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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS AND IDENTIFICATION OF BIOACTIVE CONSTITUENTS OF *CATHARANTHUS ROSEUS* AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: The main objectives of this study were analysis of the phytochemicals produced by two different *Catharanthus roseus* morphotypes, i.e., pink and white flowered and evaluate it morphologically and phytochemically in terms of total phenolic content (TPC), total flavonoid content (TFC), antioxidant properties, and gas chromatography-mass spectrometry (GC-MS) analysis.

Methods: Methanolic extracts of both morphotypes were prepared by Soxhlet apparatus. After extraction, the extracts were filtered and solvent removed by rotatory evaporator. TPC was determined by Folin–Ciocalteu reagent method and TFC was estimated by aluminum chloride colorimetric method. Antioxidant and free radical scavenging activities were estimated by superoxide dismutase and 1,1-diphenyl-2-picrylhydrazyl assay. GC-MS analysis was performed at Central Instrumentation Laboratory/ SAIF, Panjab University, Chandigarh.

Results: Pink-flowered *C. roseus* showed highest activities in terms of TPC, TFC, and antioxidant activity as compared to white-flowered *C. roseus*. 42 different bioactive compounds were detected in the methanolic extract of pink, while only 7 compounds were identified in white-flowered *C. roseus*. The identification was performed by GS-MS analysis mainly based on retention time, peak area, molecular formula, and molecular weight.

Conclusion: The finding indicated that the pink-flowered *C. roseus* was phytochemically superior then the white one.

Keywords: *Catharanthus roseus*, Total phenolic content, Total flavonoid content, Antioxidant properties, Gas chromatography-mass spectrometry.

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INTRODUCTION

Plants play a potential role, especially in the production of herbal medicine and pharmaceutical medications. A significant proportion of the world population relies on herbal and customary medicine due to the high costs and lack of orthodox medicine [1]. The modern medicine productions also rely on medicinal plant-derived therapeutic components. Many medicinal plants contain an assortment of pharmaceutical components which have an extremely fundamental role in the fields of veterinary and human prescription. Plant-based items play a dominant role in the development of novel drug leading to the treatment and prevention of diseases [2-4].

History reveals that plants are important sources of drugs and will continuously be essential for the discovery of new lead compounds [5]. The most essential bioactive constituents of the plants are phenols, alkaloids, cardiac glycoside, tannins, and flavonoid compounds. In India, medicinal plants are of great interest to the field of plant biotechnology, as most of the pharmaceutical industries depend on plants for the production of therapeutic compounds [6].

Catharanthus roseus (family: Apocynaceae) popularly known as Madagascar periwinkle or Vinca, grown as evergreen ornamental herbaceous plant in gardens throughout the world [7], flower having five petals and various colors including pink, red, white and purple. However, nowadays, this plant is mainly cultivated for its alkaloids, having antileukemic activities [8]. It is a subshrub plant growing up to 1 m tall. The plant has been used to treat a vast range of the assortment of diseases [9]. In India, leaf juice is used to treat wasp stings. In China, this plant is used as a diuretic, astringent, menstrual regulation, and cough remedy. In South America, it is used as a folk remedy for inflammation and cold. In Caribbean region, flower extract is mainly used to treat infection and irritation. In England and Brazil, entire dried plant extract is taken orally along with hot water to treat diabetes mellitus. In Australia, hot water extract of leaves is used to treat diabetes and menorrhagia [10].

A detailed literature survey about the medicinal properties of *C. roseus* leaves revealed it to possess antileukemic, antioxidant, antimicrobial, and anti-inflammatory activities [11]. The medicinal properties are attributed to the presence of alkaloids and phenolic compounds in its leaf extract. Therefore, it was essential to screen the methanolic extract of *C. roseus* leaves for the detection of phytochemicals, to identify and characterize phytoconstituents in its crude extracts for chemical profiling using gas chromatography-mass spectrometry (GC-MS) analytic technique, to evaluate its antioxidant potential using *in vitro* methods, and to correlate with the phenolic and flavonoid content.

