www.ijrasb.com

ISSN (ONLINE): 2349-8889

Vandana Publications

IJRASB

Volume-5, Issue-3, May 2018

International Journal for Research in Applied Sciences and Biotechnology

Page Number: 1-8

Phytochemical Analysis of Catharanthus Roseus L. (G.) DON.

Keshav Khilnani[1*]

[1]Department of Bio Chemistry, Xavier Research Foundation, Loyola Centre for Research & Development, St. Xavier's College Campus, Navrangpura, Ahmedabad, Gujarat, INDIA

[1]Corresponding Author: khilnani.keshav@gmail.com

ABSTRACT

Catharanthus roseus is a medicinal herb found to have two pharmaceutically important compounds viz., vinblastine and vincristine. The plant is found in many tropical and subtropical regions around the world. Therefore, the yield of these vinca alkaloids are varied at different geographical regions depending on the climatic conditions. Being a source of these important secondary metabolites, an extensive study has been carried out on this plant. The plant is in high demand worldwide due to its medicinal applications specifically the anti-cancerous. The present research work gives an account of the various phytochemicals derived from the plant at our selected location. The results highlight the biotechnological prospects of an efficient and alternative means of production of valuable metabolites from Catharanthus roseus. The present phytochemical investigations explore active constituents which are very significant in drug development. The study revealed a notable anti-microbial and anti-oxidant activity of methanolic extract of the dry leaves of this plant.

Keywords-- Vinblastine, Vincristine, Phytochemicals, Secondary metabolites, Methanolic extract.

I. INTRODUCTION

Catharanthus roseus(Madagascar periwinkle) is a medicinal plant belongs to the family Apocynaceae. Though the plant have many medicinal applications, however, due to the colourful flowers, this plant also used as an ornamental plant. The plant is also appreciated for its hardiness in dry and nutritionally deficient conditions, popular in subtropical gardens where temperatures never fall below 5 °C to 7 °C. The plant flowers throughout the year in tropical conditions and from spring to late autumn in warm temperate climates. Full sun and well-drained soil are preferred by this plant. The plant has been put to traditional use for the treatment of a wide variety of ailments worldwide since ages [1].In traditional Chinese medicine, C. roseus extracts have been used to treat numerous diseases, including diabetes, malaria, and Hodgkin's disease [2]. The plant has many important phytoconstituents which exhibits various pharmacological activities like antidiabetic, antioxidant, anti-hypertensive, antimicrobial, cytotoxic etc. Catharanthus roseus produces two important alkaloids: vinblastine and vincristine, which are known for the anticancer lead molecules.Extracts from the dried or wet flowers and leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice from the flowers of C. roseus made into a tea has been used by Ayurveda physicians in India for external use to treat skin problems, dermatitis, eczema and acne [3]. We have collected the plant from our local area and screening was done for the phytoconstituents. We have also done the antimicrobial and antioxidant analysis of the methanolic extract of plant leaf. Our results have good significance, as this plant being an essential component of traditional as well as modern pharmaceutical systems

II. MATERIALS AND METHODS

Preparation of Leaf Extracts:

Matured leaves of C. roseuswere collected from the garden of Loyola Centre for Research & Development (LCRD). Those plant leaves were washed under running tap water to remove soil and dust particles. Then the leaves were shaded, dried, and then powdered by using mechanical blender and stored in air tight bottles. The powdered plant leaves were soaked with (10g/100ml) in methanol solvent and extraction was done using Soxhlet apparatus. The extract was further concentrated to decrease the volume upto 50 ml using rotatory evaporator.

Qualitative Analysis of Phytochemicals

Saponin:

To 1 ml extract 4 ml of sterile RO water was added. The mixture was shaken vigorously. Formation of froth confirmed the presence of saponin in it.

Tannin:

To 1 ml extract 20 ml of sterile RO water was added. The mixture was kept on the boiling water bath for 45 min. It was shaken at regular intervals. The content was allowed to cool down followed by filtration through Whatman filter paper No. 1. Then 2% of Gelatine was added. Formation of white precipitates confirmed the presence of tannins in it.

