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

| Morphotypes         | Petal color | Petal arrangement | Eye color |
|---------------------|-------------|-------------------|-----------|
| *Catharanthus roseus* | Pink        | Overlap           | Red       |
| *C. 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 phosphomolybic 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 CO₃ 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 CO₃ 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, 0.2 mL of 10% aluminium chloride, 0.2 mL 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 from a stock solution of 1 mg/mL in methanol. Each stock solution diluted from 10 µ/mL to 100 µg/mL range from 1 mg/mL stock solution. Ascorbic acid was used as standard with the same concentration range 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:

\[
\text{X\% of inhibition is produced by 70 µL of sample.}
\]

\[
\text{Hence, 50\% inhibition is produced by Y µL of sample:}
\]

\[
\frac{50 \times 70}{X \times 100}
\]

**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 diphenylpicrylhydrazine. 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 concentration range 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:

\[
\frac{\text{Absorbance (sample)} - \text{Absorbance (control)}}{\text{Absorbance (control)}} \times 100
\]

**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 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 IC₅₀ 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 phytoactive 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-Pyrrrolidinone, 1-buty1 (-0.13%), Ethyl N-(o-anisyl) formimide (0.36%), 2-Pentylene-1,4-diol, 4- methyl-1-(2-thienyl)- (0.33%), Cis-Insitol (0.80%), Mucx-Insitol (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-Octadecenoic acid (3.24%), Methyl 8,11,14-heptadecatrienate (30.04%), [1,1’-Bicycloproplyl]-2-octanoic acid, 2’-hexyl-, methyl ester (0.20%). Condyfolan, 14,19-didehydo-12-methoxy-(14E)- (0.29%) and 2,20-Cycloaspidospermidine-3-carboxylic acid (1.05).

C. roseus white morphotype contained seven different components such as 4-H-Pyran-4-one (4.73%), 5-Hydroxymethylfurifural (30.86%), n-Hexadecanoic acid (6.58%), phytol (17.17%), 9,12,15-Octadecatrienoic acid, (Z, Z, Z) - (23.64%), Methyl 8,11,14-heptadecatrienate (30.04%), [1,1’-Bicycloproplyl]-2-octanoic acid, 2’-hexyl-, methyl ester (0.20%). Condyfolan, 14,19-didehydo-12-methoxy-(14E)- (0.29%) and 2,20-Cycloaspidospermidine-3-carboxylic acid (12.09%).

Antioxidant and antimicrobial activities are shown by various identified compounds such as 4H-Pyran-4-one, Benzo[b]furan, 2,3-dihydro, 1,2,5-Propantriol, 1-acetate/acetic, 1-Gala-ido-octonic lactone, desulosinigrin, 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, dasycharpidan-1-methanol, acetate (ester, Phytol, Methyl 8,11,14-heptadecatrienate, and Aspidospermide-3-carboxylic acid (4.95%), and Aspidospermidine-3-carboxylic acid (12.09%).

CONCLUSION
The study reveals the presence of bioactive compounds of the methanolic extract of C. roseus leaves. The present study can provide 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-hydroxymethylfurifural 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 acid, methyl ester (Z, Z, Z) - which is a linolic acid and reported to have an anticeruc, anti-artrthic, anti-inflammatory, anti-acne, hypocholesterolemic, hepatoprotective, antithaminic, nematicide and insecticfuge 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.

**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-heptadecatrienate, 9-octadecenoic 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-Hydroxymethylfurifural, 9,12,15-Octadecatrienoic acid, and phytol were prominent in white morphotypes. Identified compounds having numerous bioactive phytoconstituents belonging to various classes such as mannitol and sucrose are present in a considered amount in white morphotype. 5-Hydroxymethylfurifural, 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.
a good concrete base for further research to isolate the lead bioactive compounds in the leaves to develop new antioxidant and antimicrobial agent.
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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