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Phytochemical screening of plant extracts and GC-MS analysis of the n-hexane extracts of stems and roots of *Catharanthus roseus* growing in Bangladesh

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Abstract
Phytochemical screening and gas chromatography-mass spectrometry (GC-MS) methods were used to determine the phytochemical components of various extracts of stems and roots of *Catharanthus roseus*. Preliminary phytochemical screening of n-hexane, chloroform, ethyl acetate and methanol extracts of stems and roots of *Catharanthus roseus* were carried out to identify the class of compounds present in each extract. The results exhibited that steroids, terpenoids, flavonoids and phenolic compounds were found in every extract of stems and roots. Alkaloids were also found in all extracts except n-hexane. But carbohydrates were absent in all extracts of stems and roots except methanol. The GC-MS analysis of the n-hexane extract of the stems and roots of *Catharanthus roseus* was done, which allowed the identification and quantification of 30 and 22 n-hexane soluble compounds present in the stems and roots, respectively. In this experiments, D:B-Friedo-B':A'-neogammacer-5-en-3β-ol (10.23%) and Ethyl palmitate (13.63%) identified with the highest concentration in the n-hexane extract of stems and roots, respectively.

Keywords: *Catharanthus roseus*, stems, roots, phytochemical screening, GC-MS analysis

1. Introduction
Plants were the principal source of drugs when the primitive man realized that he could get relief from the suffering of disease and soothe aches and pains by using plant parts or their products [1]. This plant knowledge was passed down verbally from one generation to the next and later recorded on papyri, clay tablets, parchments, manuscripts, pharmacopoeias, and other documents. Therefore, medicinal plants have been used to eradicate human suffering since ancient times [2, 3].

The plant *Catharanthus roseus*, often known as Nayantara, is a member of the Apocynaceae family and the genus *Catharanthus*. It is found throughout Bangladesh in various regions. It is a one-meter-tall subshrub with evergreen foliage. The leaves are opposite-paired, 2.7-9.1 cm lengthy and 1-3 cm broad and hairless. The blossoms' colours range from white to dark pink with a deeper red centre. A pair of 2- 4 cm lengthy and 3 mm wide follicles make up the fruit [4]. As a decorative and therapeutic plant, *C. roseus* is grown in gardens all over Bangladesh. *C. roseus* is mainly used for anticancer [5, 6], ant diabetic [7, 8], anti-inflammatory [5], antimalarial [9], and antibacterial activities [10-11]. All sections of the plant contain indole alkaloids, making it a significant source of these compounds. Vincristine and vinblastine alkaloids are used for cancer treatment [12]. The roots contain antihypertensive alkaloids ajmalicine, reserpine and serpentine [13]. Phytochemical studies on this medicinal plant have already been conducted in several nations, including Bangladesh, India, Pakistan, Thailand, and other regions. However, the extracts from this plant's stems and roots have not yet been the subject of any comprehensive biological and phytochemical studies in Bangladesh. The current investigation aimed to determine whether the stems and roots of *C. roseus* had any therapeutic value. GS-MS analysis provides the quantitative assessment of the phytochemicals of the plant extracts, whereas phytochemical screening gives the qualitative analysis.

2. Materials and Methods

2.1 Collection & Identification of the Plant Material

Stems and roots of *Catharanthus roseus* were taken from Jahangirnagar University gardens in Bangladesh. The plant *Catharanthus roseus* was identified by the Bangladesh National.Herbarium in Dhaka using conventional taxonomical techniques. 39512 is the DACB accession number

2.2 Extraction of stems and roots of the *C. roseus*

The stems and roots were cut into small pieces and dried thoroughly underneath the shed. After that, the dried stems and roots were turned into powder by using a grinder machine. The dried powder of the stems and roots was then extracted at room temperature with n-hexane, chloroform, ethyl acetate, and methanol successively. The dried crude extracts obtained by evaporation of the solvents using a rotary evaporator and denoted as CRSH, CRSC, CRSE, & CRSM for stems and CRRH, CRRC, CRRE, & CRRM for roots of *C. roseus*.