Proanthocyanin:

2 gm of dried plant powder was taken in a test tube. 8 ml of 2N HCl was added into it. The tubes were covered with an aluminium foil and were placed in a boiling water-bath for 30 min. The mixture was allowed to cool down at room temperature and then amyl alcohol (3-methyl-butan-1-ol) was added slowly into it. The formation of a red or carmine coloured upper layer confirmed the presence of proanthocyanin. The formation of yellow coloured upper layer confirms the absence of proanthocyanin.

Cardiac Glycosides:

Kellar-Killani's Test:

To 1 ml of plant extract few drops of glacial acetic acid was added. Then 0.5 ml of 1% ferric chloride solution was added. It was mixed well and was allowed to settle for 2-5 min. Then concentrated sulfuric acid was added slowly from the side of the wall of the test tube. The formation of a brown ring at the junction confirmed the presence of glycosides.

NaOH Test:

To 1 ml of plant extract the 1 ml NaOH solution was added. The formation of yellow colouration confirmed the presence of glycosides.

Flavonoid:

$AlCl_3$ Test:

0.5 ml of concentrated crude methanolic extract was taken. 0.5 ml of 2 % $AlCl_3$ (in ethanol) was added. Tubes were then kept at room temperature for about 1 hour for colour development.

Mg Ribbon Test:

1 gm of dried plant material was taken. 10 ml of 95% ethanol was added. The mixture was then heated in boiling water bath for 15 minutes. After cooling it at room temperature, the content was filtered. Into the filtrate a small piece of Mg ribbon was placed followed by addition of 2-3 drops of concentrated HCl. The formation of red colour showed that flavonoids are present in it.

Irioides:

In 0.5 gm of dried plant powder 5 ml of aqueous HCl was added. Then it was allowed to sediment for 3-6 hours. After sedimentation, the upper clear liquid was decanted and collected it in a separate tube through centrifugation. This 0.05 ml of clear liquid was mixed with 0.5 ml of Trim-Hill reagent and heated it on a flame. Development of red colour confirmed the presence of Irioides.

Alkaloids:

Mayer's Reagent Test:

To 1 ml of extract 1 ml of Mayer's reagent was added with few drop of Iodine solution. Formation of yellow colour precipitates indicated the presence of alkaloids.

Wegner's Reagent Test:

To 1 ml of extract 1 ml of Wegner's reagent was added. Formation of orange-red colour precipitates indicated the presence of alkaloids.

Trim-Hill Reagent Test:

To 1 ml of extract 1 ml of Trim-Hill reagent was added. Formation of green colour precipitates indicated the presence of alkaloids.

Dragendorff's Reagent Test:

To 1 ml of extract 1 ml of dragendorff's reagent was added. Formation of brown colour precipitates indicated the presence of alkaloids.

Phenolics:

To 1 ml of extract 0.25 ml of Folin-Ciocalten reagent was added. Mixed well and kept at room temperature for 10 min. Then added 0.5 ml of 20% sodium carbonate ($NaCO_3$). It was mixed well. Mixture was kept in a boiling water bath for 20 min. at 40°C. Mixture was cooled down at room temp. Appearance of blue colour confirmed the presence of phenolics.

Carbohydrates:Molish's Test:

To 0.5 ml plant extract few drops of 1-naphtol was added. Then 0.2 ml conc. H_2SO_4 was added slowly along the sides of the test-tube. A reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Proteins:Bluret's Test:

To 2 ml of plant extract 1 drop of 2% $CuSO_4$ Solution was added. Then 1 ml of 95% ethanol followed by excess of Potassium Hydroxide (KOH) pellets was added. The development of pink colour indicated the presence of proteins.

Terpenoids:

To 1 ml of the plant extract,2 ml chloroform ($CHCl_3$) and 0.5 ml conc. H_2SO_4 were added. The formation of reddish-brown colour indicated the presence of terpenoid.

Anthocyanins:

To2 ml of plant extract, 2 ml 2N HCl and ammonia were added. A pink-red colour turned into blue-violet and indicates the presence of anthocyanins.