METHODS

The current study involved two different morphotypes of *C. roseus*, differing in petal color, shape, arrangement of petal and color of eye, grown naturally in the Botanical Garden and Department of Botany, Punjabi University, Patiala (Table 1 and Fig. 1). Flower petal was separated as pink and white. The voucher specimen of *C. roseus* was identified and deposited in Herbarium (PUN) at the Department of Botany, Punjabi University Patiala.

Plant material

C. roseus leaves were collected in June 2015. The collected leaf material was washed thoroughly with tap water to remove dust particles and dried under the shade at room temperature for 6–7 days. The dried leaves were ground to obtain the fine powder and kept in airtight glass bottles, until further use.

Extraction of plant material

Extraction of plant material was done with methanol: water (1:1) using Soxhlet apparatus. After the completion of extraction, the extract was filtered and solvent was removed by rotary evaporator.

Phytochemical screening of leaf extract

The freshly prepared methanolic crude leaf extract was subjected to qualitative analysis to identify various bioactive constituents present

Table 1: Morphological characteristics of two morphotypes of *C. roseus*

Morphotypes	Petal color	Petal arrangement	Eye color
Catharanthus roseus	Pink	Overlap	Red
Catharanthus roseus	White	Free	Yellow

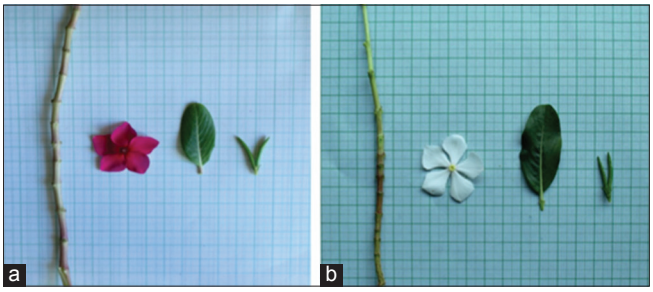

Fig. 1: Morphological features of two *Catharanthus roseus* morphotypes (a) pink and (b) white

in the leaves using standard procedures. The crude extract was also subjected for GC-MS analysis to identify the various phytochemical constituents present in the plant extracts.

Total phenolic content (TPC)

TPC was estimated by Folin–Ciocalteu reagent method. Phenols which react with phosphomolybdic acid in Folin–Ciocalteu reagent in the alkaline medium will produce a blue-colored complex (molybdenum blue) which can be estimated spectrophotometrically at 650 nm. A stock solution of plant extracts was prepared to 1 mg/mL. 5 mL of Folin– Ciocalteu and 2 mL of Na_2CO_3 were added to the 1 mL of plant sample. The solution was vortexed and incubated in the dark for 15 min. The absorbance was measured at 650 nm. Blank consisted of 5 mL Folin–Ciocalteu, 1 mL solvent, and 2 mL of Na_2CO_3 solution. Gallic acid was used as a standard of 10–100 µg/mL range from a stock solution of 1 mg/mL. The TPC was calculated from the calibration curve, and the result was expressed in terms of mg of gallic acid equivalent per gram dry weight. All tests were performed in triplicates [12].

Total flavonoid content (TFC)

TFC was estimated by aluminum chloride colorimetric method with some modifications to determine flavonoid content. 1 mL of plant sample was added to 3 mL of methanol, 0.2mL of 10% aluminium chloride, 0.2mL of 1M potassium acetate and 5.6 mL of distilled water and kept at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as standard of 10–100 µg/mL range from 1 mg/mL stock solution. All the tests were performed in triplicates. Flavonoid contents were determined from the calibration curve, and a result was expressed as mg of quercetin equivalent (per g of extracted compound) [13].

Antioxidant activity

Superoxide dismutase (SOD)

SOD levels in the cell-free supernatant were measured by the method of Kono [14]. Briefly, 1.9 mL of sodium carbonate buffer (50 mM, pH 10.0), 750 µL of nitrobluetetrazolium dye (NBT), and 240 mM and 150 µL of 0.3% Triton X-100 were 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 min, 70 µL enzyme samples from the plant tissue were added. The percentage inhibition in the rate of NBT reduction was recorded as an increase in absorbance at 560 nm. The percentage inhibition of NBT reduction was calculated as follows:

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

X% of inhibition is produced by 70 µL of sample.

