



Estimation of Bioactive Compound of *Catharanthus roseus* Leaf extract by Phytochemical Screening and GC-MS Analysis

S. Nathiya¹, N. Shaishta Jabeen¹, L. Jagapriya¹, B. Senthilkumar², K. Devi^{1*}

¹Department of Zoology, D. K. M. College for Women, Vellore, Tamil Nadu, India

²Department of Zoology, Thiruvalluvar University, Serkadu, Vellore, Tamil Nadu, India

ABSTRACT

Plant derived compounds have played a vital role in the development of several chemotherapeutic agents. *Catharanthus roseus* is an important medicinal plant of the apocynaceae family. The leaf extract of *Catharanthus roseus* have many biological activities such as *antibacterial, antioxidant and antidiabetes*. The present study was aimed to carry out the phytochemical analysis and the GC-MS analyses of *Catharanthus roseus* leaf extract ensure biological activity in the presence bioactive compounds. The leaves designated to the presence of secondary metabolites (proteins, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, sterols, terpenoids, acidic compounds, cardiac glycosides, phenols, alkaloids, flavonoids). In the GC-MS analysis the *Catharanthus roseus* extract result shows the presence of bioactive compounds which revealed a broad spectrum of many medicinal property and antioxidant activity were identified. This study is helped to identify bioactive compound formula and structure which can be used as pharmaceutical industries for drug discovery.

Keywords: *Catharanthus roseus*, *antibacterial, antioxidant antidiabetes*

INTRODUCTION

Medicinal plants are the oldest form of healthcare known to mankind. From the ancient time people are using different types of plants as the remedy for

various diseases. According to World Health Organization (WHO) approximately 80% of the World's population the herbal medicine was used to treat them traditionally (Mayuri Thanwar *et al*).

Medicinal plants have vast and diverse assortment of bioactive compounds, than can produce a definite physiological action on the human body. These bioactive compounds have been isolated from plants which could be used for the development of new drugs; pharmacists are interested in these compounds because of their therapeutic performance and low toxicity.

Catharanthus roseus is an important medicinal plant family apocyanaceae also known as Vincarosea Indian originated herb. *Catharanthus roseus* as used as medicine for traditionally treating many diseases they are Malaria, Diabetes, Leukemia, Wasp sting, Sore throat, Eye irritation, Astringent, Diuretic and Expectorant (S. Patharajan *et al*). The plant also possesses various medicinal properties such as antimicrobial, antioxidant, antihelminthic antifeedant, antiserility, antidiarrheal, antidiabetic (Gajalakshmi *et al*). These Properties of *Catharanthus roseus* due to the presence of biologically active compounds of biological importance. In this study carried out identify the phytocompounds in the ethanolic leaf extract of *Catharanthus roseus* by qualitative screening of phytochemicals and each compound with their concentration by Gas Chromatography - Mass Spectrum (GC-MS) analysis.

Materials and Methods

Collection of plant material

Catharanthus roseus leaves were collected from Vellore District. The leaves were washed with water to remove soil and dust particles, and then it was dried under shady place. The dried plant material were blended to form a fine powder and stored in airtight bottles.

Extract preparation

Plant material were used for the solvent extraction procedure, about 10gm of plant powder was soaked in 100ml of ethanol for 48 hours. The contents were then filtered through Whatman filter paper no.1. The crude extract was subjected Gas Chromatography and Mass Spectrum to find the Bioactive compounds present in plant extract.

Phytochemical analysis standard procedure (9, 10, 11)

The extract of *Catharanthus roseus* tested for the bioactive compounds by using following methods

Test for Proteins

➤ Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

➤ Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates

➤ Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

➤ Benedict's test

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate

formed which indicated the presence of the carbohydrates.

➤ Molisch's test

Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H₂SO₄ was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

➤ Iodine test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids

➤ Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

➤ Alkaline reagent test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides Libermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

➤ Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

➤ Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2ml of concentrated H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycosides.

➤ Test for steroid

Crude extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

GC-MS analysis

The GC-MS analysis of ethanol extract *Catharanthus roseus* was performed using a Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the

rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Result

The Phytochemical screening of *Catharanthus roseus* qualitatively analyzed in our lab by the standard methods exhausting identified the secondary metabolites of flavonoids, glycosides, tannins, terpenoids, phenol, steroids, alkaloids and carbohydrates were found in the ethanol leaf extract of this plant and the result are presented in Table 1.