Steroids and Triterpenoids:

Salkowski's Test:

To0.5 ml of plant extract few drops of chloroform were added. Then few drops of conc. H_2SO_4 were added. It was mixed well and allowed to settle for some time. A red colour appeared at the lower layer indicated the presence of steroids. And the formation of yellow colour at the lower layer indicates the presence of triterpenoids.

Quantitative Analysis of Phytochemicals:

Total Alkaloids:

One gm of dry leaf powder of C. roseus was in a conical flask. 40 ml of 10% acetic acid in ethanol was added

into it and the mouth of that conical flask was covered with an aluminium foil and allowed to shake for 4 hours on an orbital shaker at room temp. Then the content was filtered through the Whatman filter paper no. 1. This filtrate was concentrated on a hot plate in crucibles to 1/4th of the original volume. The concentrated extract was cooled down at room temperature and then concentrated ammonia hydroxide (NH_40H) was added drop-wise until precipitation was completed. Whole solution was allowed to settle at room temp. The precipitates were washed with 1N ammonium hydroxide through the pre-weighed Whatman filter paper no.1. The residues obtained on the filter paper were air dried and weighed. The percentage of alkaloid content in the leaf extract was calculated using the following formula:

$$\% \text{ Alkaloid} = \frac{\text{wt. of filter paper with dried content} - \text{wt. of empty filter paper}}{\text{Initial wt. of dried powder}} \times 100$$

Total Cardiac Glycosides:

In 100 ml of Erlenmeyer flasks 2 gm of dried plant powder was taken. Then 20 ml of 70% ethanol was added. Erlenmeyer flasks was covered with aluminium foil and placed on an orbital shaker at 200 rpm for 24 hours at room temperature. The content was then filtered through the Whatman paper no. 1.The final volume of the filtrate was made upto 20 ml with 70% ethanol. In this extract 4 ml of lead acetate (12.5%) was added and was kept on an orbital shaker at 200 rpm for 10 minutes. Then, 1 ml of disodium hydrogen phosphate (4.77%) was added to precipitate the excess pb++ ions. The resultant mixture was filtered through Whatman filter paper no. 1 to get a clear filtrate. Now filtrates were taken in pre-weighed crucibles and heated on a hot plate at 40°C till the solvent was completely evaporated. The dried extracts with crucible was weighed and the percentage of cardiac glycosides was calculated using the following formula:

$$\% \text{ Cardiac Glycosides} = \frac{\text{wt. of crucible with dried content} - \text{wt. of empty crucible}}{\text{Initial wt. of dried powder}} \times 100$$

Total Phenolics:

The 0.5 µl of methanolic leaf extract was taken in a test tube. In parallel, to the leaf extract sample, the standard gallic acid solution (1 mg/ml) in methanol was also taken at different concentrations viz. 30, 60, 90, 120 and 150 µl. One blank tube with only methanol was also taken. The final volume was maintained at 1 ml in all the test tubes by adding methanol. In all the tubes 500 µl Folic-Ciocalten reagent was added followed by addition of 7.5 ml of RO. Tubes were shaken gently and were kept at room temperature for 10 min. Then 10 ml of 20% sodium carbonate (Na_2CO_3) was added. All the tubes were mixed well and then kept in a water-bath for 20 minutes at 40°C. Then all the test-tubes were allowed to cool down at room temp. There was a colour development in all the tubes except in blank. Optical density (OD) was taken at 755 nm wavelength in UV-Visible Spectrophotometer. With the OD values of different concentrations of gallic acid, the standard curve was prepared, that was used to calculate the total phenolics in the leaf extract.

Total Flavonoids:

One mg rutin /ml methanol was used to prepare the standard concentrations viz., 50, 100, 150, 200 and 250 µl. In parallel to the standard tubes, the C. roseus leaf extract 250 µl, was also taken in a separate tube. One blank tube with only methanol was taken. The final volume in all the tubes were maintained at 1 ml with methanol. 4 ml of RO water was added in each tube followed by 0.3 ml of 5% $NaNO_2$ was added. All tubes were shaken to mix its content well and then 0.3 ml of NaOH was added and 2.4 ml RO water as well. After thorough mixing all the tubes were kept for few minutes to settle. There was a colour development. Then absorbance (OD) was recorded using UV-Visible spectrophotometer at 510 nm of wavelength. With the OD values of different conc. of rutin, the standard curve was prepared which was used to calculate the total flavonoids in the leaf extract.