Hence, 50% inhibition is produced by

$$Y\,\mu L \text{ of sample}=\frac{50\times 70}{X}\times 100$$

Free radical scavenging activity

The free radical scavenging activity of leaves was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [15]. This method depends on the reduction of purple color of DPPH to yellow color diphenyl-picrylhydrazine. A stock solution of leaf extracts was prepared to 1 mg/mL in methanol. Each stock solution diluted from 10 µ/mL to 100 µg/mL. 1 mL of 0.3 mM DPPH solution was added to 2.5 mL of sample solution. Ascorbic acid was used as standard with the same 1 mg/mL stock solution of different concentrations from 10 µg/mL to 100 µg/mL. The sample was incubated in the dark for 30 min. The absorbance was measured at 517 nm. The absorbance was converted into percentage antioxidant activity using the following equation:

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

Statistical analysis

A statistical analysis of data was performed in accordance with the procedure given by Gomez and Gomez [16] and was analyzed as per completely randomized design [17] to test the significance of differences between the treatments. A coefficient of variation was calculated using the method given by Burton and Devane [18].

GC-MS analysis

GC analysis was carried out at Central Instrumentation Laboratory/SAIF, Panjab University, Chandigarh.` This technique is very important for the identification of various phytochemicals of plant. The equipment used for GC-MS was QP-2010 Ultra. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min and and 1 µL of plant sample was employed (split ratio of 10:1), at injector temperature 250°C, ion-source temperature of 280°C, and total running time for a sample of about 76 min.

Identification of the components

Interpretation of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having >6200 patterns. The spectrum of the unknown component was compared with the spectrum of the known component in the repository of NIST library. The retention time, molecular weight, molecular formula, and composition percentage of the sample material were recorded.

RESULTS

TPC and TFC of leaf extracts are expressed in terms of gallic acid and quercetin, respectively, and presented in Table 2. *C. roseus* pink morphotype extract showed highest phenolic and flavonoid content as compared to the white morphotype leaf extract. The antioxidant activities were investigated by commonly used free radical scavenging methods such as DPPH and SOD. The scavenging effect of leaf extracts on the DPPH free radicals was expressed as percentage inhibition, and they were compared with standard antioxidant, ascorbic acid. Lower IC_{50} value indicates the higher antioxidant

activity. The pink morphotype showed highest while the white one showed minimum antioxidant activity. Similar trend was observed for SOD activity.

GC-MS analyzed the results which include the active principles with their molecular formula, molecular weight, retention time, peak area %, and composition of the bioactive components of *C. roseus* which are presented in Tables 3 and 4. The GC-MS chromatogram of the detected compounds is as shown in Fig. 2.

Many significant physiological active components were identified from both the samples by GC-MS. Chlorozotocin (0.17 %), 2-Pyrrolidinone, 1-butyl- (0.13%), Ethyl N-(o-anisyl) formimidate (0.36%), 2-Pentyne-1,4-diol, 4- methyl-1-(2-thienyl)- (0.33%), Cis-Inositol (0.09%), Muco-Inositol (2.38), 3-Phenylbicyclo (3.2.2) nona-3,6-dien-2-one (0.06%), 9,12,15-Octadecatrienoic acid, methyl ester (2.70 %), 9-Octadecynoic acid (3.24%), Methyl 8,11,14-heptadecatrienoate (30.04%), [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (0.20%), Condyfolan, 14,19-didehydro-12-methoxy-,(14E)- (0.29%) and 2,20-Cycloaspidospermidine-3-carboxylic acid (1.05).

C. roseus white morphotype contained seven different components such as 1-4H-Pyran-4-one (4.73%), 5-Hydroxymethylfurfural (30.86%), n-Hexadecanoicacid(6.58%),phytol(17.17%),9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (23.64%), 2,20-Cycloaspidospermidine-3-carboxylic acid (4.95%), and Aspidospermidine-3-carboxylic acid (12.09%).