2.3 Phytochemical Screening

Phytochemical analysis of the extracts of *C. roseus* was done to identify various phytochemicals, including alkaloids, steroids, flavonoids, coumarins, glycosides, quinones, anthraquinones, tannins, carbohydrates, saponins, and others [14-16]. Phytochemical components were identified using the protocols outlined by Trease and Evans [17], Harborne [18] and Sofwara [19]. The following qualitative assays were used to perform a phytochemical screening on the various extracts of stems and roots of *Catharanthus roseus* [20].

2.3.1 Test for Steroids and Terpenoids

Libermann - Burchard Test

Chloroform was added to 10 mg of the extract. After adding a few drops of Ac_2O, 1 ml of pure sulphuric acid was included. The presence of steroids is indicated by the blue chloroform layer that turned green existence of terpenoids by the emergence of the pink $CHCl_3$ layer.

2.3.2 Test for Flavonoids

Shin do's Test

Prepare extract solution (10 mg) in methanol. Then strong HCl was added, followed by magnesium turnings. Flavonoids could be seen as a pink colour.

2.3.3 Test for Phenolic compounds

A few drops of a 2.5 % $FeCl_3$ solution were included in a solution of 10 mg of extract dissolved in methanol. The red-brown colour revealed the existence of the phenolic compound.

2.3.4 Test for Coumarins

Alcoholic KOH was added after ten mg of the extract had been dissolved in methanol. The development of a yellow colour that turns gray when strong HCL is added indicated the existence of coumarins.

2.3.5 Test for Quinones

The extract, 10 mg, was dissolved in methanol and subjected to sulphuric acid treatment. Quinone was present because of the colour development.

2.3.6 Test for Alkaloids

Mayer's Test

Mayer's reagent was initially made to test for alkaloids. Mercuric chloride (1.36 g) and KI (5.0 g) were mixed in water to create the reagent (100 ml). Separately, ten mg of the extracts were dissolved in hydrochloric acid. A few solution drops were placed in the watch glass center. A glass rod was used to add Mayer's reagent along the watch glass's sides. The formation of a gelatinous white precipitate indicates a positive result.

2.3.7 Tests for Saponins

A tiny amount of the extract was dissolved in distilled water and then vigorously shaken. Foams formed during the test exhibited the saponins present.

2.3.8 Test for Carbohydrates

Molisch's Test

After vigorously shaking with water, the extracts were filtered. The aqueous filtrate was then vigorously shaken before adding a few drops of Molisch's reagent (95% ethanol + 5% naphthol). To create a layer beneath the aqueous solution, concentrated H_2SO_4 (1mL) was carefully added. The test was successful when there was a brown ring at the interface.

2.3.9 Test for Tennis

Lead Acetate Test

Aqueous extract (5 mL) were mixed with a few drips of a 1% solution of lead acetate (previously boiled in a water bath). Precipitation that was yellow or crimson denoted a positive test result.

2.4 Equipment and GC-MS analytical techniques

The n-hexane extracts of *Catharanthus roseus* stems and roots were investigated at the Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories using the Electron Impact Ionization (EI) technique on a Shimadzu GC-17A gas chromatograph coupled to an MS 2010 plus mass spectrometer. In a capillary column conveying helium, a temperature of 40 °C was kept at continuous pressure of 90 kPa. A split ratio of 10 was used to administer the samples. Chloroform was used to dissolve the sample. Here are the working conditions: The column's name is RTS5MS, and its dimensions are 30 cm in diameter and 0.25 nm in length. 10% diethylene glycol succinate was used for column packing. He was used as carrier gas at the above pressure [21].