Total Proanthocyanidines:

Three mg rutin / 3 ml methanol was used to prepare the standard concentrations viz., 200, 400, 600, 800 and 1000 µl. In parallel to the standard tubes, the C. roseusleaf extract 200 µl, was also taken in separate tube. One blank tube with only methanol was taken. The final volume in all the tubes was maintained at 1 ml with methanol. Then 3 ml of 4 % vanillin methanol (V/V) followed by 1.5 ml of HCl was added in each test-tube. All test-tubes were mixed well. Then test-tubes were allowed to stand for 15 minutes at room temperature. After settling down, absorbance (OD) readings were recorded using UV-Visible spectrophotometer at 500 nm wavelength.

Total Protein (Lowry's method):

Reagents required:

BSA Stock solution (1 mg/ml ethanol)

Analytical Reagents :

50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 gm in 100 ml distilled water) 10 ml of 1.56% copper sulphate mixed with 10 ml of 2.37 % sodium potassium tartrate solution. Prepare analytical reagents by mixing 2 ml of (b) with 100 ml of (a).

Folin-Ciocalteau reagent solution (1N) dilute commercial reagent (2N) with an equal volume of water on the day of use. (2 ml commercial reagent + 2 ml distilled water).

Procedure:

Different dilutions of BSA solutions (0.1, 0.2, 0.3, 0.4, 0.5 mg) were prepared from the stock BSA solution (1mg/ml). The final volume made upto 1 ml with 70% ethanol. In parallel, 1 ml of our each sample was also taken

in triplicate. Blank tube contained only ethanol. Then 2 ml of alkaline copper sulphate was added into all the tubes (BSA standard and test samples). The solutions were mixed well and incubated at room temperature for 10 minutes. After that, 0.2 ml of Folin-Ciocalteau reagent was added to each test tube and incubated for 30 minutes in dark. A blue colour was developed. The absorbance was taken at 660 nm. The standard linear exponential curve for the BSA concentrations was prepared and calculations for the total protein per gram of the plant leaf material was done.

Thin Layer Chromatography (TLC)

TLC was carried out to isolate the principle components that were present in the methanolic extracts of both the plants. Different solvent systems of different polarities were prepared and TLC studies were carried out to select the best solvent system capable of showing better resolution. The methanolic extracts of both the plants were applied on pre-coated TLC plates by using capillary tubes and allowed to run a glass chamber that contained the mobile solvent system. After completed the run, the TLC plates were air dried and observed under different wavelengths viz., 254 nm, 366nm and white light in CAMAG Reprostar Instrument. TLC plates were sprayed with suitable spraying reagents to get the intense band colours of different compounds present in the extracts.

Anti-microbial Activity

The following microorganisms obtained from LCRD were tested for anti-microbial activities:

Fungus:

Penicillium bravicompactum, Aspergillus niger, Rhizopus

Bacteria:

Pseudomonas arogenosa, Pseudomonas sp., Escherichia coli

Yeast:

Schizoscharomisis pombe

Preparation of Microbial Inoculum:

The glycerol stock cultures of these microorganisms were maintained at -80°C. Working cultures were stored at 4 °C and were regularly sub-cultured. To prepare the inoculum of microorganisms, a loopful of working culture was transferred into 5 ml of sterilized LB medium (for bacteria) and PDA medium (for fungi) and Nutrient medium (for yeast) and allowed to incubate on an orbital shaker. Bacteria were incubated at 37°C for overnight, whereas fungi and yeast cultures were incubated at room temperature (~ 30°C) for 2-3 days.

