharanthus roseus* extracts revealed that the n-hexane extract of the stem (CR-1) had a higher content of 209.50±1.33 mg/g of flavonoids, equivalent to quercetin. The higher flavonoid content is associated with increased antioxidant activity.

Total antioxidant capacity of Catharanthus roseus extracts

An antioxidant is any substance that, when presented at a low concentration compared to those of an oxidizable substrate (proteins, lipids, carbohydrates, and DNA), significantly delays or prevents oxidation of that substrate.[33,34] The main function of antioxidants is to protect the body against the destructive effects of free radical damage.[35] Phenolic compounds are primarily responsible for the antioxidant effect. The antioxidant capacity of phenolic compounds is mainly due to their redox properties that can play an important role in absorbing and neutralizing free radicals, quenching singlet, and triplet oxygen, or decomposing peroxides.

Table 2: Total phenolic content (TPC) in the various *Catharanthus roseus* extracts.

Sample	Abs.	Wt. of Plant Extract (g/mL)	GAE conc. (C) (µg/ml)	GAE conc. (C) (mg/ml)	V (mL)	c*V (mg)	TPC as GAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
CR-1	0.087	0.0002	5.440	0.005	1	0.005	27.20	29.53 ± 3.30
	0.094	0.0002	6.373	0.006	1	0.006	31.87	
CR-2	0.114	0.0002	9.040	0.009	1	0.009	45.20	43.87 ± 1.89
	0.11	0.0002	8.507	0.009	1	0.009	42.53	
CR-3	0.077	0.0002	4.107	0.004	1	0.004	20.53	21.87 ± 1.89
	0.081	0.0002	4.640	0.005	1	0.005	23.20	
CR-4	0.086	0.0002	5.307	0.005	1	0.005	26.53	30.56 ± 5.53
	0.098	0.0002	6.907	0.007	1	0.007	34.53	
CR-5	0.078	0.0002	4.240	0.004	1	0.004	21.20	23.53 ± 3.30
	0.085	0.0002	5.173	0.005	1	0.005	25.87	
CR-6	0.137	0.0002	12.107	0.012	1	0.012	60.53	62.20± 2.36
	0.142	0.0002	12.773	0.013	1	0.013	63.87	
CR-7	0.129	0.0002	11.040	0.011	1	0.011	55.20	57.87± 3.77
	0.137	0.0002	12.107	0.012	1	0.012	60.53	
CR-8	0.169	0.0002	16.373	0.016	1	0.016	81.87	84.87 ± 4.24
	0.178	0.0002	17.573	0.018	1	0.018	87.87	

GAE: Gallic acid equivalent

Table 3: Total flavonoid content (TFC) in the various *Catharanthus roseus* extracts.

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	QE conc. (C) (µg/ml)	QE conc. (C) (mg/ml)	V (mL)	c*V (mg)	TFC as QE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
CR-1	0.385	0.0002	42.088	0.042	1	0.042	210.44	209.50 ± 1.33
	0.382	0.0002	41.713	0.042	1	0.042	208.56	
CR-2	0.089	0.0002	5.088	0.005	1	0.005	25.44	25.75 ± 0.44
	0.09	0.0002	5.213	0.005	1	0.005	26.06	
CR-3	0.062	0.0002	1.713	0.002	1	0.002	8.56	9.50 ± 1.33
	0.065	0.0002	2.088	0.002	1	0.002	10.44	

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CR-4	0.05	0.0002	0.213	0.000	1	0.000	1.06	1.06 ± 0.00
	0.05	0.0002	0.213	0.000	1	0.000	1.06	
CR-5	0.131	0.0002	10.338	0.010	1	0.010	51.69	50.44 ± 1.77
	0.127	0.0002	9.838	0.010	1	0.010	49.19	
CR-6	0.062	0.0002	1.713	0.002	1	0.002	8.56	11.38 ± 3.89
	0.071	0.0002	2.838	0.003	1	0.003	14.19	
CR-7	0.07	0.0002	2.713	0.003	1	0.003	13.56	15.44 ± 2.65
	0.076	0.0002	3.463	0.003	1	0.003	17.31	
CR-8	0.061	0.0002	1.588	0.002	1	0.002	7.94	8.88 ± 1.33
	0.064	0.0002	1.963	0.002	1	0.002	9.81	

QE: Quercetin equivalents

Table 4: Total antioxidant capacity (TAC) of the various *Catharanthus roseus* extracts.