3. Results and discussion

3.1 Study of Phytochemical Constituents

Table 1: The results of analysing the phytochemical components in *C. roseus* stem extracts

Investigated Phytochemicals	CRSH (n-hexane extract)	CRSC (Chloroform extract)	CRSC (Ethyl acetate extract)	CRSM (Methanol extract)
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Phenolic Compounds	+	+	+	+

Coumarins	-	+	+	+
Quinones	-	-	+	+
Alkaloids	-	+	+	+
Saponins	-	-	-	+
Anthraquinones	-	-	+	-
Carbohydrates	-	-	-	+
Tannins	-	-	-	+

Note: symbol (+) indicates the presence and (-) indicating the absence

Table 2: The findings of the phytochemical constituent analysis of *C. roseus* root extract

Investigated Phytochemicals	CRRH (n-hexane extract)	CRRC (Chloroform extract)	CRRE (Ethyl acetate extract)	CRRM (Methanol extract)
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Phenolic Compound	+	+	+	+
Coumarins	-	+	+	+
Quinones	-	-	+	+
Alkaloids	-	+	+	+
Saponins	-	-	-	+
Anthraquinones	-	-	+	+
Carbohydrate	-	-	-	+
Tannins	-	-	+	+

Note: symbol (+) indicates the presence and (-) indicating the absence

Preliminary phytochemical tests of stems and roots of *C. roseus* extracts were used to identify the class of the compounds in each extract.

The results exhibited that steroids, terpenoids, flavonoids and phenolic compounds were found in every extract of stems and roots. Alkaloids were also found in all extracts except n-hexane. But carbohydrates were absent in all extracts of stems and roots except methanol.

3.2 GC-MS study of the plant extracts

Using a database of more than 62000 patterns from the National Institute of Standard and Technology (NIST), the mass spectrum of the GC-MS instrument was interpreted. The spectra of the unknown molecule were compared to the spectrum of the reported component stored in the NIST collection. The spectrum of the known component from the NIST library was compared to the spectrum of the extracts to identify the components.

3.2.1 Analysis of *Catharanthus roseus* stem n-hexane extract by GC-MS

30 components from the *Catharanthus roseus* stem n-hexane extract could be identified and quantified by the GC-MS analysis.

The results of GC-MS analysis of *C. roseus* stem n-hexane extract were presented in Fig. 1 & Table 3.