Well Diffusion Method *[4]:*

Media selected:Sabouraud Dextrose Agar (SDA) for Fungi; Mueller Hinton Agar (MHA) for Bacteria and Yeast

Procedure:

Both the media were prepared as per the instructions given on the box. Media as well as the glass petriplates were autoclaved at 121ºC and 15 Ib pressure for 20 minutes. After autoclaving, the molten media was poured into the sterile glass petriplates inside the LAF chamber and allowed to solidify. Inside laminar air flow, the 25 ml sterile media was poured in sterile Petri plates (90 mm) and allow them to cool to solidify the media. 100 µl of the homogenous spore suspension of fungi, bacteria and yeast were spread on to the respective media with the help of a sterile glass spreader. Precaution was taken while inoculating the suspension cultures that all cells were evenly spread all over the media plates. A small well was made in the centre of each plate by using sterile borer. Each well was about 6 mm diameter. All wells were filled with 100 µl ofC. roseus leaf methanolic extract and the methanol was also filled in one plate of each respective media as a negative control. All plates were kept inside the laminar air flow chamber for about one hour so as to diffuse the extract into the surrounding media. When extract was completely diffused, then all media plates were sealed with parafilm. Fungi inoculated PDA media plates were kept at 30-35^{o}C and bacteria as well as yeast inoculated NB media plates were kept at 37^{o}C for incubation. Formation of zone of inhibition was observed from one to five days of inoculation. The experiment was performed in triplicates. The inhibition zone was calculated as mean (n=3).

Anti-oxidant Activity (DPPH radical-scavenging assay):

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging reagent was used for the determination of antioxidant activity of C. roseus methanolic extracts. DPPH free radical scavenging capacity ofC. roseusextract was evaluated according to the protocol of [5] with some minor modifications. 0.1mM solution of DPPH was prepared in methanol. Ascorbic acid was taken as a positive control. The concentrations of ascorbic acid used were; 20 µg/ml, 50 µg/ml, 100 µg/ml. 100 µl methanolic extract ofC. roseus leaf was mixed with 100 µl DPPH solution and incubated in the dark for 30 minutes. Then absorbance was taken at 517 nm. Blank sample contain methanol with other ingredients except the plant extract. The experiments was performed in triplicate. Blank was also considered as the negative control and ascorbic acid concentrations were considered as the positive controls in the experiment. A low absorbance of the reaction mixture showed higher radical scavenging activity. The free radical scavenging effect (%) was calculated by using the following formula:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The percentage of scavenging effect (%) was plotted for the ascorbic acid and C. roseus methanolic leaf extract to calculate the IC50 values. IC50 value is the concentration that is required for scavenging 50% of the DPPH.

III. RESULTS & DISCUSSION

Qualitative Screening of Secondary Metabolites

The results of the phytochemical analysis of leaves of C. roseus is given in table 1. The medicinal value of this plant lies in some chemical substances that have a definite physiological action on human body. Results showed that the most important polar compound of these bioactive constituents of the plants are saponin, proanthocyanin, alkaloids, cardiac glycosides, flavonoids, Irioides, protein, terpenoids and anthocyanins present in the polar solvent i.e., methanolicextract of leaves. But the compounds like tannins, phenolics and steroids are not present in the methanolic extracts may be because of their polarity difference.

Table 1: Qualitative analysis of phytochemicals present in the methanolic extract of C. roseus leaves.

Sr. No.	Phytochemicals	Test	Indicator
1.	Saponin		+
2.	Tannins		-
3.	Proanthocyanin		+
4. i.	Alkaloids	Mayer's	+
4. ii.		Wagner's	+
4. iii		Trim-Hill	+
4. iv.		Dragendorff's	+
5. i	Cardiac Glycosides	Kellar-Killani's	+
5. ii.		NaOH	+
6.	Flavonoids		+
7.	Phenolics		-
8.	Irioides		+
9.	Carbohydrates	Molish’s	-
10.	Protein	Bluret’s	+
11.	Terpenoids		+
12.	Steroids	Salkowski's	-
13.	Anthocyanins		+

Quantitative Estimation of Primary and Secondary Metabolites:

The dry leaves of C. roseus were extracted in methanol and analysed for the presence of primary and secondary metabolites. Fig. 1a shows the percentage estimation of cardiac glycosides and alkaloids per gram of the leaf samples which was 25 % and 45 %respectively and fig. 1b shows the concentrations of phenolics, flavonoids, proanthocyanidines and protein in mg per gram pf the leaf samples.

