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Qualitative and quantitative analysis of phytochemicals by GC-MS and antioxidant activity of *Catharanthus roseus* (L.) G. Don

Manish Kapoor and Jyoti Rani

Abstract
The present study was framed to evaluate bioactive phytochemical, antimicrobial and antioxidant properties of the sequentially extracts prepared from leaves of *Catharanthus roseus* in five different solvents (Petroleum ether, Chloroform, ethanol, methanol and aqueous). Antioxidant potential of the extracts was evaluated by both enzymatic assays (Superoxide dismutase, Catalase, Glutathione peroxidase) and non-enzymatic assays (DPPH, ABTS). Lowest IC_{50} values were shown by methanolic extracts followed by ethanol and aqueous extracts. The antioxidant activity of enzymes *viz*. catalase, glutathione peroxidase and superoxide dismutase were found to be satisfactory. The antioxidant activity of the extracts exhibited positive correlation with the total phenolic and flavonoid content of the extracts. The chemical composition of methanolic extract was also analysed by GC-MS. Seven phytochemical were predominantly identified in the methanolic extract by GCMS.

Keywords: Phytoconstituents, TPC, TFC, DPPH, ABTS, DPPH and GCMS

Introduction
Men have used natural and herbal compounds for reliving various maladies since the beginning of human civilization and, for quite a while, plant, animal and mineral were the imperative sources of drugs. Medicinal plants possess a wide variety of secondary metabolites like alkaloids, flavonoids, glycosides, phenols and tannins etc. which are used for therapeutic purposes. The development of organic chemistry inclined the use of synthetic drugs for pharmacological treatment. The reason behind this was the easy isolation and structural modification of natural compounds to produce more active, potential and safer drugs [19]. Over the most recent 30 years, over 25% of newly discovered and approved drugs are based on molecules of phyto-origin [21]**.** Free radicals and Reactive oxygen species are responsible for oxidative stress and induce various degenerative diseases [8]. Recently, interest is rising among the researchers to identify and isolate new antioxidants components from various medicinal plants, vegetables and fruits. Antioxidants are a wide array of compounds that neutralize free radicals, preventing damage to cells. Anti-oxidative compounds present in natural resources play a significant role in the prevention of the oxidative stress and also play a great role in limiting the formation of ROS [5]**.** Ethnomedicinal plants are important sources of secondary metabolites and have great role drugs discovery and the curing various ROS generated diseases.
However, the potential use of large plants as a source of new drugs is still inadequately investigated. It is estimated that 2,500–5,000 plant species i.e. only a small percentage have yet studied or analysed for medicinal properties [21]. There is a large-scale international trade in the medicinal plants, one such important medicinal plant is *Catharanthus roseus.*
Catharanthus roseus is a perennial or annual evergreen sub-shrub plant belongs to family Apocynaceae, that grows up to one meter in height and furthermore secretes white milky latex *Catharanthus roseus* plant native to Madagascar Island. It has been introduced as a popular decorative plant in tropical and subtropical countries [26].

This plant is commercially cultivated in Africa, Austria, China, India and Spain for its medicinal qualities. Looking at the vast ethnomedicinal values of *C. roseus*, current study was planned to determine its phytochemical constituents, antimicrobial and antioxidant properties. Efforts were also been made to find out and analyse plant extracts with the help of GC-MS.

Materials and Methods

Collection of plant material

The leaves of *C. roseus* were collected from District Patiala, Punjab (India) in the month of June-July (2016).

Preparation of plant extracts

Young and fresh leaves of plant *C. roseus* were washed with running tap water and finally rinsed with distilled water to remove unwanted foreign materials like soil and dust. After washing, leaves were dried under shade at room temperature. Dried leaves were coarsely grounded and powdered into fine powder by electric blender and stored in an airtight glass container till further use. Percent yield of crude extract was calculated using following formula:

$$\text{Percent yield} = \frac{\text{weight of crude extract obtained (g)}}{\text{total weight of plant powder (g)}} \times 100$$

Solvent Extraction

The leaves of *C. roseus* (50 g) was extracted by maceration at room temperature in 250 mL of different solvents *viz*. Petroleum ether, Chloroform, ethanol, methanol and aqueous. All the plant extracts were filtered through a whattman filter paper and then concentrated by using a rotary evaporator. The extracts were preserved in airtight containers and stored at 4°C till further use.