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	AAE conc. (C) (µg/ml)	AAE conc. (C) (mg/ml)	V (mL)	c*V (mg)	TAC as AAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
CR-1	0.062	0.0002	13.095	0.013	1	0.013	65.48	64.29 ± 1.68
	0.061	0.0002	12.619	0.013	1	0.013	63.10	
CR-2	0.081	0.0002	22.143	0.022	1	0.022	110.71	108.33 ± 3.37
	0.079	0.0002	21.190	0.021	1	0.021	105.95	
CR-3	0.079	0.0002	21.190	0.021	1	0.021	105.95	103.57 ± 3.37
	0.077	0.0002	20.238	0.020	1	0.020	101.19	
CR-4	0.067	0.0002	15.476	0.015	1	0.015	77.38	75.00 ± 3.37
	0.065	0.0002	14.524	0.015	1	0.015	72.62	
CR-5	0.071	0.0002	17.381	0.017	1	0.017	86.90	84.52 ± 3.37
	0.069	0.0002	16.429	0.016	1	0.016	82.14	
CR-6	0.121	0.0002	41.190	0.041	1	0.041	205.95	203.38 ± 5.05
	0.118	0.0002	39.762	0.040	1	0.040	198.81	
CR-7	0.116	0.0002	38.810	0.039	1	0.039	194.05	197.62 ± 5.05
	0.119	0.0002	40.238	0.040	1	0.040	201.19	
CR-8	0.09	0.0002	26.429	0.026	1	0.026	132.14	127.38 ± 6.73
	0.086	0.0002	24.524	0.025	1	0.025	122.62	

AAE: Ascorbic Acid Equivalent

The phospho-molybdenum method is commonly based on the reduction of Mo (V1) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with maximum absorption at 695 nm. Total antioxidant capacity of plant extracts is expressed as the number of gram equivalents of ascorbic acid ($y=0.0021x + 0.0345$; $R^2 =0.9841$). The results of the total antioxidant determination are shown in Table 4 and Figure 4. Plant extracts' antioxidant activity is derived not just from phenolic compounds, but also from the presence of additional antioxidant secondary metabolites such as volatile oils, carotenoids, and vitamins, among others. It is mainly due to their redox properties.[36] The present study of the total antioxidant activity of *Catharanthus roseus* revealed that chloroform extract (CR-6) and ethyl acetate extract (CR-7) of the root have a significant total antioxidant capacity with values of 203.38±5.05 and 197.62±5.05 mg/g, respectively, as equivalent to standard ascorbic acid. Again, the total antioxidant capacity of methanol extract of the root (CR-8), chloroform extract (CR-2), and ethyl acetate extract (CR-3) of the stem were moderate, with values of 127.38±6.73, 108.33±3.37 & 103.57±3.37 mg/g, respectively. When compared to the standard, the total antioxidant capacity of the other extracts was insignificant.

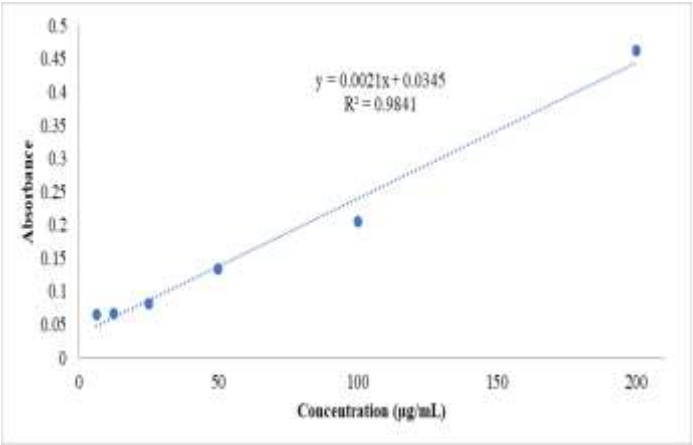

Figure 4: Calibration curve of ascorbic acid

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Table 5: Data for brine shrimp lethality bioassay for vincristine sulfate.

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_o}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
0.313	-0.50446	10	10	0	0		
0.625	-0.20412	10	10	0	0		
1.25	0.09691	13	11	2	15	0.544	3.501
2.5	0.39794	10	8	5	50		
5	0.69897	13	6	7	54		
10	1	10	2	8	80		

Table 6: Data for brine shrimp lethality bioassay for the n-hexane extract of *Catharanthus roseus* stems (CR-1).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_o}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	4	6	60		
50	1.699	10	2	8	80		
100	2.000	10	1	9	90	0.6546	4.515
200	2.301	10	0	10	100		
400	2.602	10	0	10	100		
800	2.903	10	0	10	100		

Table 7: Data for brine shrimp lethality bioassay for the chloroform extract of *Catharanthus roseus* stems (CR-2).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_o}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	10	0	0		
50	1.699	10	10	0	0		
100	2.000	10	9	1	10	2.9159	824.00
200	2.301	10	7	3	30		
400	2.602	10	6	4	40		
800	2.903	10	5	5	50		

Table 8: Data for brine shrimp lethality bioassay for the ethyl acetate extract of *Catharanthus roseus* stems (CR-3).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_o}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	10	0	0		
50	1.699	11	10	1	9		
100	2.000	10	8	2	20	2.4770	299.93
200	2.301	10	6	4	40		
400	2.602	10	5	5	50		
800	2.903	10	2	8	80		

Cytotoxic activity of Catharanthus roseus extracts

The brine shrimp lethality bioassay (BSLT) for cytotoxic activity is a general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts. BSLT is a bioassay for a bioactive compound that predicts cytotoxicity and pesticide activity.[37] The cytotoxic activity of all the extracts was assessed using the BSLT. After 24 hours, the test tubes were inspected, and the number of surviving nauplii in each tube was counted. From this data, the percentage of lethality was calculated for each concentration. The cytotoxicity test results (Table 5 and Figure 5) of the extracts demonstrated that the n-hexane extract of the stem and root have a significant cytotoxic effect, with LC_{50} values of 4.515 and 9.54 µg/mL, respectively, compared to standard vincristine sulfate with an LC_{50} value of 3.501 µg/mL. Also, the stem and root methanol extracts showed moderate cytotoxic activity with LC_{50} values of 73.67 and 106.376 µg/mL, respectively. Other extracts showed non-significant cytotoxicity compared to the standard.

