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Evaluation of bioactive compounds present in the botanical *Catharanthus roseus* (L) G. Don by GC-MS and FT-IR spectroscopic analysis

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Abstract

Catharanthus roseus is a well-known medicinal plant, a potential source of compounds with high antioxidant activity. In this study, we initially try to identify the bioactive molecule present in the whole plant of *C. roseus* by using FTIR and GCMS analysis. FT-IR analysis reveals functional groups like primary and secondary amines, amides, alkane, alkene, aromatic amine, carboxylic acid, aliphatic amine, amide, phenol, ether, sulphonamide, and halide. FT-IR analysis of plant powder, methanol extract, and aqueous extract shows that methanol was a better solvent for extraction because almost all compounds present in the plant powder are extracted by methanol compared to water. About 65 compounds were identified in the GCMS analysis, and more than 20 compounds showed the peak percentage is above 10.

Keywords: FT-IR, functional group; GCMS analysis, *Catharanthus roseus*

Introduction

The medicinal properties of different plants are due to presence of several compounds like saponins, tannins, alkaloids, alkyl phenol, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phenol ether (Pereira *et al.*, 2010) [5]. *Catharanthus roseus* is a medicinal plant and an ornamental shrub belonging to Apocynaceae family and commonly known as Madagascar periwinkle. The *C. roseus* studied as a medicinal plant as it was found to produce monoterpene indole alkaloids that contain the two major vital cytotoxic dimeric alkaloids (vincristine and vinblastine) widely used for cancer chemotherapy treatment; moreover, many alkaloids have a medicinal role. It also contains a significant number of volatiles and phenolic compounds including caffeoyl quinic acids, flavanol glycosides (Pereira *et al.*, 2010; Ferreres *et al.*, 2011) [2, 5]. Anti-oxidants are free radical scavengers that protect the human body against reactive oxygen species, which are harmful by-products generated during normal cell aerobic respiration (Bouayed *et al.*, 2009) [1].

Fourier Transform Infrared (FT-IR) is a routine analytical technique, the studies pay more attention to biological as well as biomedical investigations. The primary reason is that many common bio-molecules such as nucleic acids, proteins, lipids, and carbohydrates have characterised, which led to several essential and extensive investigations of biological samples that were analysed by IR spectroscopy. The capability to take simultaneous radiation measurements from all wavelength was the most significant feature of the FT spectrometer (Movasaghi *et al.*, 2007) [3]. Gas Chromatography-Mass Spectroscopy (GC-MS) is the most commonly used technique for identifying and quantifying compounds. The unknown compounds in a solution can be determined by matching the spectra with reference spectra.

FT-IR and GC-MS are powerful analytical techniques for identifying phytochemicals (Roberts and Xia, 1995). The present study was carried out the bioactive compounds present in the *Catharanthus roseus* with the aid of GC-MS and FT-IR techniques, which may provide an insight into its use of traditional medicine.

Materials and methods

The plant *Catharanthus roseus* (L) G. Don was collected from the Botanical garden of Calicut University, Malappuram, Kerala, India, and subsequently identified by the Department of Botany Calicut University, Malappuram, and Kerala, India.

The root, stem and leaves of fresh *C. roseus* were collected, washed, and air-dried in the shade and powdered using an electric grinder. The dried powder was extracted using a Soxhlet extractor with methanol and water.

Soxhlet extraction

The 100 grams of the shade dried sample of *C.roseus*, 50g of powder extracted in 750 ml of methanol, and 50g extracted in 750 ml of water using Soxhlet apparatus. After the 30 cycles, the final volume of 450 ml of both extracts is used for further analysis. The extract was stored in a refrigerator at 4°C for further use.

FT-IR Spectroscopic analysis

Fourier Transform Infrared (FT-IR) spectrometer is a routine analytical technique for analysing functional groups (chemical bond) of compounds present in a plant. The powdered plant, methanol extract, and aqueous extract of *C. roseus* were used for the analysis. The samples were mixed with dry potassium bromide (KBR pellet) and subjected to a pressure of about 5×10^6 Pa in an evacuated die to produce a clear transparent disc of 13 mm and thickness 1 mm. IR- spectra in frequency region 4000- 400 cm^{-1} were recorded at room temperature. For each spectrum, 100 scans were co-added at a spectral resolution of 4cm^{-1} . The frequency for all sharp bands was accurate to 0.01cm^{-1} .