The GC-MS analysis revealed the presence of 12 compounds from the ethanol extract of *Catharanthus roseus* active principles with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %) are presented in Table 2. The compounds are identified by the mass spectroscopy were presented. The total numbers of compounds identified in the ethanol extract were 1-Octadecyne (43.66%) and Tridecanoic acid (20.02%) was found as the 2 major components and other 10 minor compounds such as Hexatriacontane (1.92%), 1,2-Bis(Trimethylsilyl)Benzene (2.963), DI-N-Decylsulfone (2.09%), UnDecanoic acid (2.24%), N-Hexadecanoic acid (2.34%), Hexatriacontane (3.76%), 4-Heptanol, 2-Methyl (4.68%), Squalene (4.34%), Heotacosane (5.41%), 2h-1-Benzopyran-6-Ol, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-, Acetate (6.54%). These peak values represents phytochemicals are present in the ethanol extract of *Catharanthus roseus* leaves.

Discussion

In this study, the preliminary phytochemical screening as shown Table 1. Revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, phenols, carbohydrates, protein and amino acids. This Secondary metabolite of the ethanol extract of *Catharanthus roseus* leaves retain medicinal importance. The presence of alkaloids and phenol compounds of Secondary metabolites responsible for pharmacological activities like anti-cancer, anti-oxidant, anti-microbial, antifertility, wound healing

and anti-inflammatory, that may well this plant proving to vast valuable bioactive compounds are possess medicinal value. The GC-MS analysis to showed 12 compounds are present in the ethanol extract of *Catharanthus roseus* fragmentation pattern of N-Hexadecanoic acid have a Antioxidant, Antimicrobial, Antiinflammatory, properties, 4-heptanol, 2-methyl compound effective for Antioxidant, and 2h-1-Benzopyran-6-Ol, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyl compound involved in Antioxidant, Antimicrobial activity. The source of many medicinal plants has phytochemical compounds identified from the peak pattern of the chromatograms obtained directly from GC-MS analysis. Similarly, The GC-MS analysis will show the bioactive compound present in the ethanol extract of *Catharanthus roseus* have many biological

properties that can be used in treat to cure many diseases. In this study carried out identify the phytochemicals in the ethanolic leaf extract of *Catharanthus roseus* by qualitative screening of phytochemicals and each compound with their concentration by Gas Chromatography - Mass Spectrum (GC-MS) analysis were used in medicinal uses.

Conclusion

The ethanol leaf extract of *Catharanthus roseus* contain many phytochemical components The GC-MS analysis showed the presence of bioactive compounds in the leaves further studies are needed to detect the various other solvents of methanol, petroleum ether, chloroform and aqueous.

Table: 1 Phytochemical Constituents analysis of ethanol extract of *Catharanthus roseus*

S. No.	Name of the Test	Phytochemical analysis of <i>Catharanthus roseus</i>
1.	Test for protein Ninhydrin test	+
2.	Test for Carbohydrate Molisch's test, Iodeine test	+
3.	Test for phenols and tannins	++
4.	Test for flavonoids Alkaline reagent test	+
5.	Test for Saponins	+
6.	Test for glycosides Salkowski's test	+
7.	Test for steroid	+
8.	Test for terpenoids	+
9.	Test for alkaloids	+

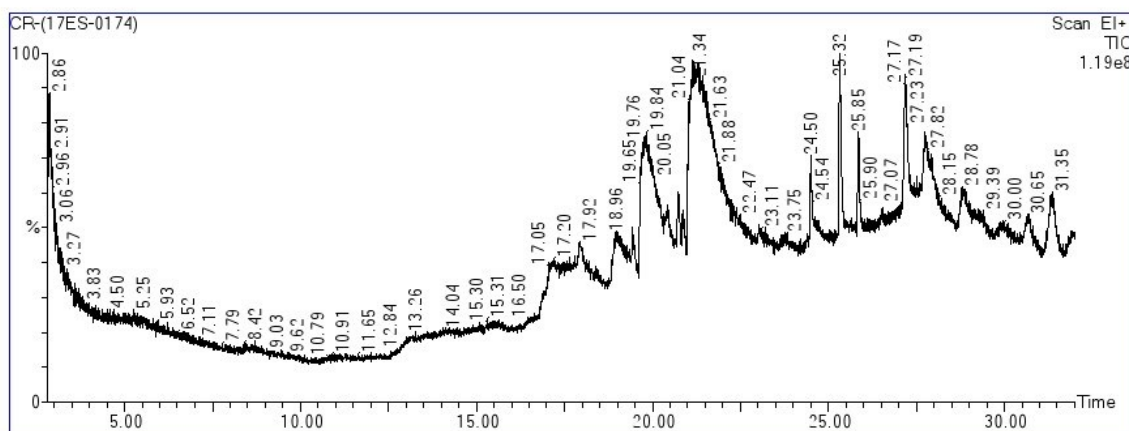
Table: 2 Phytocomponents identified in the ethanol leaf extract of *Catharanthus roseus* by GC-MS analysis