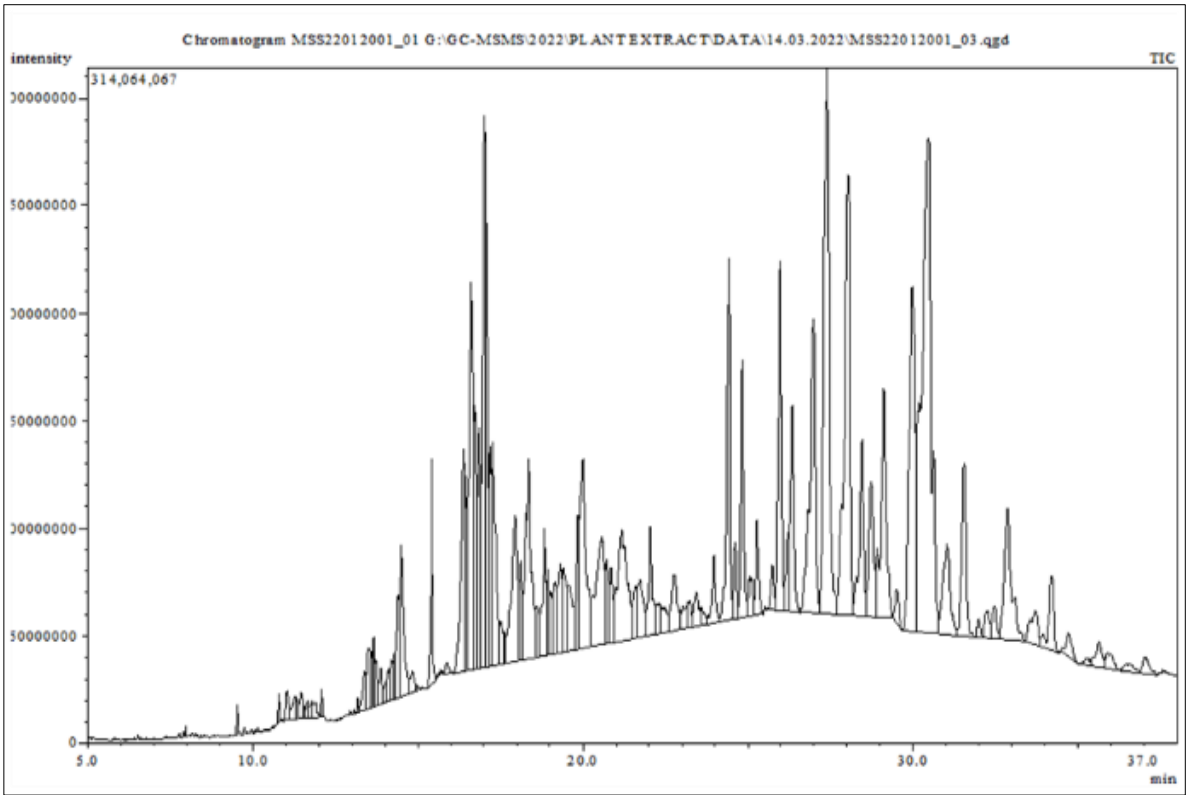


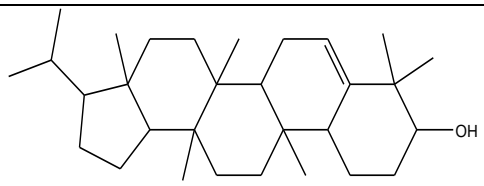
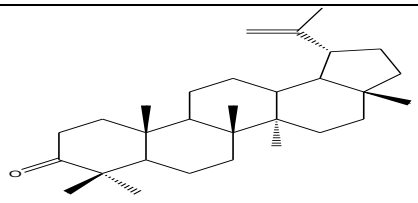
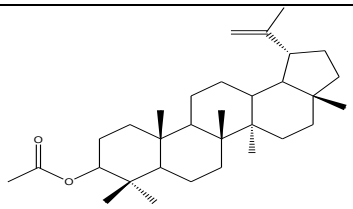
Fig 1: TIC of *C. roseus* steam n-hexane extract

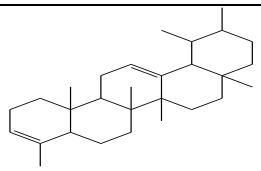
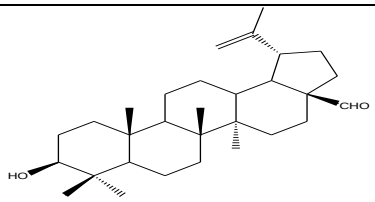
Table 3: GC-MS studies of the n-Hexane extract of the stems of *C. roseus*

