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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY STUDIES ON ETHANOLIC EXTRACT OF DRIED LEAVES OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Objective: Phytochemical screening of the ethanolic extract of *Catharanthus roseus* disclosed the presence of alkaloids, terpenoids, phenols, tannins, saponins, quinines, flavonoids, and proteins. In the present research work, we have identified and confirmed the structures of the constituents present by means of a hyphenated technique of gas chromatography-mass spectrometry (GC-MS) from the extract.

Method: Shade-dried leaves of *C. roseus* were powdered and extracted by means of Soxhlet extraction using ethanol as a solvent. Crude extract obtained was utilized for GC-MS.

Results: A total of 15 components were identified, some of which were *n*-hexadecanoic acid, ethyl ester, Vitamin E, 9,12,15-octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1-[{(trimethylsilyl)oxy}methyl]ethyl ester, phytol, 3-epivindolinine, and oleic acid.

Conclusion: GC-MS studies helped us to assess the phytochemical constituents based on retention time, molecular formula, molecular weight and the corroboration of MS libraries. In future, *C. roseus* leaves may be subjected to diverse types of extraction methodologies, and a number of various elucidated phytoconstituents can be studied for their ethnopharmacological significance and applicability citing literature.

Keywords: *Catharanthus roseus,* Gas chromatography, Mass spectroscopy, Phytol, 3-Epivindolinine, Oleic acid.

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INTRODUCTION

Discovery of natural plant-derived drugs from natural botanical herbarium has been the major breakthrough in paving the way for natural product chemistry [1]. Natural products are a substantial source of new drugs. They may have various sources or origins such as terrestrial plants, microorganisms, marine organisms, and terrestrial vertebrates, and invertebrates [2,3]. From time immemorial, they play a significant role as phytochemicals in treating and preventing a number of human diseases. Phytochemicals have been derived from the Greek word "phyto" meaning plant. Phytochemicals are biologically active, naturally occurring, non-nutritive chemical compounds found in plants having a protective and disease preventive activity. Plants produce such chemicals to safeguard themselves, but research reveals that they also have the ability to protect humans against diseases [4]. The subject of phytochemistry has been developed in recent years as a strict discipline, closely related to both natural product organic chemistry and plant biochemistry [1].

Plant metabolism has been able to separate phytochemicals in two categories, namely, primary or secondary. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, and chlorophylls. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids, and glucosides [4]. In a pharmaceutical landscape, plants with a long history of use in ethnomedicine are considered as a rich source of active phytoconstituents that provide medicinal or health benefits against various ailments and diseases. One such family with extensive traditional use is Apocynaceae family.

Catharanthus roseus is one of the most important medicinal plants belonging to this family. *C. roseus* or *Periwinkle* (Nayantara or Sadabahar) is an erect bushy perennial herb and evergreen shrub. It grows wildly in the Indian subcontinent in southern Asia and with medicinal importance in Australia, Africa, and Southern Europe. The leaves are long and they are arranged in the opposite pairs. They have oval to oblong shape, broad glossy green hairless with a pale midrib and a short petiole [5]. It has been reported to contain more than 400 types of different alkaloids. Some of the important are vinblastine, vincristine, vindesine, vindeline, tabersonine, ajmalicine, vinceine, vineamine, raubasin, reserpine, catharanthine, etc. [6]. Leaves are used in the treatment of menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, menstrual disorders, antiallergic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, antihelminthic, hypolipidemic, skin diseases, bleeding diarrhea, and antiviral properties. Currently, herbal research has been mainly focusing on isolation, characterization, identification, and quantification of bioactive constituents and secondary metabolites [7-13]. Gas chromatography-mass spectrometry (GC-MS) is one such sophisticated analytical technique used in identification, detection, and analysis of the constituents. It comprises GC coupled to a MS, by which complex mixtures of plant-related compounds may be separated, identified, and quantified [14]. In the present work, we have identified and confirmed the structures of the active constituents by GC-MS from the ethanolic extract of dried leaves of *C. roseus*.

MATERIALS AND METHOD

Collection and authentication

- The fresh leaves of *C. roseus* were collected from Mumbai, Maharashtra, and air-dried at room temperature.
- The dried leaves' sample was authenticated by Agharkar Research Institute, Pune.
- The sample was stored in an airtight container at 6°C.

Extraction

- The leaves of *C. roseus* were dried in the shade, powdered with a mechanical grinder, and passed through sieve no. 40.

