IJPSR (2023), Volume 14, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 December 2022; received in revised form, 30 July 2023; accepted 31 July 2023; published 01 August 2023

SPECTRAL ANALYSIS OF METHANOL LEAF EXTRACT OF *VINCA ROSEA* LINN. (*CATHARANTHUS ROSEUS*) USING GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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Keywords:

Catharanthus roseus, Apocynaceae, Mome inositol, Bioactivity, GC-MS

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ABSTRACT: *Catharanthus roseus* (periwinkle) is a medicinal plant that belongs to the Apocynaceae family. All parts of this plant are used as medicine according to ancient medicinal systems. In this research paper, plant leaf samples undergo phytochemical investigation through gas chromatography-mass spectrometry (GC-MS). As the result of GC-MS spectra we identified twenty-three phytochemical compounds in methanolic extract of *C. roseus* leaves. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. Compounds present to a major extent are specified with their NMR, IR, and Mass spectrometry data. This spectral analysis helps in the discovery of novel phytochemicals in the plant extract of this plant. We can guess about any novel phytochemicals that can be further used as synthetic units in organic synthesis reactions because natural synthetic units used in any process will always be environmentally friendly and follow green chemistry principles.

INTRODUCTION: *Catharenthus roseus* L. of family Apocynaceae contains about 400 genera and about 4,555 species of trees, shrubs, woody vines, and herbs. It is commonly known as *Vinca rosea* or periwinkle. It is widely distributed in Pakistan, India, West China, Nepal, Bhutan, Malaysia, and the dry hot part of India [1-2]. The plant is commonly grown in gardens for beddings, borders, and mass effect. It blooms throughout the year and is propagated by seeds or cuttings. About 150 days after sowing or transplanting, the periwinkle roots penetrate the soil up to 15-25 cm and then develop lateral rootlets.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(8).4202-08

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(8).4202-08

The crop is harvested for roots after one year. During this period, two leaf stripping are obtained *i.e.* after six months and nine months (Purohit and Vyas, 2004). Final leaf stripping is obtained at twelve months when the whole plant is harvested.

According to Bentham and Hooker (1862) it occupies the following systematic position.

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Catharanthus

Species: *roseus*

The plant has historically been used to treat various diseases [3-4]. It was used as a folk remedy for diabetes in Europe for centuries. In India, juice from the leaves was used to treat wasp stings. The plant was boiled in Hawaii to make a poultice to stop bleeding. In China, it was used as an astringent, diuretic and coughs remedy.

It was used as a homemade cold remedy in central and south America to ease inflammation. Throughout the Caribbean, an extract from the flowers was used to make a solution to treat eye irritation and infections. It also had a reputation as magic plant, as European thought it could ward off evil spirits. The French referred to it as "violet of the sorcerers." Western researchers finally noticed the plant in 1950's when they learn of a tea Jamaican was drinking to treat diabetes [5].

They discovered that the plant contains a mother lode of useful alkaloids (130 in all at last count). Some, such as catharanthine, leurosine sulphate, lochnerine, tetrahydroalstonine, vindoline and vindolinine lower blood sugar level, however, others act as haemostatics (arrest bleeding) and two others, vincristine and vinblastine have anticancerous properties [6]. Periwinkle also contains the alkaloids reserpine and serpentine, which are powerful tranquilizers.

Leaves sample of the plant was subjected to phytochemical investigation through Gas Chromatography-Mass Spectrometry (GC-MS). *C. roseus* is an important medicinal plant; the phytochemical study revealed that it is rich in phenolic compounds while mome inositol is present in large amounts in the leaves and obtained directly from the extract is the main extract compound with anti-alopecic, anti-cirrhotic, anti-neuropathic.

Twenty-three phytochemicals compounds were identified in the methanolic extract of C. *roseus* leaves **Table 1**. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *C. roseus* leaves **Fig. 1** revealed the existence of Isopropyl linoleate, 9,10,12,13-Tetrabromooctadecanoic acid, guanosine, hexadecanoic acid *etc*. **Table 2.**

Biological Activities:

Antioxidant Enzyme Activities: An experiment was carried out to determine the changes in the antioxidant enzyme activities with respect to different concentrations of sodium chloride (NaCl) in alba and rosea varieties of *C. roseus* (L.) G. Don. in pot culture at various stages of growth. Especially, Superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) enzymes antioxidant potentials were analyzed. The result revealed that the SOD activity was increased at the level of 50 mM NaCl but was reduced at further higher treatment levels. No significant changes were obtained on the POX activity at the range of 25 mM NaCl level but showed significant increases of this activity at the next, higher levels of NaCl. The above experiment proved that *C. roseus* is an ideal plant for cultivating salt-affected areas. We can obtain plants with higher antioxidant and medicinal values [7].

