ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

Comparative Studies on Cytotoxic, Antibacterial and Free Radical Scavenging Activity among Different Extracts of Leaves and Flowers of *Catharanthus roseus* Available in Bangladesh

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Article info

Article History:
Received 8 July 2014
Accepted 16 October 2014

Keywords:
Cycotoxicity, Antibacterial activity, Free radical scavenging, *Catharanthus roseus*.

Abstract

Catharnthus roseus (periwinkle) is used as an important plant in traditional medicine for a long time. The cycotoxicity of the different extracts of leaves and flowers of *Catharanthus roseus* were examined by brine shrimp lethality bioassay. However, methanol extract of leaves and methanol extract of flowers exhibited quite potent activity in brine shrimp lethality bioassay with LC_{50} 3.18 and 3.93 µg/ml respectively. On the other hand, methanol extract of leaves and flowers demonstrated strong antibacterial activity compared with steptomycin with against the tested pathogenic microorganism. The n-butanol extract of leaves exhibited quite significant activity with a zone of inhibition of 16mm against gram positive bacteria Staphylococcus sp. Further, methanol extract of leaves showed significant free radical scavenging activity with IC_{50} 68.84µg/ml.

1. INTRODUCTION

Medicinal plant products could prove useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health[1]. The increasing global interest in the medicinal potential of plants during the last few decades is therefore quite logical. General bioassays that exhibit capable of detecting board spectrum of bioactivity present in crude extracts are brine shrimp lethality bioassay (BSLT), antibacterial screening (ANBS) and free radical scavenging activity test (FRST). The techniques are easily mastered, low cost and needs small amount of test material. BSLT is predictive for cytotoxicity and pesticidal activity[2]. This test has been introduced in 1982[3] and employed for bioassay-guided fractionation of active cycotoxic and antitumor agents such as trilobacin from the bark of Animina triloba [4] and cis-annonacin from Annona muricata[5]. FRST is also predictive for antioxidant activity and introduced in 1958[6] and employed for the detection of active free radical scvengers like vitamin C, vitamin E, flavonoids, carotenes, phenolic acids, phytic acids and phytoestrogens . Theses have been recognized as having the potential to reduce disease risk [7]. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers[8].

Catharanthus roseus (Common name- Periwinkle, Vinca; Bengali- Nayantara, Synonymes-Vinca rosea; Family-Apocyanaceae) popularly known as madagascar periwinkle is a potential source for anti-leukemic alkaloids. It is cultivated mainly for its alkaloids, which are having anticancer activities[9]. It is an evergreen subshrub or harbeceous plant growing up to 1 m tall[10].

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Catharanthus roseus is administered as a cooling medicine. It is used for the treatment of diabets, fever, malaria, throat infection and chest complaints. It is also used for the regulation of menstrul cycles, and as a euphoriant [11]. The plant is an important source of indole alkaloids, which are present in all plant parts. The physically 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[12]. Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia[13,14]. These Catharanthus alkaloids are also used for the treatment of both malignant and nonmalignant diseases and in platelet and platelet associated disorder. Previous phytochemical investigations resulted in the isolation of Kaemferol [15], Kaempferol trisaccarides [16], Quercetin[14], Quercetin trisaccarides [15], Syringetin glycosides[17], Malvidin [14], Malvidin 3-*O*-glucosides [18] , Malvidin 3-*O*-(6-*O*-p-coumaroyl)[18], Petunidin [14], Petunidin 3-*O*-glucosides[18], Petunidin 3-*O*-(6-*O*-p-coumaroyl)[18], Hirsutidin [14], Hirsutidin 3-*O*-glucosides [18] , Hirsutidin 3-*O*-(6-*O*-p-coumaroyl) [18], Rutin [19]. Catharanthus Plant produce many pharmaceutically important alkaloids. They are antineoplastic medicines and the monoindole alkaloids ajmalicin and serpentine are antihypertension drugs[20-26].