Preliminary Phytochemical Screening

The extracts prepared in different solvents were analysed for the presence of different phytochemicals as per standard methods listed in Table-1.

Total phenolic content

Total phenolic content was estimated by Folin-Ciocalteu method of [3] with minor modifications. A test solution of plant extracts was prepared to 1mg/mL. 5mL of Folin-Ciocalteu and 2mL of Na_2CO_3 was added to 1mL of plant extract samples. The solution was vortexes and incubated in dark for 15 minutes Absorbance was measured at 620 nm. Blank consisted of 5 mL Folin-Ciocalteu, 1mL extraction solvent and 2mL of Na_2CO_3 solution. Tannic acid was used as standard. The total phenolic content was calculated from calibration curve ($y = 0.0164x+0.0557$, $R^2 = 0.9964$) and result was expressed in terms of mg of tannic acid equivalent (TAE) per gram dry weight of sample. All tests were performed in triplicate.

Total flavonoid content

Total flavonoid content was estimated by Aluminium Chloride method with some minor modifications [3]. A test solution of different extracts was prepared to 1mg/mL. 1mL of plant sample with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and 5.6 mL of distilled water was added and the mixture was vortexes and left at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard. All tests were performed in triplicate and flavonoid content was determined using the calibration curve ($y=0.0047x+0.0391$, $R^2 = 0.996$) and results were expressed as mg of quercetin equivalent (QE) per gram dry weight of sample.

Table 1: Standard methods for preliminary phytochemical analysis:

Phytochemicals	Test name	Methodology	Observations	Reference(s)
Alkaloid	Mayer's Test:	To 1 mL Plant extract few drops of Mayer's reagent (Potassium Mercuric Iodide) were added.	Formation of a yellow coloured precipitate	[12].
Flavonoids	Alkaline Reagent Test:	1-2 mL of Plant extract was treated with 2-3 ml of dilute NaOH, followed by addition of 3-4 mL dilute HCl.	Formation of intense yellow color, which becomes colorless on addition of dilute HCl.	[20].
Glycosides	Salkowski test:	To 1 mL of Plant extract, 2 mL of chloroform was added, followed by the 2 mL of concentrated H_2SO_4 acid.	Formation of reddish brown colored steroidal ring.	[9].
Phenols	Ferric Chloride Test:	To 2 mL of Plant extract, 3-4 drops of ferric chloride solution were added.	Formation of dark green color.	[9].
Saponins	Foam Test:	1-2 mL Plant extract, was mixed with 5 mL distilled water in a test tube and shaken vigoursly.	The formation of stable foam.	[12].
Tannins	Braemer's test:	10% alcoholic ferric chloride was added to 2-3 mL of methanolic plant extract.	Formation of dark blue or greenish grey colour of the solution.	[16].
Terpenoids		1 mL of Plant extract was dissolved in 2 mL of methanol and then evaporated to dryness followed by the addition of 3 mL of Conc. H_2SO_4.	Formation of reddish brown color.	[16].

Antioxidant Enzymatic assays

Plant extraction for enzymatic Assays

Freshly collected leaves (0.5 g) were homogenized in pestle and mortar with 5.0 ml of 100 mM potassium phosphate buffer (pH 7.0) under ice cold conditions. The homogenate was centrifuged at 15,000 g for 20 minutes at 4°C. and the supernatant was used for analysis of protein content and activities of antioxidative enzyme.

Protein Estimation

Protein estimation was done by following method using Reagent A – 2.0 % sodium carbonate in 0.1N sodium hydroxide, Reagent B – 0.5% copper sulphate in 1.0 % potassium sodium tartarate, Reagent C – 50 ml of reagent A and 1.0 ml of reagent B, Reagent D – FC reagent. Protein solution (stock standard) – 50 mg of BSA dissolved in distilled water and the final volume was made to 50 ml.