Anthelminthic Activity: Helminthes infections are chronic illnesses affecting humans and cattle. *C. roseus* was found to be used from the traditional period as an anthelminthic agent. The anthelminthic property of *C. roseus* has been evaluated using *Pherithema posthuma* as an experimental model and with Piperazine citrate as the standard reference. The ethanolic extract of the concentration of 250 mg/ml was found to show significant anthelminthic activity with a death time of 46.33 min, whereas the standard drug at 50 mg/ml was found to show a death time of 40.67 min. This investigation supported the ethnomedical claims of *C. roseus* as an anthelminthic plant [8] .

Anti-hyperglycemic Effect: The effect of the daily oral administration of *C. roseus* (CR) leaf dichloromethane: methanol (1:1) extracts (500 mg/ body weight) for 20 days was tested on the blood glucose and hepatic enzymes in the normal and Alloxan induced diabetic rats. The extract showed a significant increase in body weight and a decrease in the test animals' blood glucose, urea, and cholesterol levels. The activity of the hepatic enzymes such as hexokinase was increased whereas glucose 6-phosphatase and fructose 1,6-bisphosphatase were found to be decreased significantly [9].

Antineoplastic and Antidiabetic Effect: Different percentages of the methanolic crude extracts of *C. roseus* showed significant anticancer activity against numerous cell types in the in-vitro condition. The greatest activity was found against the multidrug-resistant tumour types. Several animal studies have proved that the ethanolic extracts of the leaves and flowers of *C. roseus* has lowered blood glucose levels. The aqueous extract was found to lower the blood glucose of about 20% in diabetic rats when compared to that of the dichloromethane and methanol extracts which lowered the blood glucose level to 49-58%. The hypoglycaemic effects have appeared due to increased glucose utilization in the liver [10-14].

***In-vivo* Antidiarrheal Activity:** *In-vivo* antidiarrheal activity of *C. roseus* ethanolic leaf extract was tested in the Wistar rats with castor oil as an experimental diarrhea-inducing agent in addition to the pre-treatment of the extract. Loperamide and atropine sulphate was used as the standard drugs. The antidiarrheal effect of ethanolic extract of C. *roseus* showed the dose-dependant inhibition of the castor oil-induced diarrhea at 200 and 500 mg/kg 15 doses.

Antioxidant Properties: Free radicals are found to be fundamental to any biochemical process and hence represent an essential part of aerobic life and metabolism and could show a dual role in our body as both the deleterious and beneficial species. The antioxidant potential of the ethanolic extracts of the roots of the two varieties of *C. roseus* L. namely '*rosea*'(pink flowers) and '*alba*'(white flowers) was obtained by using different systems of assay such as Hydroxyl radical-scavenging activity, superoxide radical-scavenging activity, DPPH radical- scavenging activity and nitric oxide radical inhibition method. The results obtained proved that the ethanolic extracts of the roots of Periwinkle varieties extracts has exhibited a satisfactory scavenging effect in all the radical scavenging assays in a concentration-dependent manner, but *Catharanthus rosea* was found to possess more antioxidant activity than that of *Catharanthus alba* [16].