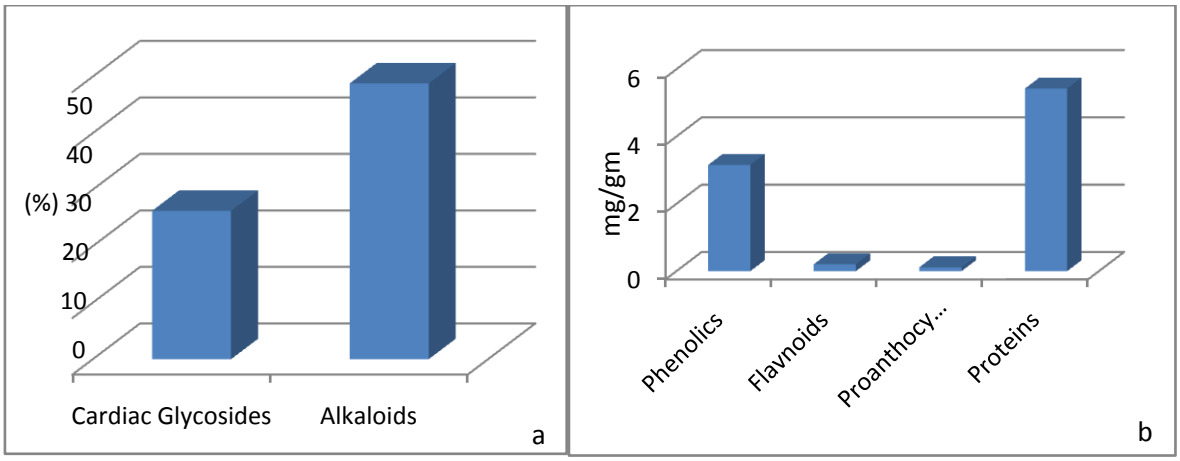

Fig. 1: Quantitative analysis of phytochemicals present in the methanolic extract of C. roseus leaves. (a) cardiac glycosides and alkaloids and (b) phenolics, flavonoids, proanthocyanidines, protein.

Since the plant is well known for the anti-cancerous properties, therefore, the leaf extract is rich in alkaloids and phenolics in comparison to other secondary metabolites. Almost all anticancer compounds are chemically alkaloids and phenolics groups. Table 2 shows the antimicrobial activity of the leaf extract. Methanolic extract shows the inhibition of E. coli when treated with leaf extract for 16 hours at 37 ^{O}C (Fig. 3). Rest of the other microbes shows no inhibition of growth while treated with leaf extract.

Table 2: Zone of inhibition of microorganisms treated with methanolic extract of C. roseusleaves.

Sr. No.	Microorganism	Action	Diameter (cm)
1.	Pseudomonas aeruginosa	No	--
2.	Pseudomonas sp.	No	--
3.	Schizosaccharomyces pombe	No	--
4.	Escherichia coli	Yes	1.1
5.	Aspergillus niger	No	--
6.	Penicillium bravicompactum	No	--