Therefore, the present study was undertaken with an objective to evaluate the cycotoxic, antibacterial and antioxidant activities of the different extracts of leaves and flowers of *Catharanthus roseus* .

2. MATERIALS AND METHODS

2.1 Instrumentation

The UV absorbance was performed with a Perkin Elmer Shelton, CT 06484 USA, Almbda 25 UV/VIS spectometer. Vacuam rotary evaporator (BUCHI, Rotavapor R-210 Switzerland) was used for evaporating solvents. All solvents were of analytical grade and obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA).

2.2 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.

2.3 Preparation of the solvent extracts (Cold Extraction)

Freshly collected leaves and flowers of *C. roseus* were dried in an oven at 38˚C and powdered by using a grinding machine. The powder of leaves (510g) 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 s 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 (11.0g), ethyl acetate extract (9.0g), n-butanol extract (8.5g). The residual methanol soluble part (11.5 g) was finally denoted as methanol extract. Powder of the flower (200g) was extracted successively different solvent at room temperature. At first it was extracted with n-hexane for 5 days and the extract was dried to get a gummy mass (7.15g) 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 (5.80g). Again the residual part was extracted with methanol and the filtrate was dried under reduced pressure to gummy mass (22.69 g).

2.4 Bioassays

2.4.1 Cycotoxicity

The cycotoxic activity was performed by brine shrimp lethality bioassay method. The test samples for crude extracts were dissolved in DMSO as following serial dilutions 150, 75, 37.5, 18.75, 9.375, 4.684, 2.344, 1.172, 0.586 and 0.292 µg/mL . Then each of these test solutions was added to the test tube containing 10 shrimps in simulated brine water (5mL) and incubated at room temperature for 24h. After 24h, the median lethal concentration (LC_{50}) of the test samples was determined by a plot of percentage of the shrimps against the logarithm of the sample concentrations (Finney Method). Vincristine sulphate (LC_{50}=0 .57) was used as positive control in this assay to compare the cycotoxicity of the test samples.

2.4.2 Antibacterial Screening

The test samples were dissolved separately in specific volume of choloform or methanol depending their solubility. The antibacterial screening was then carried out by the disk diffusion method [27,28]. The diluted samples were applied on the sterile discs (Oxford, UK) at a concentration of 100 µg/disc for this test where Streptomycin (10 µg/disc, Oxford, UK) used as standard.

2.4.3 Free radical scavenging activity

The free radical scavenging activity was assayed spectro-photometrically by DPPH method[29].The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical has a deep violet color due to its unpaired electron, and radical scavenging activity can be followed spectrophotometrically by a loss of absorbance at 517 nm. Different concentrations (20, 40, 60, 80, 100, 200, 400, 800 µg/ml in methanol) of ascorbic acid solution (1ml) as well as *C. roseus* leaf and flower extract solutions (1ml) were mixed separately with 3 ml of 0.4 mM DPPH solution. The mixture were kept in dark for 30 minutes to measure the absorbance at 517nm using UV-Visible Spectrophotometer (Cintra, Australia) and ascorbic acid was used as a positive control. The whole procedure was performed three times for each test solution. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The degree of decolorization of DPPH from purple to yellow indicated the scavenging activity of the extract. The scavenging activity against DPPH was calculated using the equation: Scavenging activity (%)=[(A-B)/A]x100. Where A is the absorbance of control (DPPH solution without sample), B is the absorbance of DPPH solution in the presence of sample (extract/ascorbic acid). The scavenging activity (%) was then plotted against concentration and from the graph IC_{50} (Concentration 50% inhibition)value was calculated by linear regression analysis. Each of the method was performed three times and the results were averaged.