TABLE 1: PHYTOCHEMICALS IN THE METHANOL EXTRACT OF THE LEAF OF *C. ROSEUS* BY GC-MS

Peak#	R. Time	Area	Area%	Name
1	10.123	756361	1.17	Guanosine
2	10.887	436590	0.67	Diethyl Phthalate
3	13.100	47508268	73.24	Mome Inositol
4	13.445	661793	1.02	Neophytadiene
5	13.849	119865	0.18	1,2-Benzenedicarboxylic Acid, Bis(2-Methyl
6	13.901	149967	0.23	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
7	14.402	1134901	1.75	Hexadecanoic Acid, Methyl Ester
8	14.834	218620	0.34	Dibutyl Phthalate
9	15.027	522979	0.81	N-Hexadecanoic Acid
10	16.039	281329	0.43	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
11	16.103	1250711	1.93	9,12,15-Octadecatrienoic Acid, Methyl Ester
12	16.220	328327	0.51	Phytol
13	16.325	208550	0.32	Octadecanoic Acid, Methyl Ester
14	16.781	394407	0.61	1-(4-Isopropylcyclohexyl)Ethanol
15	17.739	44607	0.07	Carbonic Acid, 2-Dimethylaminoethyl Neopentyl Ester
16	18.092	151887	0.23	Eicosanoic Acid, Methyl Ester
17	20.279	1021188	1.57	1,2-Benzenedicarboxylic Acid
18	21.093	359374	0.55	2,20-Cycloaspidospermidine-3-Carboxylic Acid, 6,7-Didehy
19	21.312	165139	0.25	2,20-Cycloaspidospermidine-3-Carboxylic A
20	27.273	3070058	4.73	Isopropyl Linoleate
21	27.899	3239602	4.99	9,10,12,13-Tetrabromooctadecanoic Acid
22	29.871	412252	0.64	Dl-.Alpha.-Tocopherol
23	31.190	2433865	3.75	1h-Indolizino[8,1-Cd]Carbazole, Aspidosper
		64870640	100.00	

MATERIAL AND METHODS:

Plant Material: Sample of *C. roseus* leaves was collected and identified by the Department of Botany, University of Rajasthan, Jaipur. Leaves were further washed with distilled water and transferred to our laboratory to dry. These dried

leaves were stored and sealed in polythene bags for further experimental purpose.

Extraction: The shade-dried leaves (3Kg) were finely grinded and converted into a fine powder. This grinded leaves powder (500 g) was taken in Soxhlet apparatus to extract in two litter methanol solvent for 24 hours in the heating mental. The excess solvent was removed by the help of rota-vapor (R-300) at 45°C temperature. Further it was treated with sodium sulphate to make this moisture free. This extract was filtered and stored in cold place until further analysis. This methanol extract was pronounced because of less work done in methanolic solvent extraction.

GCMS Analysis: Gas Chromatography combined with Mass Spectrometry is a preferred methodology for routine analysis of compounds. The GC-MS analysis of the above-mentioned extracts was performed with a Gas Chromatography unit Shimadzu GCMS-QP2010 Plus comprising AOC-20i+s auto-sampler. Various components were identified by different retention times, which were detected by a mass spectrophotometer.

Fig. 1 is a chromatogram plot of intensity against retention time recorded by the software attached. From the graph, the compounds are identified, comparing the data with the existing software libraries like WILEY8.lib, NIST11.lib, NIST11s.lib, FFNSC2.lib, and mass spectra of standard. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULT AND DISCUSSION: The GC spectrum of the methanolic extract shows a total 23 compound present in the methanolic extract, which were determined by the chromatographic method with the help of NIST and WILLEY library, as shown in **Table 1.** Compound mome inositole was found to be in highest concentration (73.24%) followed by 9, 10, 12, 13-Tetrabromooctadecanoic acid(4.99%), Isopropyl linoleate (4.73%), 1H-Indolizino[8,1-CD] Carbazole (3.75%) and Methyl linolinate (1.93%) **Table 2**.

Other compounds were found in trace amounts **Table 1**. One or all identified compounds may be responsible for the biological activity of the methanolic extract. Further separation of the identified compounds will be due in the course.

Inositol can stimulate glucose uptake in skeletal muscle cells, allowing blood sugar levels to decrease. This effect is later seen as a reduction in urine glucose concentration and indicates a decrease in high blood sugar levels. In PCOS, the administration of inositol has produced the remission of symptoms as well as a reduction in male hormone secretion, a regulation of the cholesterol level, and a more efficient fat breakdown which significantly reduces body mass and appetite.