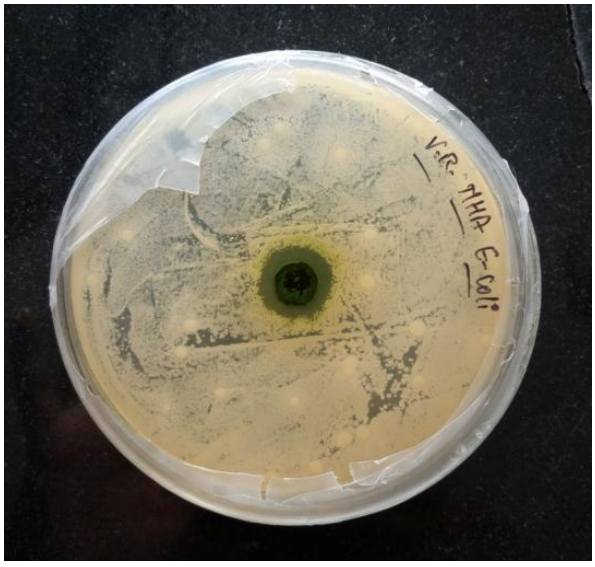

Fig. 3: Escherichia coliculture plates showing zone of inhibition

For further analysis, thin layer chromatography (TLC) technique was employed. Three different mobile phases were used to run the 5 µl methanolic leaf extract and their Rf values were calculated (Fig. 4). Table 5 shows the Rf valuesof the compounds observed on three TLC plates.

Table 5: Rf values of methanolic extract of C. roseus leaves calculated after TLC.

Sr. No.	Plate number	Compounds	Mobile Phase	R_f Value
1.	1	Saponin	Ethyl acetate : Ethanol : Water : Ammonia (6.5:2.5:0.9:0.1)	0.4166
2.				0.5833
3.	2	Anthocyanidines	Ethyl acetate : Glacial acetic acid : Water : Formic acid (10.0:1.1:1.1:2.6)	0.8285
4.	3	Tannins	Chloroform : Ethyl acetate : Ethanol (6:4:4)	6811

Fig. 4: TLC plates showing bands of secondary metabolites present in C. roseus leaves extract.

The methanolic leaf extract of the C. roseus were undergo for the analysis of percentage scavenging by DPPH. Ascorbic acid was used as a standard to compare the result. Our results confirmed that the plant exhibit the antioxidant properties (Fig. 5).

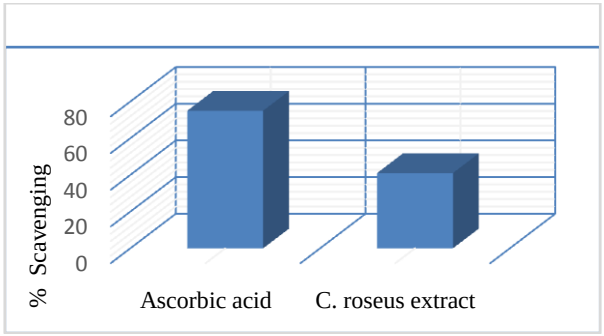

Fig. 5: Percentage scavenging by DPPH with ascorbic acid and methanolic extract of C. roseus leaves.

The plant possesses various medicinal properties due to the presence of various secondary metabolites. To determine the functional groups of the compounds present the leaf extract, the FTIR assay was done using methanolic leaf extract. Fig 6 shows the cardiogram of the FTIR assay and table 6 shows the range of peaks that resembles a specific functional group compounds present in the extract.