GC-MS analysis

Methanol extract of *C.roseus* was analysed with the help of a GC-MS analyser (Perkin Elmer Gas Chromatography-Mass Spectrum). On the Elite-1 column, the data was generated. The carrier gas helium (99.999%) was used at a flow rate of 1 ml per min in split mode (10:1). 8 μ of methanol sample was

injected into the column at 250°C injector temperature. Injection temperature of oven starts at 40°C and hold for 6min, and then it was raised at a rate of 10°C per min to 300°C without holding. Holding was allowed for 6 min at program rate of 5°C per min. the temperature of ion sources was maintained at 240°C. The injector temperature was set at 250°C and detector temperature was set at 260°C. The mass Spectrum of compounds present in samples was obtained by electron ionization at 70eV and detector operates in scan mode 50 to 600Da atomic units. A 0.5 seconds of scan interval and fragments from 50 to 600Da was maintained. Total running was 40 minutes. The compound identified by NIST (National Institute of Science and Technology).

Result

After the Soxhlet extraction of the whole part of the plant with aqueous and methanol medium, the preliminary phytochemical study of both extract and plant powder is carried out by FT-IR spectroscopic analysis.

FT-IR Spectroscopy

The FT-IR analysis of dry plant powder (Fig.1A), methanol extract (Fig.1B), an aqueous extract (Fig.1C) of *C. roseus* were carried out; it represents the following functional group's compounds in which the frequency ranges from 3445.69 to 1017.27. Alcohol and phenol (OH bond) and alkynes ($\text{C}\equiv\text{C}$ or $\text{C}\equiv\text{N}$) functional groups are common in both extract and plant powder. The functional groups like 1°, 2° amines, amides, alkane, aromatic amine, aliphatic amine, etc. are present in the plant powder, most of them are presented in the methanol extract but absent in the aqueous extract (Table.1).

Table 1: FTIR spectral analysis of *C. roseus*

Functional groups	Components (peaks)	Frequency of Plant powder(cm^{-1})	Frequency of Aqueous extract(cm^{-1})	Frequency of Methanol extract(cm^{-1})
Alkanes	C-H (Stretch)	2851.24		2863.52 2949.59
	-CH ₂ (bend)	1418.39		1645.95 1454.55
Alkene	C=C(Stretch)	1644.98		1645.95
Aldehydes	C-H (Stretch)			2949.59
Quinones	O-H(stretch)		3445.69	3419.17
	C-O(stretch)	1243.86		
Carboxylic acids and their salts	O-H(stretch)	3396.09	3445.69	3419.17 2949.59 2863.52
	C=O (stretch)	1644.98		1645.95
	C-O (stretch)	1243.86		1454.55
Ammonium salts	N-H ₄ (stretch)	3396.09	3445.69	3419.17
	C=O (stretch)	1644.98		1645.95 1454.55
Amides	C=O (stretch)	1644.98		1645.95
	N-H(stretch)	3396.09	3445.69	3419.17
Anhydride	C=O (stretch)			
	C-O (stretch)	1243.86 1029.32		1054.39
	O-H(stretch)	3396.09	3445.69	3419.17
Alcohols	O-H(stretch)	3396.09	3445.69	3419.17
H- bonded	C-O(stretch)	1029.32		1454.55 1054.39
Carbohydrate	O-H (stretch)		3445.69	3419.17
Phenols	O-H (stretch)	3396.09	3445.69	3419.17
Ethers	C-O (stretch)	1243.86 1029.32		1054.39
1° and 2° amines	N-H(stretch)	3396.09	3445.69	3419.17
Aromatic amine	C-N(stretch)	1326.99		1054.39

		1029.32		
	N-H(bend)	1644.98		1645.95
Nitriles	C=N (stretch)			
Isocyanides	N=C (stretch)	2021.63	2060.57	2075.99
Nitro compounds	N=O	1326.99		
Sulphonic acids	O-H(stretch)			
	S=O(stretch)	1243.86		
Sulphonate esters	S=O(stretch)	1243.86		
Sulphonamides	N-H(stretch)	3396.09	3445.69	3419.17
Halides	C-F	1029.32		1054.39
	C-Cl	1243.86

(A)

(B)

Fig 1: FT-IR spectrum of *Catharanthus roseus* (A) Plant powder (B) Methanol extract and (C) Aqueous extract.