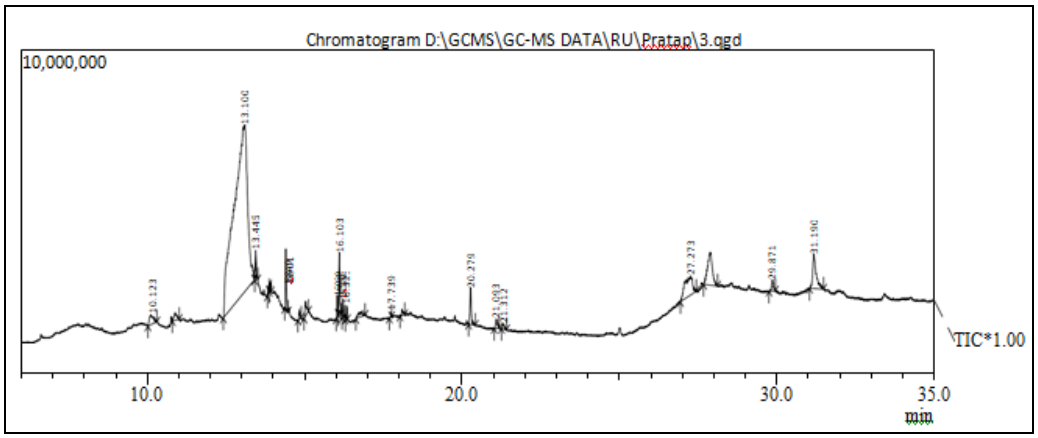
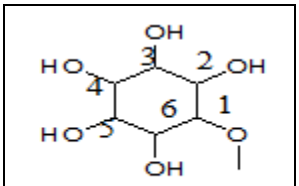


FIG. 1: GC-MS SPECTRUM OF METHANOLIC LEAF EXTRACT OF *C. ROSEUS*

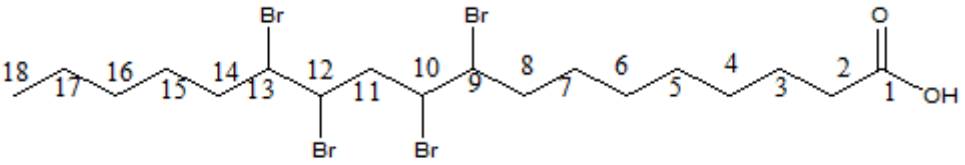
TABLE 2: MAJOR PHYTOCHEMICALS IDENTIFIED IN THE METHANOL EXTRACT OF *C. ROSEUS* L.

S. no.	Phytochemical Compound	Rt min	Molecular Formula	Molecular Weight	MS Fragment –ions	Area %
1	Mome inositol	13.100	C_7 H14 O_6	194	27, 31, 45, 60, 73, 87, 103, 116, 127, 144, 158	73.24

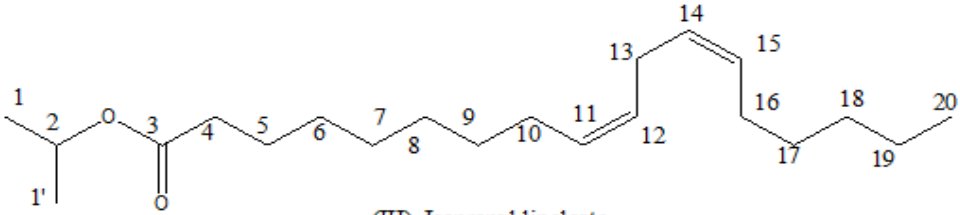
2	9,10,12,13-Tetrabromo octadecanoin acid	27.899	$C_{18}H_{32}BrO_2$	596	17, 27, 41, 55, 67, 81, 95, 109, 123, 137, 149, 163, 181, 195, 209, 223, 235, 259, 277, 280, 297, 321, 341, 357, 379, 419, 439, 517	4.99
3	Isopropyl linoleate	27.273	$C_{21}H_{38}O_2$	322	51, 55, 67, 81, 95, 109, 123, 137, 151, 163, 181, 195, 209, 223, 237, 263, 279, 280, 322	4.73
4	1H-indolizino[8,1-CD]carbazole, Aspidospern	31.190	$C_{25}H_{32}N_2O_6$	456	106, 121, 135, 144, 161, 174, 188, 222, 240, 265, 282, 296, 309, 397, 456	3.75