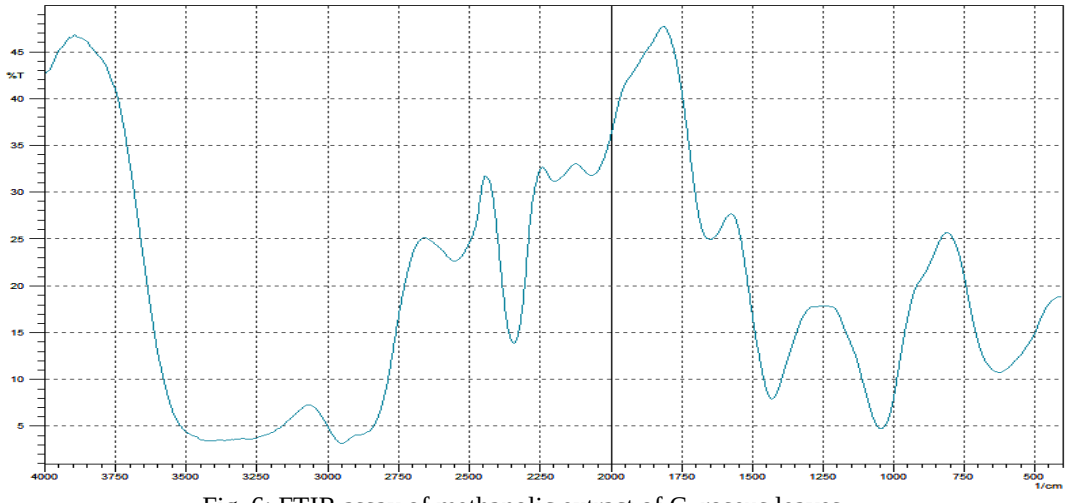

Fig. 6: FTIR assay of methanolic extract of C. roseus leaves.

Table 6: Secondary metabolites functional groups present in the C. roseus leaves.

Sr. No.	Range	Strength	Area	Functional Group
1	3000-2950	Strong	Broad	N-H
2	2650-2600	Weak	Slightly broad	S-H
3	2350-2300	Strong	Sharp	O=C=O
4	2250-2200	Weak	Slightly broad	C≡C
5	2100-2050	Weak	Slightly broad	C≡C
6	1700-1650	Strong	Slightly broad	C=O
7	1450-1400	Medium	Sharp	C-H
8	1100-1050	Strong	Sharp	C-O
9	700-650	Strong	Broad	C=C

FTIR results indicated the presence of amine (3000-2950 N-H), aldehyde (2350-230 O=C=O), alkyne (250-2200 and 2100-2050), ketone (1700-1650 C=O), hydrocarbons (1450-1400 C-H) and alkene (700-650 C=C) groups present in the leaf extract.

IV. CONCLUSION

C. roseus is an important medicinal plant with a wide range of uses. The dried plant leaf extract contain many alkaloids of medicinal use. The plant has been proven useful not only in the field of medicine but has also been recently put into use for the phytoremediation heavy metal waste. The leaves of this plant have been found to be of immense medicinal use as most of the pharmacological activities of this plant are attributed to its leaves. So the cultivation as well as the conservation of this plant must be promoted on a large scale. The methods that are less time consuming, sustainable and more economical, must be developed and adopted for the production of the active constituents present in this plant. In the present times, when the emphasis is being placed on the use of natural materials in the control and treatment of various diseases and infections because of the undesirable side effects of synthetic drugs there is a need for further research especially on the bioactive compounds, their production from alternative sources, methods for increasing their production, herbal remedies, effectiveness of plants for various uses and bioprospecting new sources of natural bioactive products which can provide unlimited scope for the development of new drug need to be explored for their huge potential of being used as the sources of pharmacologically active therapeutic lead compounds.

REFERENCES

[1] J. Aslam, S.H. Khan, Z.H. Siddiqui, Z. Fatima, M. Maqsood, M.A. Bhat, S.A. Nasim, A. Ilah, I.Z. Ahmed, S.A. Khan, A. Mujib & M.P. Sharma. (2010). Catharanthus Roseus (L.) G. Don an Important Drug: Its Applications and Production. *Pharma Globale, 4*(12), 1-16.

[2] C. Abdul Jaleel, R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan & R. Panneerselvam (2007). Studies on Germination, Seedling Vigour, Lipid Peroxidation and Proline Metabolism in Catharanthus Roseus Seedlings Under Salt Stress. *South African Journal of Botany, 73*(2), 190-195.

[3] S.S. Maithili & C. Mekala. (2014). Preliminary Phytochemical Screening and Antibacterial Activities of Methanolic Extract of Catharanthus Roseus Leaves Against Pathogenic Strains. *World Journal of Pharmacy and Pharmaceutical Sciences 4*(1), 934-944.

[4] J.R. Hood, J.M. Wilkinson & H.M.A. Cavanagh. (2003). Evaluation of Common Antibacterial Screening Methods Utilized in Essential Oils Research. *Journal of Essential Oil Research, 15*(6), 428-433.

[5] W. Brand-Williams, M.E. Cuvelier & C. Berset. (1995) Use of Free Radical Method to Evaluate the Antioxidant Activity. *Journal of Food Science and Technology, 28*, 25–30.