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Phytochemical Analysis and Antioxidant Properties of the Various Extracts of *Catharanthus roseus*

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ABSTRACT

The estimation of various phytochemicals and the antioxidant power of various extracts of Catharanthus roseus was carried out by various chemical and spectroscopic methods. Various types of phytochemicals have been identified both qualitatively as well their quantification had been carried out. The observed results so for obtained showed that the plant in reference posses all types of phytochemical in a good quantity which are essential for the human consumption, like alkaloids, flavonoids and saponins. Catharanthus roseus also has been found to have the capacity to neutralize the free radicals from which various types of disease visit humans from time to time. Three solvents namely Acetone, DMSO and Water) have been used for extraction of various phytochemicals from the plant parts. Among the various extracts of the plant, water extract had been found more potent as per the phytochemical analysis and the antioxidant properties of the plant are taken into consideration.

Keywords: Phytochemicals; Antioxidant; *Catharanthus roseus*; Plant extracts

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INTRODUCTION

Catharanthus roseus is native to the Indian Ocean Island of Madagascar. In wild particularly, it has found an endangered plant and the major cause of its decline is due to the destruction of the habitat and agricultural burn however, it has been found commonly in many tropical and subtropical regions worldwide, including the Southern United states. *Catharanthus roseus* (L) G. Don (formerly *Vinca rosea* L., Apocynaceae) is commonly called a Madagascar periwinkle. It is perennial ever green herb, 30-100 cm tall commonly native to the island of Madagascar but now has been found wildly dispersed in the tropics [1]. The significance of the concerned plant is mainly because it could synthesize many types of terpenoids, alkaloids which bear good medicinal properties. The compounds mentioned above have a wide range of applications mostly in the treatment of lymphocytic cancer, Wilkins's cancer, neuroblastoma and reticulum cell tumor, Hodgkin's disease besides lymphosarcoma, choriocarcinoma [2]. Alkaloids have been identified and isolated to large extent as compared to, other natural compounds in *C. roseus* [3]. *Catharanthus roseus*, [4] which is a potent medicinal plant many of the pharmacological actions such as antimicrobial, antioxidant, anthelmintic, antifeedant, antisterility, antidiarrheal, antidiabetic effect etc. Methanolic extract of leaves of *Catharanthus roseus* [5] shows anti-bacterial action against three pathogenic micro-organisms, (Klebsiella pneumonia, Staphylococcus aureus, and Escherichia coli).

MATERIAL AND METHODS

Study area and plant collection

The *Catharanthus roseus* has been collected from FRI Dehradun (UK). The plant parts were segregated and dried under shade, finally the mixture was powdered.

Extraction Procedure for *Catharanthus roseus*

Nearly 60 gms of the plant material powder was weighed accurately and the extraction process was carried out by using Soxhlet Apparatus in which thimble was used to get the pure form of the extract. Only three solvents have been used in the order of their increasing polarity viz (Acetone, DMSO and Water).

Extraction A

The extraction process was carried out with lowest polarity solvent (Acetone) among the concerned solvents, in a Soxhlet apparatus, in which the extraction was carried out, and continued up to which the extract comes out of the material, which was examined by taking a small amount of the extracted solvent from the Soxhlet apparatus over the watch glass and was studied for precipitate formation. After the Extraction with acetone, the extract solution was collected and was filtered to remove the residue from extract. The filtrate so obtained was evaporated to 1/4 of its volume on water bath. The whole filtrate was then solidified (powdered) after being kept in an oven. Then the residue was collected and was kept for further extraction.

Extraction B

The residue after first extraction was collected and was treated in the same manner for extraction process by using DMSO.

Extraction C

The residue after second extraction was then extracted with water by decoction technique, in which the residue so obtained was dissolved in nearly 500 of water and was boiled for time period in which the whole water got evaporated. Then after in the same residue nearly 300 ml of water was added and the whole mixture was boiled to ½ of its original volume. Finally the extract mixture was cooled and filtered via ordinary filter paper and then by whatman filter paper. The filtrate so obtained was evaporated to ¼ of its original volume. . The whole filtrate was then made in solid form (powdered) after being kept in an oven. Then the residue was collected.