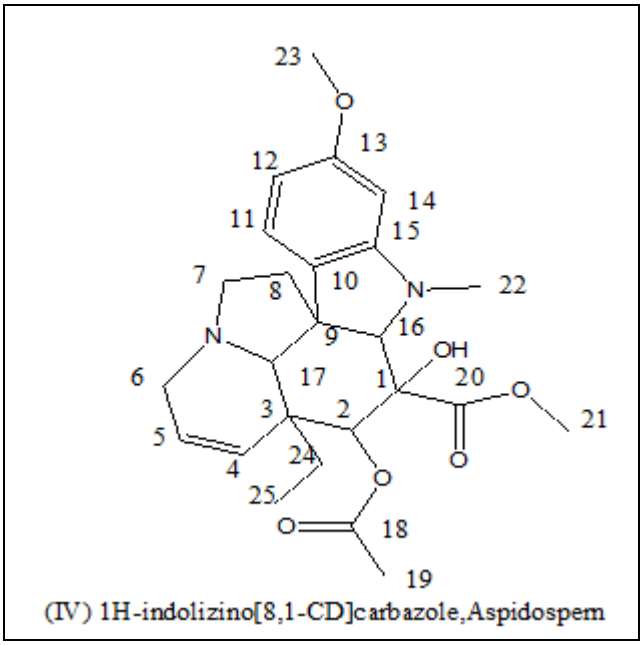
(I) Mome inositol



(II) 9,10,12,13-Tetrabromooctadecanoin acid



(III) Isopropyl linoleate



(IV) 1H-indolizino[8,1-CD]carbazole,Aspidospern

In the cases of infertility, inositol has been proven to increase sperm count and motility and the overall quality of oocytes and embryos. In the brain, inositol has been shown to produce an increase in serotonin receptor sensitivity. This activity produces an increase in GABA release. Some of the effects observed in the brain produced relief in symptoms of anxiety and obsessive-compulsive disorders. In high doses, it has been shown to even reduce panic attacks. In cancer research, inositol has gained interest as it can act as an antioxidant, anti-inflammatory and it seems to enhance immune properties.

Experimental Section:

Characterization Data:

Compound 1: Mome Inositol: White crystalline powder; IR (KBr):-3370, 3010, 2825, 2890 cm^{-1} 1H NMR (400 MHz, CDCl3): δH 3.33(3H, d, C-3, 4, 5); 3.57(2H, d, C2, 6); 2.95 (1H, s, C-1), 2.22 (5H, OHx5), 3.29 (3H, s,CH_3); 13C NMR (300 MHz, CDCl3): δC 67.3(C-2), 67.5(C-3), 64.9(C-4), 73.1(C-5), 67.8(C-6), 64.5(C-1), 52.5(CH_3) Mass (MS Fragment ion): m/z 27, 31, 45, 60, 73, 87, 103, 116, 127, 144, 158.

Compound 2: 9, 10, 12, 13-Tetrabromo-octadecanoin Acid: Light yellow amorphous powder; IR (KBr):-2885, 2990, 1780, 3320 cm^{-1} 1H NMR (400 MHz, CDCl3): δH 11.1(1H, s, -OH), 2.25 (2H,t, H-2), 1.60 (2H,tt, H-4) , 1.31(2H, tt, H-4), 1.27(2H, tt,H-5), 1.29(2H, tt,H-6), 1.30(2H, tt, H-7), 1.78(2H, dt, H-8), 3.82(1H,dt,H-9), 3.85(1H,dt,H-10),1.80(2H,dd,H-11),3.84(1H,dt,H-12),3.78(1H,dt,H-13),1.75(2H,dt,H-14), 1.33 (2H, tt, H-15), 1.30(2H,tt,H-16),1.28(2H,tt,H-17), 0.96(3H,t,H-18) 13C NMR (400 M Hz, CDCl3): δC 176(C-1), 36.2(C-2), 25.6(C-3), 29.1 (C-4), 30.5 (C-5), 29.5 (C-6), 25.3(C-7), 34.5 (C-8), 48.1 (C-9), 44.2 (C-10), 39.1 (C-11), 47.3(C-12), 35.5 (C-13), 25.2 (C-14), 32.3 (C-15), 26.2(C-16), 23.1 (C-17), 15.4 (C-18).

Mass (MS Fragment Ion): m/z 17, 27, 41, 55, 67, 81, 95, 109, 123, 137, 149, 163, 181, 195, 209, 223, 235, 259, 277, 280, 297, 321, 341, 357, 379, 419, 439, 517.