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Table 9: Data for brine shrimp lethality bioassay for the methanol extract of *Catharanthus roseus* stems (CR-4).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_0}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	8	2	20	1.867	73.67
50	1.699	10	7	3	30		
100	2.000	10	3	7	70		
200	2.301	10	2	8	80		
400	2.602	10	0	10	100		
800	2.903	10	0	10	100		

Table 10: Data for brine shrimp lethality bioassay for the n-hexane extract of *Catharanthus roseus* roots (CR-5).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_0}\times 100$	LogLC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	4	6	60	0.9798	9.45
50	1.699	10	3	7	70		
100	2.000	10	2	8	80		
200	2.301	10	1	9	90		
400	2.602	10	0	10	100		
800	2.903	10	0	10	100		

Table 11: Data for brine shrimp lethality bioassay for the chloroform extract of *Catharanthus roseus* roots (CR-6).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_0}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	10	0	0	2.7674	585.33
50	1.699	10	8	2	20		
100	2.000	10	8	2	20		
200	2.301	10	7	3	30		
400	2.602	10	6	4	40		
800	2.903	10	4	6	60		

Table 12: Data for brine shrimp lethality bioassay for the ethyl acetate extract of *Catharanthus roseus* roots (CR-7).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_0}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	10	0	0	3.386	2433.55
50	1.699	10	9	1	10		
100	2.000	10	9	1	10		
200	2.301	10	8	2	20		
400	2.602	10	7	3	30		
800	2.903	10	6	4	40		

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Table 13: Data for brine shrimp lethality bioassay for the methanol extract of *Catharanthus roseus* roots (CR-8).

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality $M=\frac{N_0-N_1}{N_0}\times 100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
25	1.398	10	9	1	10	2.0268	106.376
50	1.699	10	8	2	20		
100	2.000	10	5	5	50		
200	2.301	10	3	7	70		
400	2.602	10	0	10	100		
800	2.903	10	0	10	100		

Table 14: LC_{50} values of the various extracts of *Catharanthus roseus* as determined by the brine shrimp lethality bioassay

Sample	LC_{50} value
Vincristine sulfate	3.571
n-Hexane extract of the stem (CR-1)	4.515
Chloroform extract of the stem (CR-2)	824
Ethyl acetate extract of the stem (CR-3)	299.93
Methanol extract of the stem (CR-4)	73.67
n-Hexane extract of root (CR-5)	9.54
Chloroform extract of root (CR-6)	585.33
Ethyl acetate extract of root (CR-7)	2433.55
Methanol extract of root (CR-8)	106.376

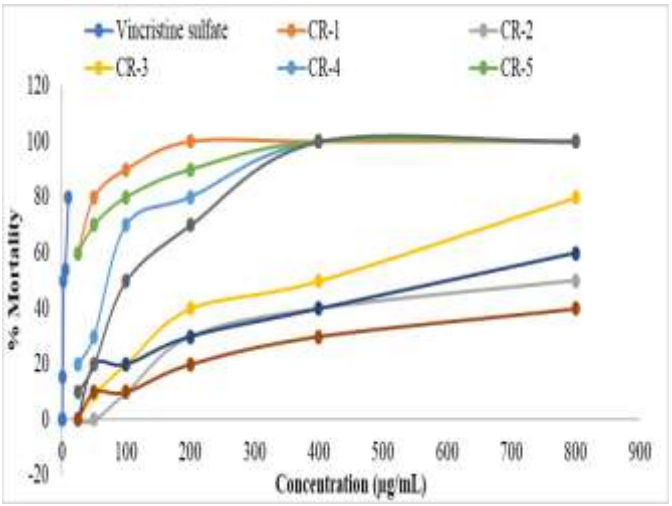

Figure 5: Comparative percentage mortality of the various *Catharanthus roseus* extracts.

Conclusion

The antioxidant testing using various methodologies revealed that the chloroform extract of the root had significantly higher antioxidant potential than the other extracts. However, the total flavonoid content indicated a higher value for n-hexane extract of the stem of *C. roseus*. Also, the n-hexane extracts of the stems and roots are significantly cytotoxic compared to the other extracts and standards. The findings of this study suggest that the stems and roots of *Catharanthus roseus* growing in Bangladesh contain potent natural antioxidants and anticancer potentials.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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