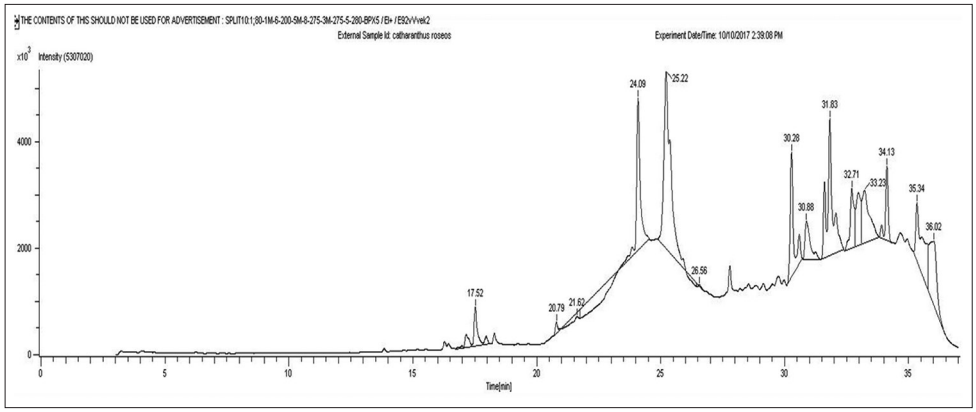

Fig. 1: Gas chromatography spectrum of *Catharanthus roseus* ethanolic extract

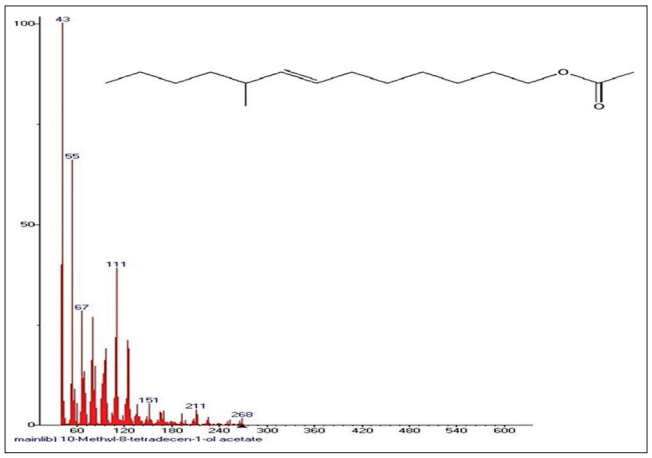

Fig. 2: Mass spectrum showing the presence of 10-methyl-8-tetradecen-1-ol acetate

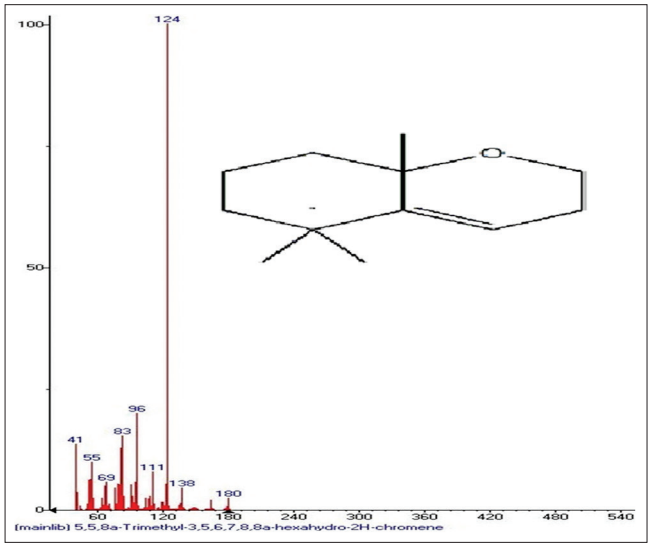

Fig. 3: Mass spectrum showing the presence of 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene

- The dried powdered material (25 g) was extracted with 80% ethanol using Soxhlet apparatus at a temperature of 50°C for 21 h.
- The solvent was then evaporated on a water bath at temperature maintained at 70°C.