GC-MS analysis

Based on the retention time, we identified the compounds present in the methanolic extract of *C. roseus* (Fig.2). A total of 65 compounds were identified in the methanolic section of

C. roseus, among the 65, 20 compounds showed the plot area percentage above 1.00. Some compounds peak area is above 10%; such as Arginine, n-Decanoic acid, and (Methylthio)-acetonitrile or (2-methylsulfanyl acetonitrile) (Table 2).

Fig 2: GCMS chromatogram of methanolic extract of *C. roseus*.

Table 2: Peak report of methanolic extract of *C. roseus*.

No.	RT	Name of Compounds	Molecular Formula	Molecular Weight	Plot Area %
1	2.592	Methyl glyoxal	C ₃ H ₄ O ₂	72.0627	0.32
2	9.331	Propanoic acid	C ₃ H ₆ O ₂	74.0785	0.77
3	9.331	Oxalo acetic acid	C ₄ H ₄ O ₅	132.07	0.77
4	9.331	Malonic acid (propanedioic acid)	C ₃ H ₄ O ₄	104.0615	0.77
5	10.287	2- methylcyclo pentanone	C ₆ H ₁₀ O	98.143	0.83
6	10.287	Propylamine	C ₄ H ₁₀ N ₂ O	102.1350	0.83
7	16.110	Cyclopentanone	C ₅ H ₈ O	84.1164	0.59
8	16.110	Melamine	C ₃ H ₆ N ₆	126.1199	0.59
9	16.399	Acetic acid	C ₂ H ₄ O ₂	60.0510	0.59
10	17.885	Pyranone	C ₆ H ₈ O ₄	144.1265	2.61
11	20.120	Ery thritol	C ₄ H ₁₀ O ₄	122.1198	0.87