0.1 mL of the sample and standard were pipetted into a series of test tubes. The volume of 1.0 mL was made up in all test tubes with distilled water. A tube with 1.0 mL of distilled water was used as blank. 5.0 mL of reagent C was added to each tube. After mixing it, it was allowed to stand for 10

minutes. Then, 0.5 ml of reagent D was added, mixed and incubated at room temperature in the dark for 30 minutes. Blue colour developed. The absorbance was recorded at 550 nm. A graph of absorbance to concentration for standard solutions of proteins was plotted and the amount of protein in the samples was calculated from the graph. The amount of proteins was expressed as mg/g FW.

Catalase (CAT) Assay

Catalase activity was determined as per the method of [2] 300 µL of enzyme extract was taken in cuvette and to this 1.5 mL phosphate buffer (10 mM) and 1.2 mL of hydrogen peroxide (150 mM) was added. The absorbance was taken at 240 nm and decrease in absorbance/minute was recorded. One unit of the enzyme activity was calculated as the amount of enzyme required to liberate half the peroxide oxygen from H_2O_2 and calculated using the following equation:

$$\text{Unit activity (Units/minute/gram FW)} = \frac{\text{Change in absorbance per minute} \times \text{Total volume (mL)}}{\text{Extinction coefficient} \times \text{Volume of sample taken (mL)}}$$

Where, extinction coefficient = 39.4 $mM^{-1}cm^{-1}$

$$\text{Specific activity (mol UA/mg protein)} = \frac{\text{Unit activity (Units/minute/gram FW)}}{\text{Protein content (mg/gram FW)}}$$

Superoxide dismutase (SOD) Assay

Superoxide dismutase levels in the cell free supernatant were measured by the method of [17]. Briefly 1.9 mL of sodium carbonate buffer (50 mM, pH 10.0), 750 µL Nitrobluetetrazolium dye (NBT), 240 mM and 150 µL of 0.3% Triton X-100 was added to the test cuvette. The reaction was initiated by the addition of 150 µL of hydroxylamine hydrochloride (10 mM, pH 6.0). After 2 minutes, 70 µL enzyme sample from the plant tissue was added. The percent inhibition in the rate of NBT reduction was recorded as increase in absorbance at 560 nm. The percent inhibition of NBT reduction was calculated as:

$$\text{X (\%)} = \frac{\text{Change in absorbance/minute (Control)} - \text{Change in absorbance/minute (Test)}}{\text{Change in absorbance/minute (Control)}} \times 100$$

X% of inhibition is produced by 70 µL of sample.
Hence, 50% inhibition is produced by

$$\text{Y µL of sample} = \frac{50 \times 70}{\text{X}} \times 100$$

One unit of the enzyme activity is defined as the enzyme concentration required for inhibiting the absorbance at 540 nm of chromogen production by 50% in one minute under the assay conditions. SOD activity was expressed as SA = mol UA/mg protein.

Guaiacol peroxidase (POD) Assay

Peroxidase was estimated according to the method given by [24]. In the test cuvette, the reaction mixture composing of 3 mL phosphate buffer (100 mM), 30 µL guaiacol solution (20.1 mM), 100 µL enzyme sample and 30 µL H_2O_2 solution (12.3 mM) was taken. The rate of formation of guaiacol dehydrogenation product was followed spectrophotometric ally at 436 nm. One unit of enzyme activity was expressed as units/minutes/gram tissue. Enzyme activity was calculated as following:

$$\text{Enzyme activity} = \frac{\text{Change in absorbance/minute} \times \text{Total volume}}{\text{Extinction coefficient} \times \text{Volume of sample taken}}$$

Where extinction co-efficient = 26.60 $mM^{-1}cm^{-1}$

$$\text{Specific activity (m mol UA/mg protein)} = \frac{\text{Unit activity (Units/minute/gram FW)}}{\text{Protein content (mg/gram FW)}}$$

DPPH Assay

The free radical scavenging activity of plant extracts was measured using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay [6]. A stock solution of plant extract was prepared to 1 mg/mL in their respective solvents. Each stock solution was diluted from 10-100 µg /mL. One ml of 0.3 mM DPPH solution was added to 1mL of the plant extracts of different concentrations. Ascorbic acid was used as standard with same concentration (10-100 µg/mL). All the steps were performed in dark and sample was incubated in dark for 30 minutes. The absorbance was measured at 517 nm by UV-Vis spectrophotometer. Experiment was performed in triplicate and mean was recorded. Antioxidant activity was calculated using the following formula:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