3. RESULTS AND DISCUSSION

The cytotoxic activity of the extracts of leaves and flowers of *C. roseus* were determined by using brine shrimp lethality bioassay. The LC_{50} for vincristine sulphate (positive control), methanol, ethyl acetate, n-butanol and n-hexane extract form leaf and n-hexane, DCM, methanol extract from flower obtained by Finney method were found to be 0.57 (Vincristin Sulphate) 3.18 ,4.25, 7.84, 9.70 (for leaf) and 12.57, 14.93, 3.93 µg/mL for flower respectively.(Table1). In comparison with the positive control (vincristine sulphate), it is clear that all the test samples were lethal to brine shrimp nauplii. However methanol and ethyl extract of leaves and methanol extract of flowers demonostrated quite potent activity in brine shrimp lethality bioassay but methano extract of leaves of *C. roseus* showed more potent activity than other extracts. These positive results suggested that they may contain antitumor or pesticidal activity.

The antibacterial activity of all extracts of leaves and flowers were subjected to screening at100 µg/disc by using disc diffusion method. The moderate to good zone of inhibition exhibited by methanol extracts of leaves and flowers and n-butano extract of leaves against almost all pathogenic microorganisms (Table-2). Here methanol extract of leaves also showed more potent activity than methanol extract of flower. This implicates that if a lead molecule is identified from such studies, plant tissue culture techniques can be harnessed for the production of plant secondary metabolitie[30]. Furthermore, gram positive bacteria were found to have more susceptibility as compared to gram-negative bacterial species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms. Therefore, a study with large number of clinical pathogens with phytochemicals is expected to provide a hint to fish-out an effective lead molecule.

Table 1: Cycotoxic effect of the solvent extracts of C. *roseus* leaf and flower on brine shrimp nauplii.

Material tested	LC_{50}(µg/ml)
Vincristine Sulphate	0.571
LM	3.188
LE	4.251
LBn	7.852
LH	9.703
FM	3.930
FH	12.579
FD	14.934

LH:Leaf n-Hexane Extract, LE: Leaf Ethyl Acatate Extract, LBu-Leaf butanol Extract, LM:Leaf methanol extract FH: Flower nhexane Extract, F:Flower Dichloromethane Extract, FM:Flower methanol extract.

Table 2: Antibacterial screening of the solvent extracts of C. *roseus* Leaf and Flower.

	Diameter of Zone of inhibition (mm)								
Test microorganism	LM	LE	L-Bu	LH	LBu	FM	FD	FH	Steptromycin
Grum Positive bacteria									
Bacellius cereus	12	5	7	5	7	8	NA	NA	18
Staphylococus aureus	15	5	7	5	7	9	NA	NA	20
Bacillus megaterium	13	NA	7.0	0.5	7.0	13	NA	NA	25
Staphylococcus sp.	19	NA	16	NA	16	13	NA	5	23
Gram Negative bacteria									
Vibreo colera	10	4	NA	NA	NA	6	NA	NA	17
Escherichia coli	6	NA	3	NA	3	4	NA	NA	10

LH:Leaf n-Hexane Extract, LE: Leaf Ethyl Acatate Extract, LBu-Leaf butanol Extract, LM:Leaf methanol extract FH: Flower nhexane Extract, F:Flower Dichloromethane Extract, FM:Flower methanol extract. NA: No activity observed, Steptomycin (Std.) (10.0 µg/disc).

On the other hand, the free radical scavenging activity of the extracts of leaves and flowers were assayed by using DPPH method. The IC_{50} of Vit-C was found to be 24.14 µg/ml and the extracts of leaves and flowers shown in Table-3. In comparison with the positive control (Vit-C), it is evident that significant antioxidant activity exhibited by the crude methanol extract of leaves. This findings suggests that methanol extract of leaves may contain flavonoid/phenolic compounds which have the antitumor potentials. ROS (reactive oxygen species) are important effectors in ageing and lifespan determination [31] as well as cell proliferation and cell differentiation and apoptosis.The methanol extract of leaves may contain antioxidants which can minimize more reactive oxygen species than other extracts to prevent diseases. Finally it is evident that methanol extract of leaves have formed more potent as cycotoxic, antibacterial and antioxidant in all assays employed.