Compound 3: Isopropyl Iinoleate: Colourless liquid; IR (KBr):-1710, 1180, 2205, 720, 3070, 2980, 2890 cm^{-1} 1H-NMR (400 MHz; CDCl3): δH 1.38 (6H,d,H-1), 4.35(1H,m,H-2), 2.30(2H,t,H-4), 1.65(2H,m,H-5), 1.28(2H,m,H-6), 1.27(2H,M,H-7), 1.30(2H,m,H-8), 1.34(2H,m,H-9), 1.91(2H,dt,H-10), 5.30(1H,dt,H-11), 5.41(1H,dt,H-12), 2.65(2H, dd,H-13), 5.44(1H, dt, H-14), 5.34(1H, dt, H-15), 1.93(2H,dt,H-16), 1.30(2H,M,H-17), 1.28(2H,m,H-18), 1.36(2H,m,H-19), 0.92(2H,t,H-20).

13C-NMR (100 MHz; CDCl3): 21.9(C-1), 68.1(C-2), 176(C-3), 33.2(C-4), 26.3(C-5), 28.6(C-6), 30.5(C-7), 30.2(C-8), 30.1(C-9), 27.5(C-10), 133.2(C-11), 123(C-12), 25.2(C-13), 123(C-14), 133.2(C-15), 27.4(C-16), 31.1(C-17), 32.4(C-18), 23.5(C-19), 14.4(C-20).

Mass (MS Fragment Ion): m/z 51, 55, 67, 81, 95, 109, 123, 137, 151, 163, 181, 195, 209, 223, 237, 263, 279, 280, 322.

Compound 4 1H-Indolizino [8, 1-CD]Carbazole, Aspidospern: Yellowish brown amorphous solid; IR (KBr):-2820, 1590, 1430, 2785, 1720, 1185, 3410cm^{-1} 1H NMR (400 MHz, CDCl3): δH 1.29(1H,s,C-2), 5.72(1H,d,C-4), 5.77(1H,m,C-5), 2.83(2H,d,C-6), 2.25(2H,t,C-7), 1.84(2H,t,C-8), 7.11(1H,d,C-11), 6.20(1H,d,C-12), 6.01(1H,s,C-14), 3.65(1H,s,C-16), 2.01(3H,s,C-19), 3.67(3H, s,C-21), 2.85(3H,s,C-22), 3.73(3H,s,C-23), 4.64 (2H, q,C-24), 0.96(3H,t,C-25), 2.0(1H,s,-OH) 13C NMR (400 MHz, CDCl3): δC 80.4(C-1), 77.0(C-2), 24(C-3), 132.4(C-4), 125.4(C-5), 51.5(C-6), 45.2(C-7), 29.4(C-8), 35.4(C-9), 121.7(C-10), 128.6(C-11), 103.3(C-12), 160.1(C-13), 98.4(C-14), 43.7(C-15), 59.3(C-16), 35.4(C-17), 171(C-18), 17.6(C-19), 176.0(C-20), 51.0(C-21), 39.8(C-22), 56.0(C-23), 21.3(C-24), 9.7(C-25).

Mass (MS Fragment Ion): m/z 106, 121,135, 144, 161,174, 188, 222, 240,265, 282, 296, 309, 397, 456.

CONCLUSION: Present study of the methanolic extract of *C. roseus* leave indicated that it contains biologically active compounds. The properties of these compounds probably contribute, at least to some extent, to the pharmacological and traditional uses of *C. roseus*. One or all identified compounds may be responsible for the biological activity of the methanolic extract. Further separation of the identified compounds will be in due course.

ACKNOWLEDGEMENT: Financial support from CSIR, New Delhi, India, is highly acknowledged. Further, the authors are grateful to JNU Delhi for providing GC-MS spectral analysis/ data of plant extract.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Singh P, Bhargava S and Rishi H: Spectral analysis of methanol leaf extract of *Vinca rosea* linn. (*Catharanthus roseus*) using gas chromatography- mass spectrometry. Int J Pharm Sci & Res 2023; 14(8): 4202-08. doi: 10.13040/IJPSR.0975-8232.14(8).4202-08.