12	20.120	1,2, -elthane dial	C ₂ H ₆ O ₂	62.0678	0.87
13	20.389	2-methy 1,3-clithiolane	C ₄ H ₈ S ₂	120.236	0.26
14	20.389	Coumaran	C ₈ H ₈ O	120.1485	0.26
15	20.625	Furfural	C ₅ H ₄ O ₂	96.0841	3.61
16	20.625	1,4-dimethyl-5-ethyl 2-pyrozoline	C ₇ H ₄ N ₂	126.1995	3.61
17	21.203	2,2-diacetoxy pentane	C ₉ H ₁₆ O ₄	188.2209	2.46
18	21.203	3-methyl-2-hipta non	C ₈ H ₁₆ O	128.2120	2.46
19	21.539	Lysine	C ₆ H ₁₄ N ₂ O 2	146.1876	1.85
20	21.539	3-hydroxy-3-methylbu tanaceacid	C ₅ H ₁₀ O ₃	118.1311	1.85
21	22.609	3,6-droxa octamethylene	C ₆ H ₆ N ₂ O ₂	148.2034	2.33
22	22.609	Dodecyl-3-mercaptopropionate	C ₁₅ H ₃₀ O ₂ S	274.462	2.33
23	22.609	3,6-dioxaocta methylene diamine	C ₆ H ₁₆ N ₂ O ₂	148.2034	2.33
24	22.971	Dichloropropylp hophine	C ₃ H ₇ Cl ₂ P	144.967	0.96
25	22.971	3-auto propyl autale 4-oxypentyn autate	C ₇ H ₁₂ O ₃	144.168	0.96
26	23.733	L-1,2,3,4-butane teteaol	C ₄ H ₁₀ O ₄	122.1198	0.13
27	24.889	1-octanamino,n-odgl	C ₁₆ H ₃ N	241.455	0.50
28	26.917	N,N-dimethylpropanamide	C ₅ H ₁₁ NO	101.1469	0.44
29	26.917	Ethane sulfonamide	C ₄ H ₁₁ N OS ₂	137.201	0.44
30	27.360	Lethane	C ₉ H ₁₇ N OS ₂	203.302	4.87
31	27.970	Alloxan	C ₄ H ₂ N ₂ O ₄	142.0697	0.80
32	28.422	1,4E-D-Galactonotactone	C ₆ H ₁₀ O ₁₁	342.29	0.11
33	28.422	B- lactose	C ₁₂ H ₂₂ O ₁₁	342.29	0.11
34	28.935	Stilbene	C ₁₄ H ₂	180.2451	0.17
35	30.315	Benzene, 1-isocyano-2- methyl	C ₈ H ₇ N	117.1479	0.13
36	30.315	Benzonitrile, 2-methyl-	C ₈ H ₇ N	117.1479	0.13
37	30.826	Benzyl Nitrile	C ₈ H ₇ N	117.1478	0.22
37	31.159	Decane, 5,6-diprpyl	C ₁₆ H ₃₄	226.4412	1.9
38	31.159	Pentane, 1-butoxy-	C ₉ H ₂₀ O	144.2545	1.9
39	32.212	n- Decanoic acid	C ₁₀ H ₂₀ O ₂	172.2646	11.02
40	32.212	Arginine	C ₆ H ₁₄ N ₄ O ₂	174.2010	11.02
41	34.657	(Methylthio)-acetonitrile or (2-methylsulfanyl acetonitril)	C ₃ H ₅ NS	87.143	31.20
42	35.328	Silane, methyltripropyl-	C ₁₀ H ₂₄ Si	172.3831	1.14
43	36.643	Pentadecanoicacid, 14-methyl-,methyl ester	C ₁₇ H ₃₄ O ₂	270.4507	0.39
44	37.456	Palmitic acid (n-Hexadecanoic acid)	C ₁₆ H ₃₂ O ₂	256.4241	3.66
45	38.033	Penta decanoic acid	C ₁₅ H ₃₀ O ₂	242.3975	0.17
46	38.033	Trehalose	C ₁₂ H ₂₂ O ₁₁	342.2965	0.17
47	38.033	Tri decanoicacid	C ₁₃ H ₂₆ O ₂	214.3443	0.17
48	38.204	Tetra decannoic acid	C ₁₄ H ₂₈ O ₂	228.3709	0.3
49	38.204	Lactose	C ₁₂ H ₂₂ O ₁₁	342.2965	0.3
50	39.805	9, 12-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.4721	0.17
51	40.111	Phytol	C ₂₀ H ₄₀ O	296.5310	0.31
52	40.428	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5038	0.10
53	40.733	Linolenic acid	C ₁₈ H ₃₀ O ₂	278.4296	3.97
54	41.163	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.4772	0.59
55	46.616	Methoxamine	C ₁₁ H ₁₇ NO ₃	211.2576	0.21
56	46.925	Undecanal	C ₁₁ H ₂₂ O	170.2918	0.16
57	46.925	Methoxamine	C ₁₁ H ₁₇ NO ₃	211.2576	0.16
58	47.123	Thiocyanic acid, phenylmethyl ester	C ₈ H ₇ NS	149.213	0.18
59	47.721	Epinephrine	C ₉ H ₁₃ NO ₃	183.2044	0.34
60	49.177	Ethyl isopropyl dimethylphosphoramidate	C ₇ H ₁₈ NO ₃ P	195.1965	0.52
61	61.630	Alpha-Amyrin	C ₃₀ H ₅₀ O	426.7174	1.01
62	60.977	Cyclotrisiloxane, hexamethyl-	C ₆ H ₁₈ O ₃ Si ₃	222.4618	0.24
63	59.498	β-Sitosterol	C ₂₉ H ₅₀ O	414.7067	1.26
64	58.091	Stigmasterol	C ₂₉ H ₄₈ O	412.6908	0.54
65	57.533	Campesterol	C ₂₈ H ₄₈ O	400.6801	1.24

Discussion

Most of the study focusing on the particular plant parts, but in our study, we analysed and documented the phytochemicals present in the *C. roseus* whole plant (root, stem, and leaves). The initial preliminary screening of vibrational functional group identifications was done by using FT-IR spectrum. Plant powder, aqueous, and methanol extract revealed the presence of alkanes, alkenes, Quinone, carboxylic acids, amides, ammonium, alcohol, phenol, ether, primary and secondary amines, aromatic amines, isocyanides, sulphonamide, etc. (Fig. 1A-C and Table.1). Some impressive peaks were found in the FTIR analysis found in methanolic

extracts; it indicated the presence of some bioactive molecules. Apart from this, methanolic extracts showed several peaks in between the range of 3000-1000 cm⁻¹. This indicated the more functional groups present in the methanolic extracts than aqueous extracts. In the GCMS analysis, most compounds can be used for a therapeutical application like antimicrobial, antidiabetic, anticancer and antioxidant activity (Panday-Rai *et al.*, 2006; Thanwar *et al.*, 2017) [4, 6]. More studies are needed to be addressing the biological activity of each compound identified in the GCMS spectrum.

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Conflict of interest

No conflict of interest is associated with this work.

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