Antioxidant and antimicrobial activities are shown by various identified compounds such as 4H-Pyran-4-one, Benzofuran, 2,3-dihydro, 1,2,3-Propanetriol, 1-acetate/acetin, l-Gala-l-ido-octonic lactone, desulfosinigrin, 3',5'-Dimethoxyacetophenone, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-d-fru, 1,2,3,5-Cyclohexanetetrol, 2-Methyl-9-a-d-ribofuranosylhypoxanthine, Myo-Inositol, 4-C-methyl-, Hexadecanoic acid, methyl ester, pentadecanoic acid, dasycarpidan-1-methanol, acetate (ester, Phytol, Methyl 8,11,14-heptadecatrienoate, [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester, and Phthalic acid, di(oct-3-yl) ester.

DISCUSSION

In the present study, a total of 42 compounds were identified in pink morphotypes and seven in white morphotype of *Catharanthus*. In terms of percentage amount, methyl 8,11,14-heptadecatrienoate, 9-octadecynoic acid, 9,12,15-octadecatrienoic acid, methyl ester, pentadecanoic acid, 1,2,3,5-Cyclohexanetetrol, muco-inositol, and sucrose were prominent in pink morphotype, whereas 5-Hydroxymethylfurfural, 9,12,15-octadecatrienoic acid, and phytol were prominent in white morphotypes. Identified compounds having anti-inflammatory, antimicrobial, antioxidants, and antiproliferative activity have been identified. The plant is mainly used due to its antineoplastic properties.

Among the identified compounds, 5-hydroxymethylfurfural is a sugar component and showed antioxidant and antiproliferative activity previously reported by Hussein [19]. 9,12,15-Octadecatrienoic acid (Z, Z, Z)- and 9,12,15-Octadecatrienoic, methyl ester (Z, Z, Z)- which is a linoleic acid and reported to have an anticancer, anti-arthritic, anti-inflammatory, anti-acne, hypocholesterolemic, hepatoprotective, antihistaminic, nematicide and insectifuge properties. Similarly, the presence of 9-octadecenoic acid was observed in the ethanolic root extract of *Plumbago zeylanica* by Ajayi *et al*. [20]. Hexadecanoic acid methyl ester is also known as palmitic acid ester and effectively used as an antioxidant, pesticide, anti-androgenic, nematicide, flavoring agent, hypocholesterolemic, and lubricant [21,22]. Carbohydrates such as mannitol and sucrose are present in a considered amount in methanolic extracts of *Catharanthus*. The GC-MS analysis revealed that the methanolic extract of *C. roseus* pink morphotype is composed of more oxygenated hydrocarbons and predominantly phenolic hydrocarbons as compared to the white morphotype. These phytochemicals are responsible for various pharmacological actions such as antimicrobial, antioxidant, and antiproliferative activity. Results showed that components from both of the two morphotypes are a complex mixture of numerous compounds, many of which are found in trace amount. It is worth monitoring that there is a great variation in the chemical composition of these two morphotypes of *C. roseus*. This confirms that the reported variation in phytoconstituents is due to morphological variation between the two accessions.

The results of GC-MS testing indicated that *C. roseus* leaves contained numerous bioactive phytoconstituents belonging to various classes such as tannins, glycosides, alkaloids, flavonoids, and steroids. The leaf extract quantification, by colorimetric methods, was found to be rich in phenolic compounds (flavonoids) and therefore exhibited very good scavenging activity against DPPH and SOD free radicals. Based on the results, it can be concluded that *C. roseus* leaves could be used as a natural source of antioxidants and its regular consumption in diet could provide health benefits to humans by protecting against oxidative stress. Further detailed *in vitro* and *in vivo* correlation studies along with isolation of active constituents are needed to unravel novel treatment strategies for free radical-induced diseases.