ABTS Assay

ABTS **(**2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid**)** assay was carried out as per the slightly modified method of [28]**.** ABTS radical cation ($ABTS^+$) was produced by reacting 7 mM ABTS solution with 2.45 mM ammonium per sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The ABTS solution was diluted with ethanol to get an absorbance of 0.700 at 745 nm. A stock solution of each plant extract was prepared to 1mg/mL in their respective solvent. Each stock solution diluted from 10- 100 µg/mL. One mL solution of ABTS was added to 1mL of sample solution of different concentration. Ascorbic acid was used as standard with same concentration (10-100 µg/mL). The absorbance was measured at 745 nm by UV-Vis spectrophotometer. Antioxidant activity was calculated as below:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

GC-MS Analysis

Methanolic extract was also analysed by GC-MS equipment. The temperature was set as, 70°C for 2 minutes, hold increased at 7°C/minute up to 200°C and then accelerated at 5°C/minute up to 220°C with 5 minutes hold. Injector temperature was set at 220°C. The scanning of mass range was from 35 to 400 (m/z). The control of the GC-MS system and data peak processing was controlled by means of Excalibur software. Phytoconstituents identification was verified based on the relative retention time and their peak area with the NIST and LIB database of the GC-MS system. The spectrum of the unknown constituents was compared with the spectrum of known constituents stored in the NIST library and also compared with the available literature. The compound name, molecular weight, molecular formula, peak area percentage and their potent biological functions of the methanolic extract was determined.

Statistical Analysis

Statistical analysis of data was done accordance with the procedure given by [10] and were analysed as per completely

randomized design (CRD) [29] to test the significance of differences between the extracts. Coefficient of variation was calculated using method given by [7].

Results and Discussions

Percentage yield

Percentage yield of the five different extracts obtained was calculated and the results are presented in Table 2, reveal that the maximum yield was observed in methanol extract (8.8 %), followed by ethanol extract (6.7 %), aqueous extract (5.6 %) and chloroform extract (3.5 %); while minimum yield was obtained in petroleum ether extract (2.3 %).

Table 2: Percentage yield of the leaves extracts of *Catharanthus roseus* prepared in different solvents:

S. No.	Extract	Method of extraction	Colour	Physical nature	Yield % W/V
1	Methanol	Extraction using maceration method	Brownish green/ Thick solid mass	Solid	8.8
2	Ethanol		Green sticky	Solid	6.7
3	Aqueous		Brownish/ sticky mass	Semi-solid	5.6
4	Chloroform		Greenish brown/ Sticky mass	Semi-solid	3.5
5	Petroleum ether		Light green	solid	2.3

Phytochemical Analysis

The plant extracts of *C. roseus* prepared in five different solvents were evaluated for the presence of different phytochemicals and the result obtained are presented in Table 3, perusal of the results reveal that all the five extracts, showed the presence of alkaloids, phenols and flavonoids. Methanolic extract showed the maximum number of phytoconstituents, followed by the ethanol and aqueous extract. Glycosides were detected in all the extracts, except petroleum ether extract. Terpenoids were detected in both methanolic and chloroform extracts. Saponins were detected in the aqueous extract only.

Table 3: Phytochemical analysis of plant extracts of *C. roseus* prepared in different solvents.

S. No	Phytochemicals	Plant extracts				
		Methanol	Ethanol	Aqueous	Chloroform	Petroleum ether
1	Alkaloids	+	+	+	+	+
2	Flavanoids	+	+	+	+	+
3	Glycosides	+	+	-	-	-
4	Phenols	+	+	+	+	+
5	Saponins	-	-	+	-	-
6	Terpenoids	+	+	-	-	-
7	Tannins	+	+	+	-	-

** (+) indicate the presence, (-) indicate the absence of phytoconstituents.

Total phenolic content

Total amount of phenols obtained in the plant extracts is depicted in Table 4. Maximum phenolic content was estimated by methanolic extract (29.96±0.55) followed by ethanol extract (27.11±0.59). The least value of TPC was observed in petroleum ether extract (14.04±0.58) followed by Chloroform (18.50±0.29).