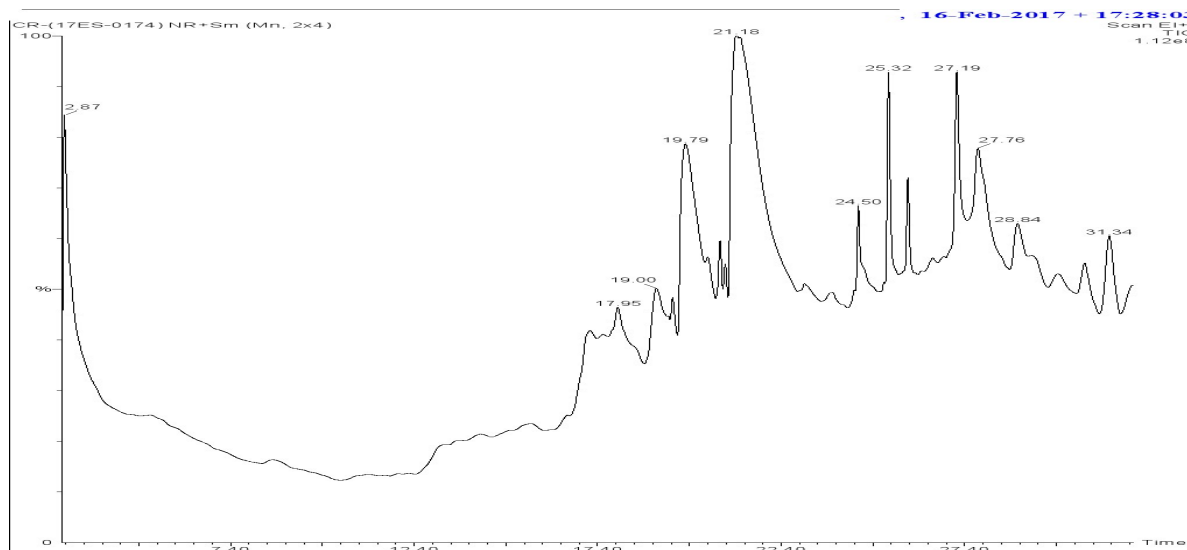
S. No.	Rt	Peak Value	Compound Name	Molecular Weight	Molecular Formula	Bioactivity
1.	17.199	2.342	N-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	Antioxidant, Antimicrobial , Antiinflammatory
2.	18.965	4.683	4-heptanol, 2-methyl	130	C ₈ H ₁₈ O	Antioxidant
3.	19.84	20.023	Tridecanoic acid	214	C ₁₃ H ₂₆ O ₂	No activity found
4.	20.431	2.24	Undecanoic acid	186	C ₁₁ H ₂₂ O ₂	No activity found
5.	21.146	43.66	1-Octadecyne	250	C ₁₈ H ₃₆ O ₂	No activity found
6.	24.497	3.762	Hexatriacontane	506		No activity found
7.	25.318	4.349	Squalene	410	C ₃₀ H ₅₀	Antioxidant, chemopreventive, antitumour and Hypocholesterolemic
8.	25.848	1.923	Hexatriacontane	506	C ₃₆ H ₇₄	No activity found
9.	27.178	5.412	Heptacosane	380	C ₂₇ H ₅₆	No activity found
10.	27.744	6.549	2h-1-Benzopyran-6-Ol, 3,4-Dihydro- 2,5,7,8-Tetramethyl- 2-(4,8,12-Trimethy	472	C ₃₁ H ₅₂ O ₃	Antioxidant, Antimicrobial
11.	28.784	2.095	Di-N-Decylsulfone	346	C ₂₀ H ₄₂ O ₂ S	No activity found
12.	31.37	2.963	1,2-Bis(Trimethylsilyl)Benzene	222	C ₁₂ H ₂₂ S ₁₂	No activity found

Figure2. GC-MS analysis of ethanolic leaf extract of *Catharanthus roseus*

Qualitative Report

File: C:\TURBOMASS\2016.PRO\Data\CR-(17ES-0174).raw
 Acquired: 16-Feb-17 05:28:03 PM
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 GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP
 Sample ID: CR-(17ES-0174)
 Printed: 07-Mar-17 04:29 PM
 Page 1 of 1
 Vial Number: 45





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