Symbol	Retention Time	Name of the Compound	Molecular Weight (g/ml)	Molecular Formula	Conc.%
C-1	15.422	cis-11-Eicosenamide	309.312	$C_{20}H_{39}NO$	0.72
C-2	16.728	3,3,3',3',5,5,5',5'-octamethyl- Bi-1-cyclohexen-1-yl	274.272	$C_{20}H_{34}$	1.65
C-3	17.019	Betulinaldehyde	440.700	$C_{30}H_{48}O_2$	3.53
C-4	17.194	4,4-dimethyl-Cholestan-3-one	414.400	$C_{29}H_{50}O$	1.01
C-5	17.27	Lanosta-8,24-dien-3-one	424.384	$C_{30}H_{48}O$	1.88
C-6	18.11	Hexacontane	842.976	$C_{60}H_{122}$	0.67
C-7	18.837	(Z)-13-Docosenamide	337.582	$C_{22}H_{43}NO$	0.92
C-8	19.154	Tris (2,4-di-tert-butylphenyl) phosphate	662.921	$C_{42}H_{63}O_4P$	0.52
C-9	19.59	1,54-dibromo-Tetrapentacontane	788.864	$C_{54}H_{108}O_2$	0.67
C-10	19.85	Tetracosane	338.653	$C_{24}H_{50}$	0.72
C-11	20.003	1-(1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl-1,3a-ethano-3aH-inden-6-yl)-Ethanone	246.208	$C_{17}H_{26}O$	2.46
C-12	20.55	3,3'-thiobis-didodecyl Propanoate	514.844	$C_{30}H_{58}O_4S$	1.93
C-13	21.176	Ursolic aldehyde	440.700	$C_{30}H_{48}O_2$	2.27
C-14	21.735	10-Methylundec-2-en-4-olide	196.286	$C_{12}H_{20}O_2$	0.68
C-15	22.045	Tetrapentacontane	759.451	$C_{54}H_{110}$	0.87
C-16	22.778	9,19-Cyclolanostan-3β-ol, acetate	470.770	$C_{32}H_{54}O_2$	0.72
C-17	24.427	Campesterol	400.680	$C_{28}H_{48}O$	2.53
C-18	24.883	Stigmasterol	412.690	$C_{29}H_{48}O$	1.56
C-19	25.27	Cholestan-3-one	386.653	$C_{27}H_{46}O$	0.62
C-20	25.977	γ-Sitosterol	414.706	$C_{29}H_{50}O$	2.31
C-21	26.21	Stigmastanol	416.416	$C_{29}H_{52}O$	0.56
C-22	26.343	4,4,6a,6b,8a,11,11,14b-Octamethyl 1,4,4a,5,6,6a, 6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	424.384	$C_{30}H_{48}O$	1.43
C-23	26.979	24-Norursa-3,12-diene	394.675	$C_{29}H_{46}$	3.53
C-24	27.398	Lup-20(29)-en-3-one	424.701	$C_{30}H_{48}O$	6.07
C-25	28.043	Lup-20(29)-en-3β-ol, acetate	468.754	$C_{32}H_{52}O_2$	5.53
C-26	28.736	Olean-12-en-3β-ol, acetate	468.754	$C_{32}H_{52}O_2$	1.42
C-27	29.12	Lanosterol	426.717	$C_{30}H_{50}O$	2.45
C-28	30.467	D:B-Friedo-B':A'-neogammacer-5-en-3β-ol	426.717	$C_{30}H_{50}O$	10.23
C-29	32.861	Stigmastane-3,6-dione	428.690	$C_{29}H_{48}O_2$	2.09
C-30	33.708	A'-Neogammacer - 22(29) -en-3-one	424.384	$C_{30}H_{48}O$	0.56

From Table 3, the total number of identified components can be observed. The total amount of identified compounds was approximately 62%, and nearly 38% remained unidentified. Here the major compounds were identified as D:B-Friedo-B':A'-neogammacer-5 – en - 3β-ol (C - 28, 10.23%), Lup- 20 (29) -en-3-one (C-24, 6.07%), Lup-20 (29) - en-3β- ol, acetate (C- 25, 5.53%), 24-Norursa-3, 12-diene (C-23, 3.53%), Betulinaldehyde (C - 3, 3.53%). All identified major compounds are the class of triterpenoids.

Table 4: Structures of significant compounds discovered from stem of *C. roseus* n-hexane extracts

Name of the Compound	Chemical structure
D: B-Friedo-B':A'-neogammacer-5-en-3β-ol- (C- 28, 10.23%)	
Lup-20 (29)-en- 3-one (C-24, 6.07%)	
Lup-20(29)-en-3β-ol, acetate (C-25, 5.53%)	

24-Norursa-3, 12-diene (C- 23, 3.53%)	
Betulinaldehyde (C- 3, 3.53%)	

3.2.2 Analysis of *Catharanthus roseus* root n-hexane extract by GC-MS: Using GC-MS, 22 components from the n-hexane extract of *Catharanthus roseus* roots could be identified and quantified. Fig. 2 and Table 5 showed the outcomes of the GC-MS investigation of the *C. roseus* root n-hexane extract.