Table 3: Free radical scavenging activity of the solvent extract of leaf and flower of C.*roseus*

Sample	% Scavenging activity								IC_{50} (µg/ml)
	800 (µg/ml)	400 (µg/ml)	200 (µg/ml)	100 (µg/ml)	80 (µg/ml)	60 (µg/ml)	40 (µg/ml)	20 (µg/ml)	
Vit-C	90.43	86.10	76.86	71.29	63.57	57.48	53.52	49.91	24.14
LM	88.53	79.65	75.11	54.76	50.00	42.64	36.58	35.28	68.84
LE	92.21	86.80	76.41	50.65	39.18	30.52	23.16	18.83	98.30
LBn	76.41	68.83	56.28	45.02	40.91	36.58	28.79	24.89	134.64
LH	82.47	62.99	59.09	38.74	33.12	26.84	24.46	26.19	157.27
FM	95.02	73.16	70.35	47.62	41.77	37.66	34.85	30.30	108.20
FH	66.88	56.06	50.43	49.57	40.69	32.47	28.35	26.19	191.87
FD	74.24	56.28	50.87	46.10	43.29	38.96	34.85	33.12	162.13

LH:Leaf n-Hexane Extract, LE: Leaf Ethyl Acatate Extract, LBu-Leaf butanol Extract, LM:Leaf methanol extract FH: Flower nhexane Extract, F:Flower Dichloromethane Extract, FM:Flower methanol extract.

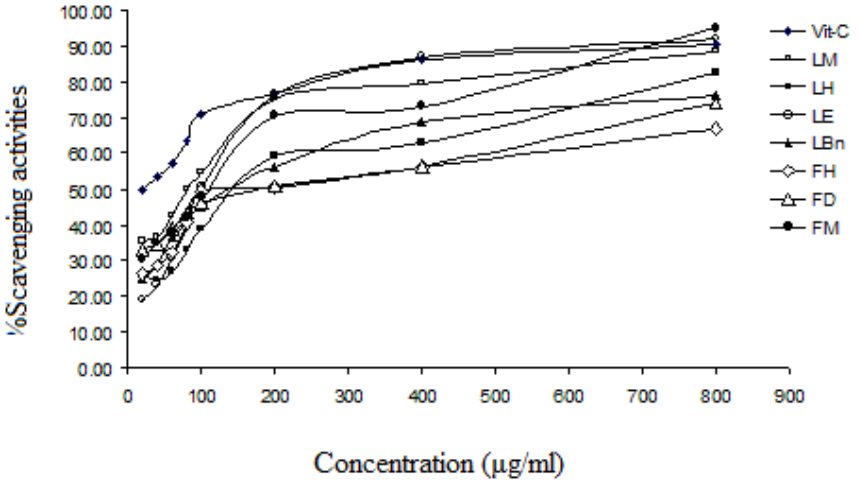

Figure 1: Comparative % scavenging of DPPH showed by standard antioxidant (Vit-C) and *C. roseus* leaf and flower extracts

4. CONCLUSION

Cycotoxicity, antibacterial and antioxidant activity of the different extracts of leaves and flowers of *C. roreus* were found to be consistent with the folk uses of this plant by local people. In the present study, methanol extracts of both leaf and flower of *C. roreus* showed significant cytotoxic, antibacterial and free radical scavenging activity. But in case of methanol extracts, leaf showed more potent activity in all the bioassays than flower. Further investigation of the extracts to evaluate active phytoconstituents for both leaf and flower of *C. roreus* is now going on.

5. ACKNOWLEDGEMENT

We are grateful to department of Pharmacy, Dhaka University for giving us the opportunity to do the bioassays of the crude extract of plant materials. We are also thankful to the former Director, BCSIR Laboratories, Dhaka, Mr. Abu Anis Jahangir for providing necessary facilities to carry out this research work.

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