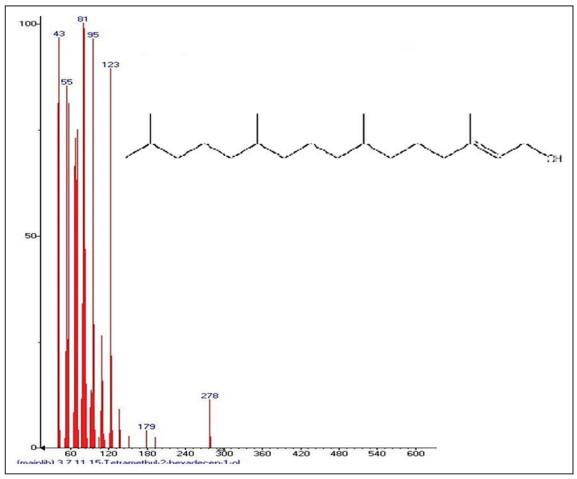

Fig. 4: Mass spectrum showing the presence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

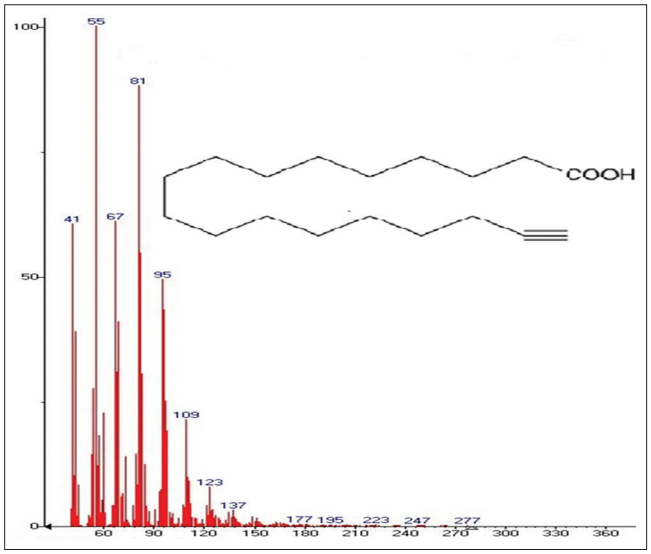

Fig. 5: Mass spectrum showing the presence of 17-octadecynoic acid

GC-MS

- The instrument used in the experimentation purpose was Joel, USA, with model of Accu Time-of-Flight GCV. The column details comprise capillary (type), semi-standard non-polar (class), and 30m/60m (length)