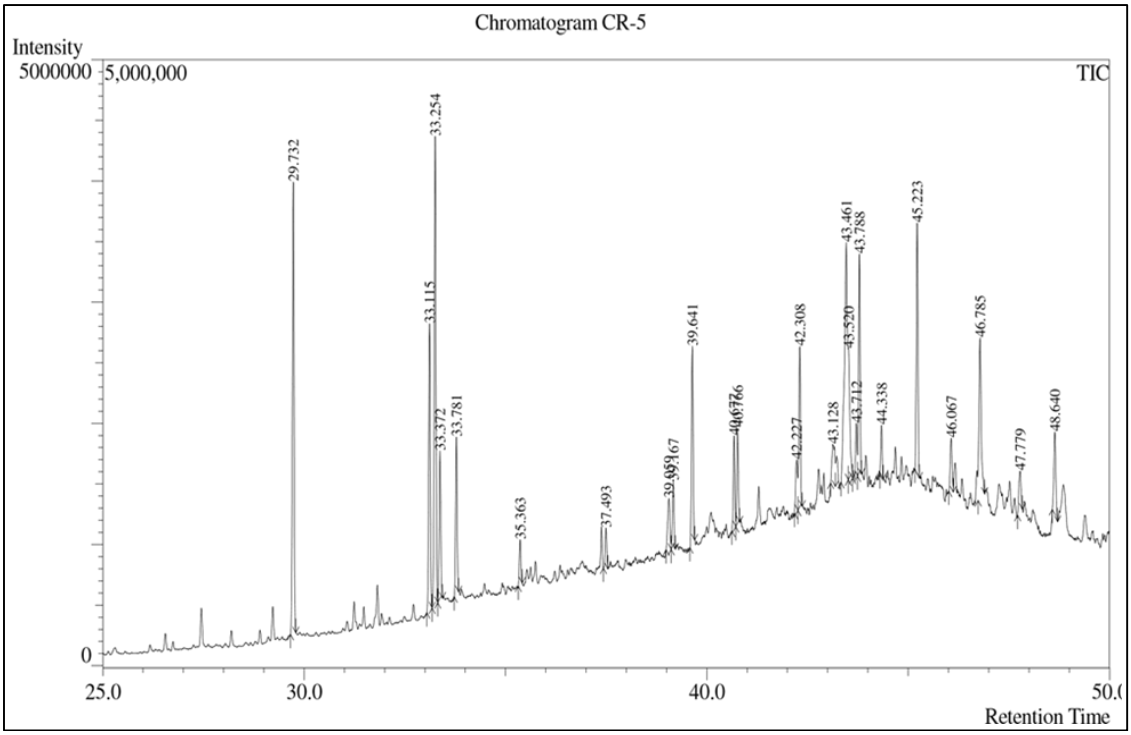


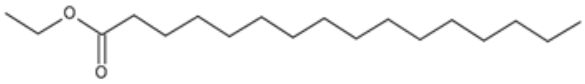
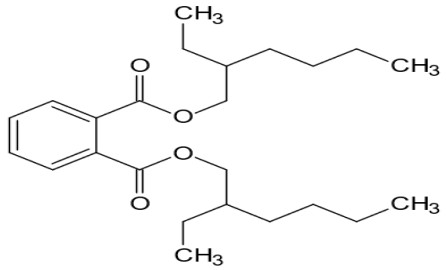
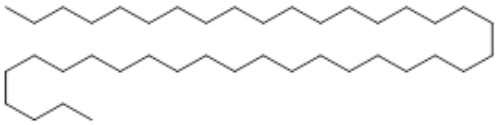
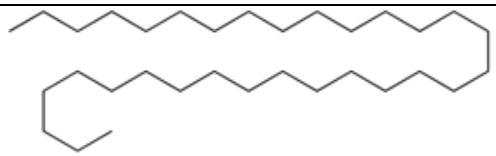
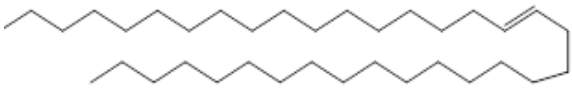
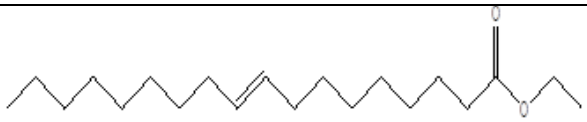
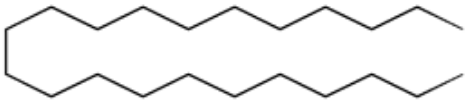
Fig 2: TIC of *C. roseus* root n-hexane extract