CONCLUSION

The study reveals the presence of bioactive compounds of the methanolic extract of *C. roseus* leaves. The present study can provide

Table 2: TPC, TFC, and antioxidant activities of two morphotypes of *Catharanthus roseus*

Morphotypes	DPPH assay (IC_{50}) µg/ml	SOD	TPC (mg gallic acid equivalent per g of leaves)	TFC (mg quercetin equivalent per g of leaves)
Catharanthus roseus (Pink)	10.62±2.42	123±0.42	40.8±0.52	12.4±0.77
Catharanthus roseus (White)	25.95±1.32	88±1.05	15.3±1.12	9.82±0.67

Values are the average of triplicate experiments and values expressed as mean±SEM, SEM: Standard error of mean, SOD: Superoxide dismutase, TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: 1,1-diphenyl-2-picrylhydrazyl

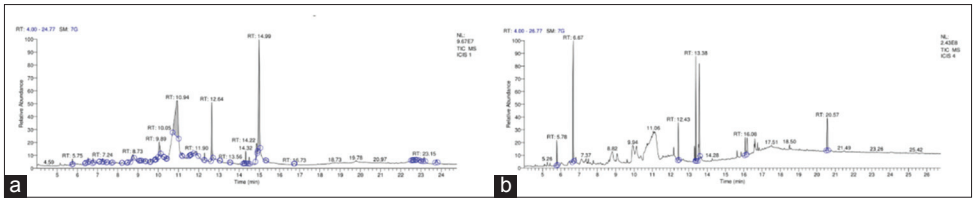

Fig. 2: Chromatogram of the leaf methanolic extract of *Catharanthus roseus* (a) pink and (b) white

Table 3: Results of the GC-MS analysis from the leaves of *Catharanthus roseus* (pink)