Total flavonoid content

All the four plant extracts were evaluated for the quantitative determination of total flavonoid content, the results obtained are represented in the Table 4. The perusal of the data reveals that TFC was highest in the methanolic extract (30.59±1.44) followed by the ethanol extract (23.87±0.12), while least flavonoid content was observed to be in the plant extract prepared in the petroleum ether (11.92±0.59).

Table 4: Mean phenolic and flavonoid content (mg/g) in the leaves of *Catharanthus roseus* L (G.) Don

	Methanol extract	Ethanol extract	Aqueous extract	Chloroform	Petroleum ether
TPC	29.96±0.55	27.11±0.59	21.74±0.26	18.50±0.29	14.04±0.58
TFC	30.59±1.44	23.87±0.12	15.49±0.86	14.46±0.29	11.92±0.59

Enzymatic antioxidant activity

The activity results obtained for antioxidant enzymes *viz*. SOD, POD and Catalase, are presented in Table 5. The critical observation of the data reveals that, *C. roseus* had good enzymatic antioxidant activities for all the three enzymes tested. The enzyme activity was found to be 0.53±0.23 µmole H_2O_2/minute/g for Catalase; 1.54±0.15 µmol H_2O_2/min/g of POD and SOD activity is 28.05±0.08 unit/minute/g of FW.

Table 5: Antioxidant activity in term of SOD, POD and CAT of leaves extracts of *Catharanthus roseus* L (G.) Don

Enzyme activity (units/minute/g)		
SOD	**POD**	**CAT**
28.05±0.08	1.54±0.15	0.53±0.23

DPPH Assay

The results obtained from the DPPH radical scavenging activity of all the four extracts are graphically represented in the Fig. 1. The highest antioxidant activity was obtained with the methanolic extract followed by ethanol extract and least activity was obtained with of petroleum ether extract.

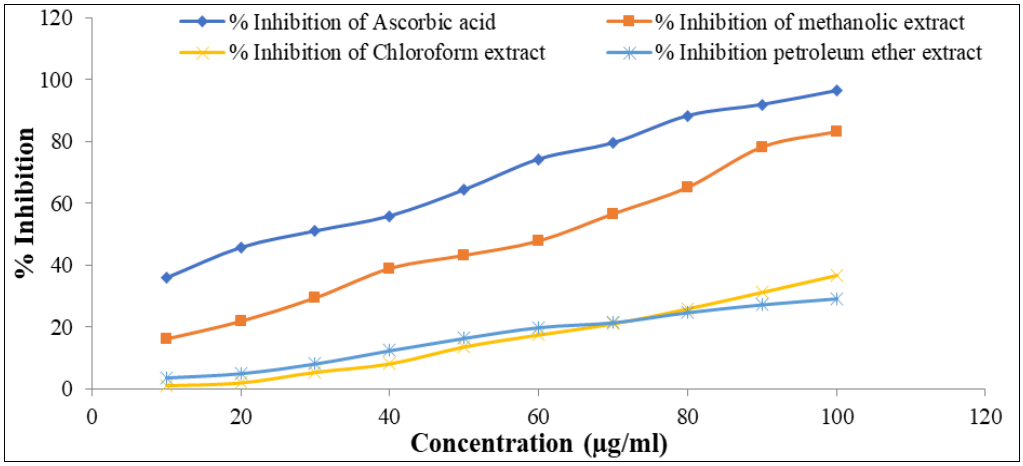

Fig 1: Inhibition Percentage of DPPH radical by plant extracts of *C. roseus*

A low IC_{50} value shows high antioxidant activity and *vice versa*. Methanolic extract showed the least value of IC_{50} (53.60 µg/mL) hence highest antioxidant activity followed by the chloroform extract (82.22 µg/mL) and least antioxidant activity was shown by petroleum ether extract having highest IC_{50} value (160.30 µg/ml**)** showed in Fig. 2.