Phytochemical screening was done in which test for various chemical constituent were performed namely:-

1. Test for Flavonoids
2. Test for Tannins
3. Test for Saponin
4. Test for Terpenoids(Salkowski test)
5. Test for Carbohydrates (Benedict's test)
6. Test for Ferric chloride
7. Test for Cardiac glycosides (keller-Killani test)
8. Test for phlobatannins.

Chemical Tests

Test for flavonoids: The method used to determine the presence of flavonoids in the plant sample was given by Sofowara, 1993; Harbrone, 1973, in which 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H2SO4. A yellow colouration observed in each extract indicated the presence of flavonoids.

Test for Tannins: In this method nearly about 2.5 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as the positive test for the presence of tannins (Trease and Evans, 1989).

Test for Saponins (Kokate)

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

Test for terpenoids (Salkowski test): Nearly 5 ml of the extract was mixed in two ml of chloroform, and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration at the inter face was formed to show positive test for the presence of terpenoids.

Test for Carbohydrates

Benedict's test: To nearly 0.5 ml of the extract filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes; a characteristic red colored precipitate indicates the presence of sugar.

Test for Phenolic compounds (Mace, 1963)

Ferric chloride test: The plant was diluted to 5 ml with distilled water and to this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

Antioxidant Power Determination

DPPH method: DPPH Scavenging activity was measured by spectrophotometric method, in which a stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it was in 3 ml of methanol. Decrease in the absorbance in presence of sample extract at different concentration (10-125 µg/ml) was noted after 15 min. IC50 was calculated from % inhibition.

Protocol for DPPH free radical scavenging activity: Preparation of stock solution of the sample:

1. Nearly 100 mg of extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.
2. Dilution of test solution: 10, 20, 30, 40, 50,60,70,80,90,100 µg/ml solution of test was prepared from stock solution.
3. Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The resulting solution was then covered with aluminium foil to protect from light.
4. Estimation of DPPH scavenging activity: from the prepared DPPH solution an exactly 75 µl of DPPH solution was taken and the total volume was made up to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Finally the decline in the absorbance of DPPH with sample of different concentrations was measured after 15 minute at 517 nm.

Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction=Control absorbance – Test absorbance/ Control absorbance x 100 Calculation of IC50 value using Figure ical method (Figures 1-8; Tables 1-8).

OBSERVATIONS AND RESULTS

Antioxidant power of *Catharanthus roseus*

Table 1. DPPH Free Radical Scavenging potential of Ascorbic Acid

S. No.	Conc. (µg/ml)	Absorbance of Ascorbic acid	% Reduction	IC_{50} (µg/ml)
1.	10	0.292	40.63	26
2.	20	0.269	45.90	
3.	30	0.244	50.60	
4.	40	0.226	54.45	
5.	50	0.202	59.10	
6.	60	0.181	63.33	
7.	70	0.162	67.21	
8.	80	0.141	71.45	
9.	90	0.122	75.30	
10.	100	0.088	82.16	

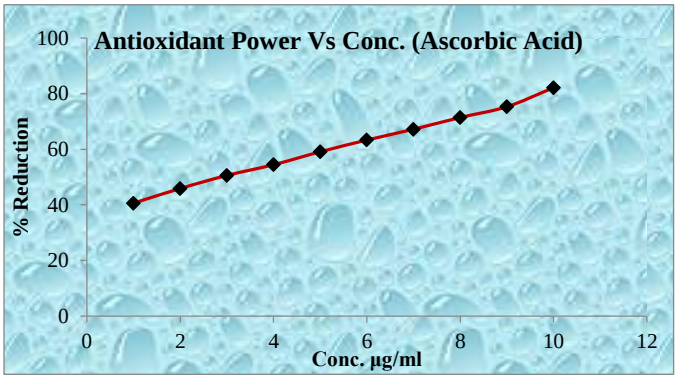

Figure 1. DPPH Free Radical Scavenging Activity of Ascorbic Acid

Table 2. Antioxidant Activity of Acetone Extract of *Catharanthus roseus*

S. No	Conc. (µg/ml)	Absorbance of Acetone extract	% Reduction	IC_{50} Value
1.	10	0.378	22.85	48.5
2.	20	0.361	26.32	
3.	30	0.355	30.55	
4.	40	0.352	45.16	
5.	50	0.341	50.41	
6.	60	0.339	60.81	
7.	70	0.315	69.71	
8.	80	0.210	71.14	
9.	90	0.140	79.42	
10.	100	0.095	80.61	