S. No	RT	Name of the compound	Molecular formula	MW	Peak area %	Pharmacological actions
1	5.75	4H-Pyran-4-one	$C_6H_8O_4$	144	0.63	Antioxidant, Antimicrobial, Anti-inflammatory, and Antioxidant
2	6.39	Catechol/Resorcinol	$C_6H_6O_2$	110.1	0.06	Dermatological treatment/Acne
3	6.53	Benzofuran, 2,3-dihydro	C_8H_8O	120.1	0.61	Antioxidant, Analgesic, Antimutagenic
4	6.77	1,2,3-Propanetriol, 1-acetate/acetin	$C_5H_{10}O_4$	134.1	0.84	Antimicrobial
5	7.09	Chlorozotocin	$C_9H_{16}C_1N_3O_7$	313.6	0.01	Antineoplastic
6	7.24	1,3-Diazacyclooctane-2-th	$C_6H_{12}N_2S$	144.2	0.82	Antimicrobial, Antioxidant
7	7.44	Ascaridole epoxide	$C_{10}H_{16}O_3$	184	0.14	Anticarcinogenic
8	7.71	Chlorozotocin	$C_9H_{16}C_1N_3O_7$	313.6	0.17	Antineoplastic
9	8.21	l-Gala-l-ido-octonic lactone	$C_8H_{14}O_8$		0.05	Antibacterial activity against *Pseudomonas aeruginosa*
10	8.48	Limonen-6-ol, pivalate	$C_{15}H_{24}O_2$	236.3	0.06	Anti-stress
11	8.73	Sucrose	$C_{12}H_{22}O_{11}$	342.2	3.14	Antioxidant
12	9.07	Desulfosinigrin	$C_{10}H_{17}NO_6S$	279.3	0.06	Antioxidant
13	9.16	2-Pyrrolidinone, 1-butyl-	$C_8H_{15}NO$	141.2	0.13	Unknown
14	9.38	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180.2	0.17	Antioxidant
15	9.61	2H-1-Benzopyran-3,4-diol	$C_{18}H_{20}O_5$	316.3	0.35	Antihypertensives
16	9.83	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw. 3)-ß-d-fru	$C_{18}H_{32}O_{16}$	504	0.06	Antidiabetic activity and antitumor
17	9.89	Ethyl N-(o-anisyl) formimidate	$C_{10}H_{13}NO_2$	179.2	0.36	Unknown
18	10.05	1,2,3,5-Cyclohexanetetrol	$C_6H_{12}O_4$	148.1	5.12	Polyhydroxy compound, Analgesic, Anesthetic, Antioxidant, Antiseptic, Antibacterial, Antiviral cancer preventive
19	10.28	2-Methyl-9-á-d-ribofuranosylhypoxanthine	$C_{11}H_{14}N_4O_5$	282	0.03	Antimicrobial
20	10.42	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343.3	0.19	Antiasthmatic, Anti-inflammatory and Antipyretic
21	10.94	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194.1	30.45	Antioxidant and Antimicrobial
22	11.18	2-Pentyne-1,4-diol, 4- methyl-1-(2-thienyl)-	$C_{10}H_{12}O_2S$	196.2	0.33	Unknown
23	11.54	Psicofuranine	$C_{11}H_{15}N_5O_5$	297.2	0.11	Antibiotic and Antitumor
24	11.61	Cis-Inositol	$C_6H_{12}O_6$	180.6	0.09	Chemopreventive
25	11.90	Muco-Inositol	$C_6H_{12}O_6$	180.1	2.38	Chemopreventive
26	12.29	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	370	0.97	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, 5-Alpha-reductase inhibitor
27	12.64	Pentadecanoic acid	$C_{15}H_{30}O_2$		10.41	Antimicrobial, Antioxidant, Anticancer
28	13.05	3-Phenylbicyclo (3.2.2) nona-3,6-dien-2-one	$C_{15}H_{14}O$	210.2	0.06	No activity reported
29	13.56	Dasycarpidan-1-methanol, acetate (ester	$C_{20}H_{26}N_2O_2$	326.4	0.11	Antimicrobial
30	14.22	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4	0.67	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective
31	14.32	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.2	2.70	Anticancerous, Nematicides,
32	14.50	Phytol	$C_{20}H_{40}O$	296.5	1.38	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer
33	14.86	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280.4	3.24	Inhibit fibrosis
34	14.99	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278.4	30.04	Antioxidant
35	15.33	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	0.20	Antimicrobial
36	16.73	9,12,15-Octadecatrienoic acid,	$C_{27}H_{52}O_4Si_2$	496.2	0.04	Anti-inflammation
37	22.56	N-desmethyltramadol	$C_{15}H_{23}NO_2$	249.3	0.17	Rheumatoid arthritis, Restless legs syndrome and fibromyalgia
38	22.61	Prednisolone hemisuccinate	$C_{25}H_{32}O_8$	460.5	0.20	Anti-inflammatory
39	22.69	Condyfolan, 14,19-didehydro-12-methoxy-, (14E)-	$C_{19}H_{24}N_2O$	322.4	0.29	Antimicrobial
40	22.76	Aspidospermidine-3-carboxylic acid	$C_{22}H_{28}N_8O_5$	400.4	0.11	Anticancer
41	22.97	Phthalic acid, di (oct-3-yl) ester	$C_{24}H_{38}O_4$	390.5	0.54	Antimicrobial and antifouling
42	23.76	2,20-Cycloaspidospermidine-3-carboxylic acid	$C_{21}H_{42}N_2O_2$	336	1.05	Anticancer

GC-MS: Gas chromatography-mass spectrometry

a good concrete base for further research to isolate the lead bioactive compounds in the leaves to develop new antioxidant and antimicrobial agent.

AUTHORS' CONTRIBUTIONS

Manish Kapoor conceptualized the research and framework and supervised it. Jyoti Rani performed all the experimental work. Both the

Table 4: Results of the GC-MS analysis from the leaves of *Catharanthus roseus* (white)

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	5-Hydroxymethylfurfural	$C_3H_6O_3$	126	30.86	Antioxidant, Antiproliferative activity
3	12.43	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.58	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor
4	13.38	Phytol	$C_{20}H_{40}O$	296	17.17	Hypocholesterolemic, Antimicrobial, Anticancer, Diuretic, Anti-inflammatory
5	13.57	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278	23.64	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge
6	16.08	2,20-Cycloaspidospermidine -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

GC-MS: Gas chromatography-mass spectrometry

authors analyzed the data and interpreted the results. Jyoti Rani and Manish Kapoor wrote and finalized the manuscript.

CONFLICTS OF INTEREST

Both the authors declare that there are no conflicts of interest regarding the publication of this research.

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