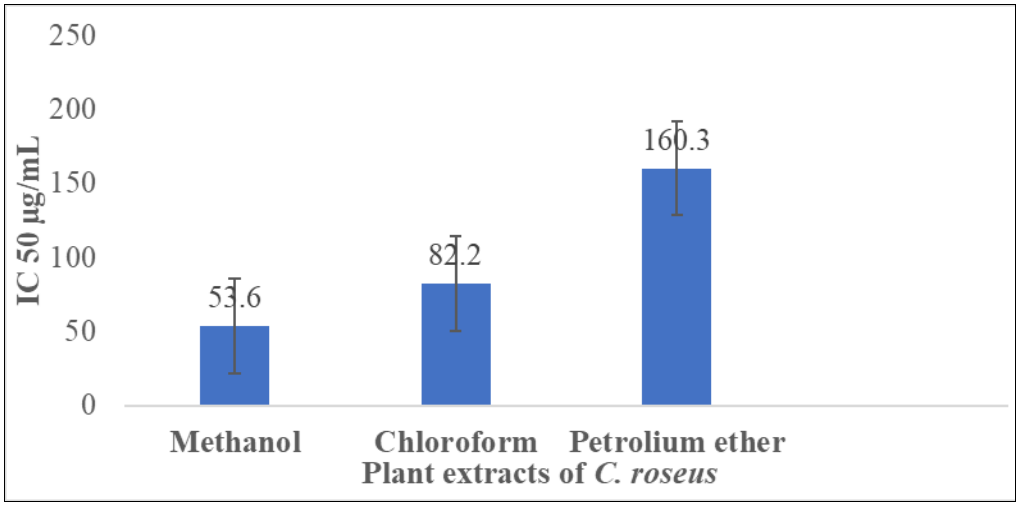

Fig 2: IC_{50} values of plant extracts.

ABTS assay

The antioxidant activities of the plant extracts towards ABTS radical were also determined and results are presented in Fig. 3. All the four extracts showed the ability to neutralise the ABTS radical. The maximum activity was obtained with the methanolic extract followed by chloroform extract and similarly like DPPH assay lowest was calculated in case of petroleum ether extract.

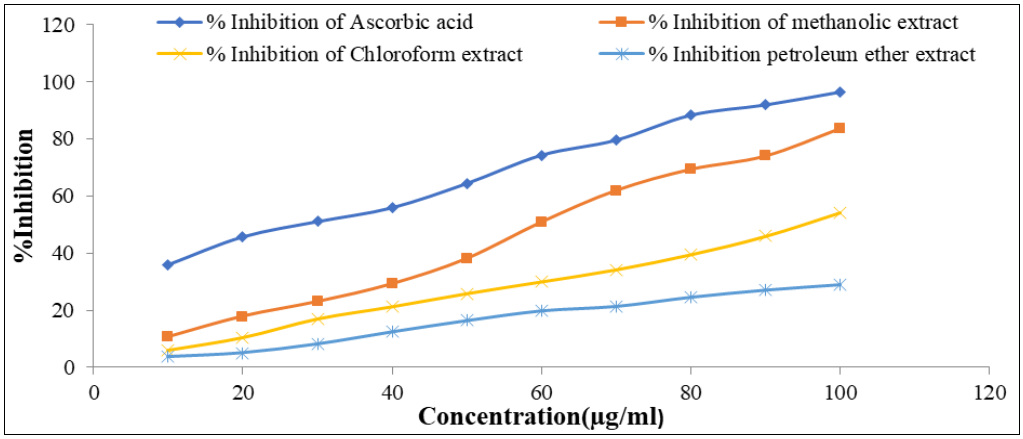

Fig 3: Inhibition Percentage of ABTS radical by plant extracts of *C. roseus*

IC_{50} values were also calculated for all the four extracts and are shown in Fig-4. Low IC_{50} represented the high antioxidant potential so in the case of methanolic extract least IC_{50} value (60.32µg/ml) followed by chloroform extract (98.76 µg/ml) and petroleum extract showed the poorest antioxidant activity having highest IC_{50} (142.54 µg/ml**)** value.