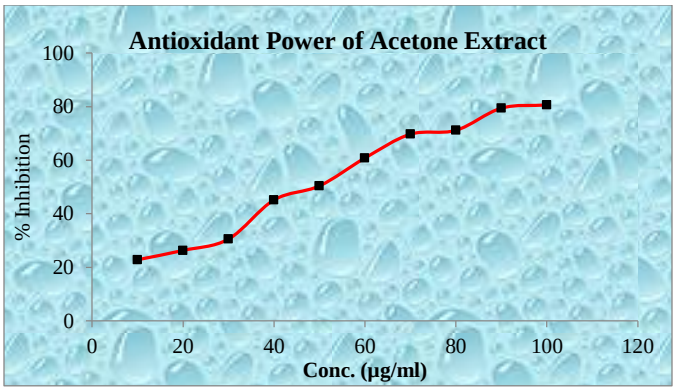

Figure 2. Antioxidant Activity of Acetone Extract of *Catharanthus roseus*

Table 3. Antioxidant Activity of DMSO Extract of *Catharanthus roseus*

S. No.	Conc. (µg/ml)	Absorb. of DMSO Extract	% Reduction	IC_{50} Value
1	10	0.355	41.63	38.5
2	20	0.341	44.48	
3	30	0.286	45.69	
4	40	0.272	52.91	
5	50	0.271	59.53	
6	60	0.265	69.38	
7	70	0.262	79.61	
8	80	0.150	82.83	
9	90	0.046	87.67	
10	100	0.040	91.23	

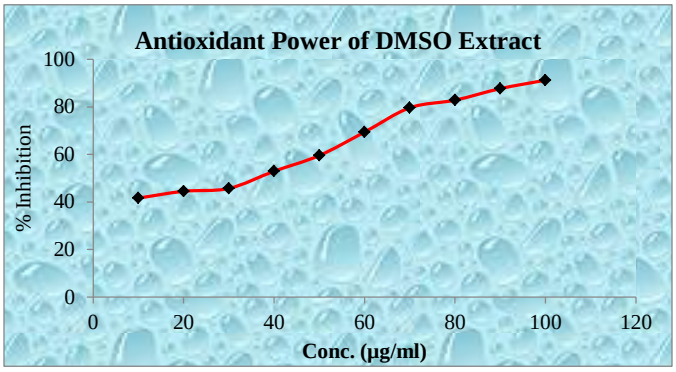

Figure 3. Antioxidant Activity of DMSO Extract of *Catharanthus roseus*

Table 4. Antioxidant Activity of Water Extract of *Catharanthus roseus*

S. No.	Conc. (µg/ml)	Absorbance of water extract	% Reduction	IC_{50} Value
1	10	0.400	28.36	37.1
2	20	0.399	31.42	
3	30	0.378	34.89	
4	40	0.365	52.61	
5	50	0.351	65.46	
6	60	0.336	72.73	

7	70	0.319	79.89	
8	80	0.193	86.61	
9	90	0.032	91.46	
10	100	0.015	95.73	

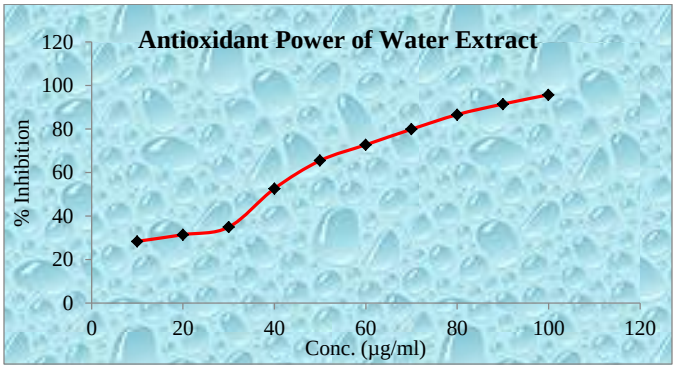

Figure 4. Antioxidant Activity of Water Extract of *Catharanthus roseus*

Qualitative Analysis

Table 5. Phytochemical Tests for *Catharanthus roseus*

S. No	Phytochemical tests	Acetone	DMSO	Water
1.	Flavonoids Test	-ve	-ve	+ve
2.	Tannins Test	-ve	+ve	+ve
3.	Saponin Test	-ve	+ve	+ve
4.	Terpenoids Test	-ve	+ve	+ve
5.	Carbohydrates	-ve	-ve	-ve
6.	Ferric chloride Test	-ve	-ve	+ve
7.	Cardiac glycosides	-ve	+ve	+ve