Table 5: GC-MS studies of the *C. roseus* root n-hexane extract

Symbol	Retention Time	Name of the compound	Molecular weight (g/ml)	Molecular Formula	Conc.%
R-1	29.733	Ethyl palmitate	284.477	$C_{18}H_{36}O_2$	13.63
R-2	33.115	Ethyl Linoleic	308.498	$C_{20}H_{36}O_2$	4.01
R-3	33.255	Ethyl (E)-9-Octadecenoate	310.514	$C_{20}H_{38}O_2$	5.47
R-4	33.372	Ethyl Oleate	310.514	$C_{20}H_{38}O_2$	1.90
R-5	33.782	Methyl 17-methyl-octadecanoate	312.530	$C_{20}H_{40}O_2$	4.43
R-6	35.363	Ethyl 9-hexadecenoate	282.461	$C_{18}H_{34}O_2$	0.59
R-7	37.495	Heneicosane	296.574	$C_{21}H_{44}$	1.65
R-8	39.167	Eicosane	282.547	$C_{20}H_{42}$	2.50
R-9	39.640	Bis (2-ethylhexyl) phthalate	390.556	$C_{24}H_{38}O_4$	8.56
R-10	40.677	Ethyl docosanoate	368.636	$C_{24}H_{48}O_2$	2.01
R-11	40.766	Pentacosane	352.680	$C_{25}H_{52}$	3.46
R-12	42.222	Ethyl eicosanoate	340.583	$C_{22}H_{44}O_2$	0.997
R-13	42.308	Docosane	310.600	$C_{22}H_{46}$	5.30
R-14	43.132	Tetrapentacontane	759.451	$C_{54}H_{110}$	2.57
R-15	43.461	(Z)-13-Docosenamide	337.582	$C_{22}H_{43}NO$	4.14
R-16	43.516	17-Pentatriacontene	490.930	$C_{35}H_{70}$	6.52
R-17	44.337	Henicosanal	310.557	$C_{21}H_{42}O$	0.78
R-18	45.224	Tetracontane	563.079	$C_{40}H_{82}$	8.20
R-19	46.061	Ergosta-5,7,9(11),22(E)-tetraen-3β-ol	394.632	$C_{28}H_{42}O$	1.11
R-20	46.784	Dotriacontane	450.866	$C_{32}H_{66}$	7.11
R-21	47.775	4a,7,7,10a-Tetramethyldodecahydrobenzo[f]chromen-3-ol	265.232	$C_{17}H_{29}O_2$	1.01
R- 22	48.640	Hexatriacontane	506.972	$C_{36}H_{74}$	4.84

From Table 5, the total number of identified components can be observed. The total amount of identified compounds was approximately 91%, and nearly 9% remained unidentified.
Here the major compounds were identified as Ethyl palmitate (R- 1, 13.63%), Bis (2-ethylhexyl) phthalate (R- 9, 8.56%), Tetracontane (R-18, 8.20%), 17-Pentatriacontene (R- 16, .52%), Ethyl- (E)-9-Octadecenoate (R-3, 5.47%), Docosane (R-13, 5.30%). All the compounds identified from n-hexane extract of roots are fatty acids of esters and hydrocarbons.

Table 6: Structures of important compounds revealed from the root of *C. roseus* n-hexane extracts

Name of the compound	Chemical Formula
Ethyl palmitate (R-1, 13.62%)	
Bis (2-ethylhexyl) phthalate (R-9, 8.56%)	
Tetracontane (R-18, 8.21%)	
Dotriacontane (R-20, 7.11%)	
17-Pentatriacontene (R-16, 6.52%)	
Ethyl-(E)-9-Octadecenoate (R-3, 5.47%)	
Docosane (R-13, 5.30%)	

4. Conclusion

The current study gives a general review of the abundance of secondary metabolites throughout the entire *Catharanthus roseus* plant. It shows that many classes of chemicals may be present with potential therapeutic value. Therefore, additional research on this plant material is needed to identify and clarify the structures of the bioactive chemicals.

5. Acknowledgement

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