Table 6: IC_{50} value for DPPH and ABTS assay of different extracts of *C. roseus*

	IC_{50} (µg/mL)		
	Methanol	**Chloroform**	**Petroleum ether**
DPPH	53.60	82.2	160.30
ABTS	60.32	98.76	142.54

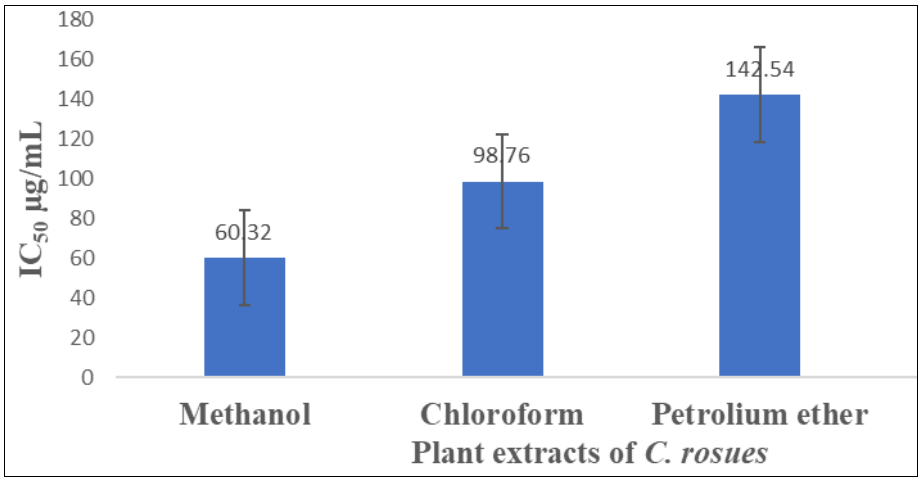

Fig 4: ABTS scavenging activity of plant extracts of *C. roseus.*

GC-MS Analysis

Methanolic extract showed highest TPC, TFC and antioxidant activity that's why methanolic extract was further analysed by GC-MS. GC-MS analysed results, which included the active principles with their retention time, molecular formula, molecular weight, peak area % components of *C. roseus* are presented in Table-7 and Fig. 5. Methanolic extract showed predominantly presence of seven different phytoconstituents with antioxidant and antimicrobial properties. 5-Hydroxymethylfurfural is a sugar component and showed antioxidant and antiproliferative activity, showed highest peak area % 30.86, followed by 9,12,15-Octadecatrienoic acid (23.64%) which showed Anti-inflammatory, Hypo-cholesterolemic and Cancer preventive properties.