Quantitative Analysis

Alkaloid determination was carried out by Harborne method [6] in which 5 g of the sample was weighed in a 250 ml beaker and to it 200 ml of 10% acetic acid in ethanol was added and the whole mixture was covered and allowed to stand for about 4 h. The whole mixture was filtered and the filtrate was concentrated on a water bath to 1/4 of its original volume. Concentrated ammonium hydroxide solution was added drop by drop to the extract until the precipitation was complete. The whole extract was allowed to get settled, and the precipitated was collected and washed with dilute ammonium hydroxide and then it was filtered. The residue was the alkaloid, and was weighed after complete drying.

Table 6. Alkaloid Content in Various Plant Extracts

Acetone extract	**DMSO extract**	**Water extract**
Weight of extract taken=5 g	Weight of extract taken=5 g	
Weight of empty watch glass=34.43g	Weight of empty watch glass=34.32 g	Weight of extract taken=5 g Weight of empty watch glass=35.22g
Weight of watch glass with extract=34.32g	Weight of watch glass with extract=34.37 g	Weight of watch glass with extract=35.25g
Weight of sample=(34.43-34.49)=0.06 g	Weight of sample=34.32-34.37=0.05 g	Weight of sample=35.22-35.25g=0.03g

Flavonoids were determined by Bohm and Kocipai- Abyazan method: An exactly 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight [7].

Table 7. Flavonoid Content in various Plant Extracts

Acetone extract: Weight of extract taken=10 g	**DMSO extract:** Weight of extract taken=10 g	**Water extract:** Weight of extract taken=10 g
Weight of empty crucible=32.84 g	Weight of empty crucible=35.16 g	Weight of empty crucible=32.38g
Weight of crucible with extract=32.87g	Weight of crucible with extract=35.22g	Weight of crucible with extract=32.43g
Weight of sample =32.84-32.87g =0.03g	Weight of sample= 35.16-35.22g=0.06g	Weight of sample=32.38-32.43=0.05g

Saponin determination: The method used was given by Obadoni and Ochuko [8]. In this method the sample was grounded and 20 g of each were put into a conical flask and to it 100ml of 20% aqueous ethanol was added. The whole mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was filtered and the residue was re-extracted with another 200ml of 20% ethanol. The combined extract was reduced to 40ml over water bath at about 90ºC.The concentrated mixture was transferred into a 250ml separating funnel and then 20ml of diethyl ether, was added and the separating funnel was shacked vigorously. The aqueous layer was collected and the ether layer was discarded. The purification process was repeated by adding 60ml of n-butanol. The combined n- butanol

extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as percentage.

Table 8. Saponin content in various plant extracts

Acetone Extract: Weight of extract taken=20 g	**DMSO leaves extract:** Weight of extract taken=20 g	**Water Extract:** Weight of extract taken=20 g
Weight of empty china dish =52.03 g	Weight of empty china dish=53.89 g	Weight of empty watch glass=57.24g
Weight of china dish with extract=52.50g	Weight of china dish with extract=54.24 g	Weight of watch glass with extract=57.62g
Weight of sample=52.03-52.50=0.47g	Weight of sample =53.89-54.24=0.35g	Weight of sample=57.24-57.62=0.38g

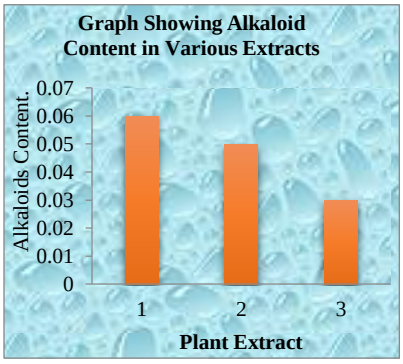

Figure 5. Alkaloid Content in various extracts of the plant

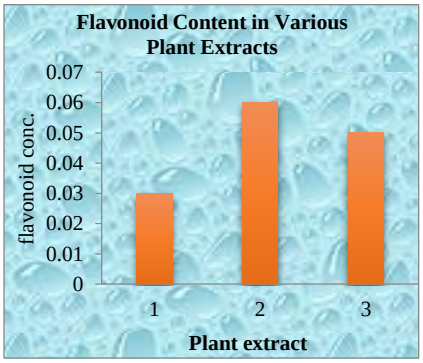

Figure 6. Flavonoid content in various extracts of the plant

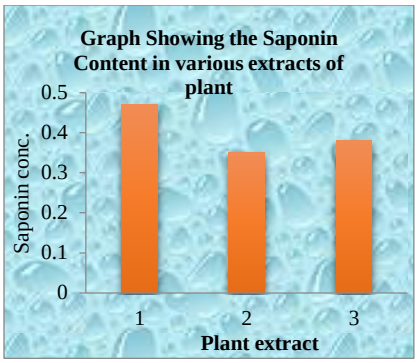

Figure 7. Saponin content in various extracts of the plant

DISCUSSION

The study which was carried out on the "*Catharanthus roseus*" showed the presence of bioactive compounds which are important both biologically as well as medicinally. The phytochemically active components/ metabolites have been analyzed on the concerned plant, using different solvents having different ionic potential. In the identification process, saponins, flavonoids and alkaloids showed different concentration in different solvent extracts. The medicinal value of plants lies on these hidden phytochemicals and they have a definite physiological action on life of an organism. These phytochemicals possess a wide range of activities, and help in protection against chronic diseases. For example, Saponins have a profound effect against hypercholesterolemia and have antibiotic properties. Steroids and triterpenoids possess analgesic properties. The steroids and saponins were responsible for central nervous system activities. The alkaloid content of "*Catharanthus roseus*" in acetone and DMSO extracts were found to be (0.06 and 0.05), and (0.03) were found respectively in the water extract. The flavonoid content of "*Catharanthus roseus*" in acetone and DMSO extracts were found to be (0.03 and 0.06), and (0.05) were found respectively in water extract. The composition of Saponin content in Acetone and DMSO extracts of "*Catharanthus roseus*" were found to be (0.47 and 0.35), and (0.38) were found respectively in water extract.

The antioxidant power of the Acetone, DMSO and water extracts of *Catharanthus roseus* being carried out by DPPH method showed that all plant extracts show increase in % reduction in their antioxidants potential, with the increase in the concentration of the extracts. The water extract of the plant showed higher antioxidant potential than the acetone and DMSO extracts of the plant**.** The antioxidant powers of Ascorbic acid have been found to be more than the Plant extracts. The IC_{50} value of all the extracts (Acetone, DMSO and Water) have been determined and the corresponding values have been found to be (48.5, 38.5, 37.1 µg/ml) respectively. From the above results it could be concluded that the IC_{50} value showed decline with the decrease in the polarity of the solvents. Less is the IC_{50} value more is the free radical scavenging potential of the concerned plant extract. Among all the extracts, the water extract was found to have more potent in inhibiting the free radicals followed by methanol extracts and least activity was noticed for the DCM extracts.

The ascorbic acid exhibited the lowest IC_{50} value (26.0µg/ml) with the highest antioxidant potential in relation to all the extracts of *Catharanthus roseus*.

CONCLUSION

Medicinal plants are the most important source of life providing medicines for majority of the world's population. Plants continued to be important therapeutic agents for relieving the ailments of human kinds. The search or defence mechanism, longevity and remedies to relieve pain and discomfort lead early man to explore the immediate natural resources against various ailments. Today, renewal interest gets developed in traditional medicine, so increasing demand for more drugs from plant sources because green medicine are safe, cheap without side effects than synthetic drugs. *Catharanthus roseus* was investigated for their phytochemical components and their therapeutic effect. The plant contains enormous phytochemical constituents of various medicinal applications which may act directly or in connection with other metabolites to overcome a particular disease. The plant also possesses a good property such as antioxidant which in turn is because of various poly phenols engulfed within the identified phytochemicals. Hence more work is possible on the above plant to reveal the unknown importance which would be helpful which is the need of an hour for the present pharmaceutical world.

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