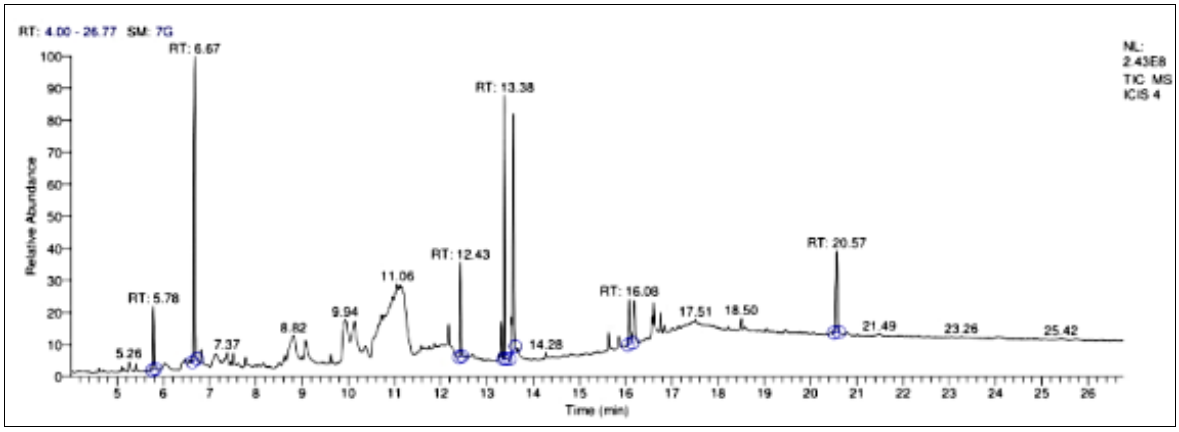

Fig 5: Chromatogram of methanolic leaf extract of C. roseus

In the current study, *C. roseus* was selected for the investigation, as it is a widely distributed plant throughout India. The plant also has been utilized as ethnomedicine in various traditional systems of medicine. The crude extract of leaves prepared by maceration method in five different solvents were analysed to validate its traditional uses. The study provides information on the phytochemical analysis, total phenolic and flavonoid contents of *C. roseus* in relation to the antioxidant activities. Efforts were also made to analyse the extract by GC-MS. Phytochemical analysis of the extracts showed the presence of phenols, flavonoids, terpenoids, glycosides, alkaloids. Out of these, phenols, flavonoid and alkaloids were present in all the plant extracts. Similar observations have also been reported [11] investigated different solvent extracts of *C. roseus* leaves for the screening of different phytoconstituents and reported the presence of alkaloids, flavonoids, phenols, saponins, carbohydrates and glycosides. Similarly, least number of components were detected in chloroform and Petroleum ether. Similarly, [13] used methanolic extract of *C. roseus* and reported the presence of most of the components. A recent study by [14] reported phenolic content in methanolic extract (178 µg/g) and in aqueous extract (52µg/g) of *C. roseus* leaf samples [18], reported that the total flavonoid content in *C. roseus* leaves ranged from the (148.31±1.3 to 115.31±1.32 µg/g DW). Further [27] used different solvent extract for TFC which ranged between 1.8 to 5.4 CE (g/100g dry weight). Recently [4] founded similar trend of enzymatic antioxidant activities in *Rumex obtusifolius* L. POD activity was observed to be maximum, followed by CAT and SOD.

The outcomes of GC-MS analysis, indicated that *C. roseus* leaves contained numerous bioactive phytoconstituents

belonging to various classes such as tannins, glycosides, alkaloids, flavonoids, steroids etc. Enzymatic antioxidants serve as an intrinsic defence tool to resist oxidative damage in plants. Catalase, POD and SOD are the key enzymes that are involved in cellular defence against reactive oxygen species in living organisms; hence these are an important indicator of antioxidant capacity [30]. According to the enzyme activity results, we can conclude that *C. roseus* has good antioxidant capacity**.**

Table 7: Phytocompounds detected from the methanolic extract of leaf of *C.roseus* by GC-MS.

S. No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Pharmacological actions
1	5.78	4H-Pyran-4-one	$C_6H_8O_4$	144	4.73	Antioxidant, Antimicrobial, Anti-inflammatory and Antioxidant
2	6.67	5Hydroxymethylfurfural	$C_3H_6O_3$	126	30.86	Antioxidant, Antiproliferative activity
3	12.43	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.58	Antioxidant, Hypocholester-olemic, Nematicide, Pesticide, Lubricant, Anliandrogenic, Flavor
4	13.38	Phytol	$C_{20}H_{40}O$	296	17.17	Hypocholesterolemic, Antimicrobial, Anticancer, Diuretic, Anti inflammatory
5	13.57	9,12,15Octadecatrienoic acid	$C_{18}H_{30}O_2$	278	23.64	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge
6	16.08	2,20-Cycloaspido-spermidine-3-carboxylic acid Vindolinine	$C_{21}H_{24}N_2O_2$	336	4.95	Anticancer
7	20.57	Aspidospermidine-3-carboxylic acid vindoline	$C_{22}H_{28}N_2O_5$	400	12.09	Anticancer

Phytol, Hexadecanoic acid, 9,12-Octadecadienoic acid are reported to have antimicrobial properties from some previous findings [1, 15, 22]. Other compounds like Octadecadienoic acid have the property of anti-inflammatory and antiarthritic activity as reported by the earlier workers [25, 23]. Due to the presence of these compounds in the leaves of methanolic extract of *C. roseus*, which may be used for various pharmaceutical applications.

Conclusion

Five plant extracts prepared in various solvents from leaves of *C. roseus* were analysed for their qualitative, quantitate phytochemical analysis and antioxidant potential. Out of the five extracts, methanol extract of *C. roseus* showed maximum properties. The extract also showed highest phenolic and flavonoid content. The antioxidant potential of the extract was also found to be highest as it showed minimum IC50 values in both cases DPPH as well as ABTS methods. The plant is thus a potential source of novel herbal drug that can be beneficial for health maintenance. However, more detailed research is required to explore its potential by isolating components that have antioxidant properties.

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