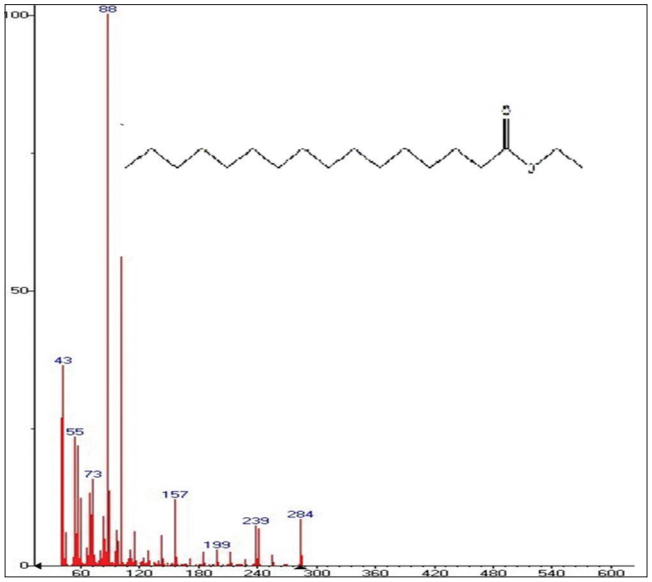

Fig. 6: Mass spectrum showing the presence of hexadecanoic acid, ethyl ester

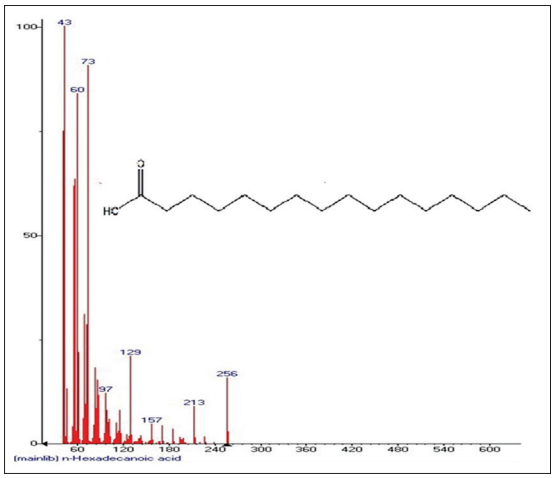

Fig. 7: Mass spectrum showing the presence of *n*-hexadecanoic acid

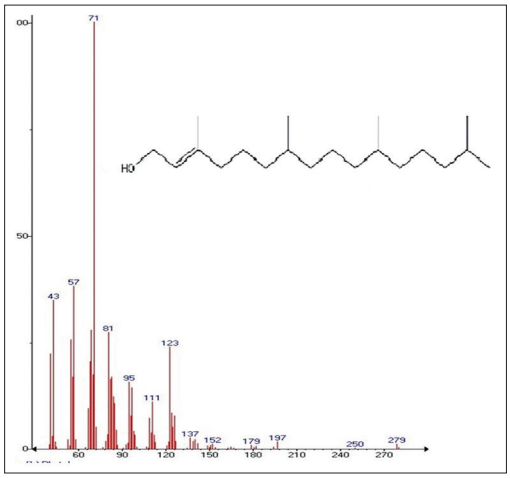

Fig. 8: Mass spectrum showing the presence of phytol

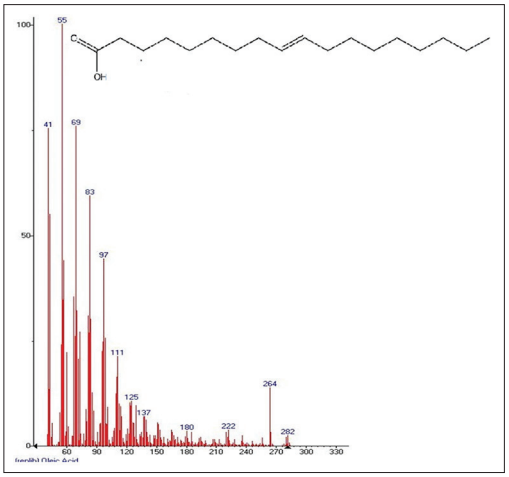

Fig. 9: Mass spectrum showing the presence of oleic acid

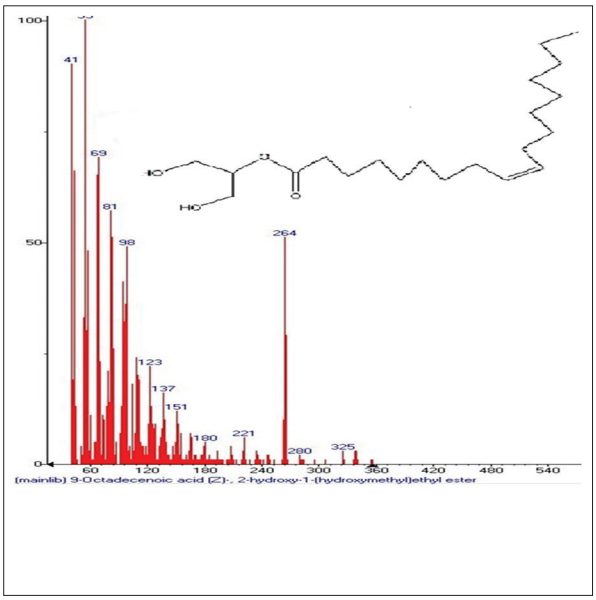

Fig. 10: Mass spectrum showing the presence of 9-octadecenoic acid(Z),2-hydroxy-1-(hydroxymethyl)ethyl ester

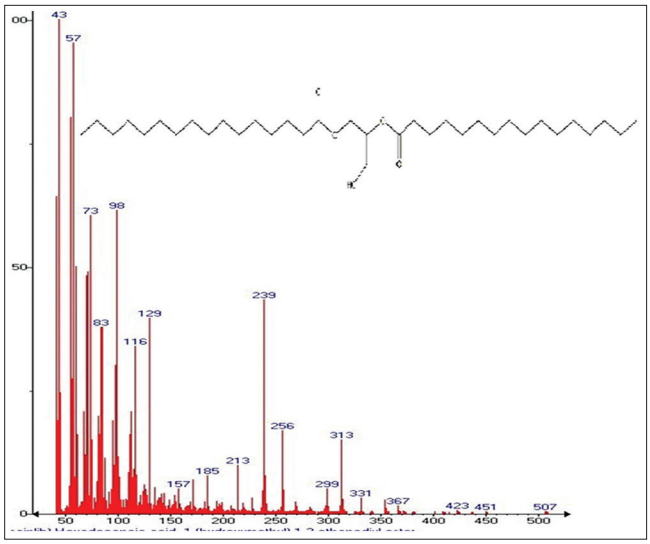

Fig. 11: Mass spectrum showing the presence of hexadecanoic acid-1-(hydroxymethyl)-1,2-ethanediyl ester

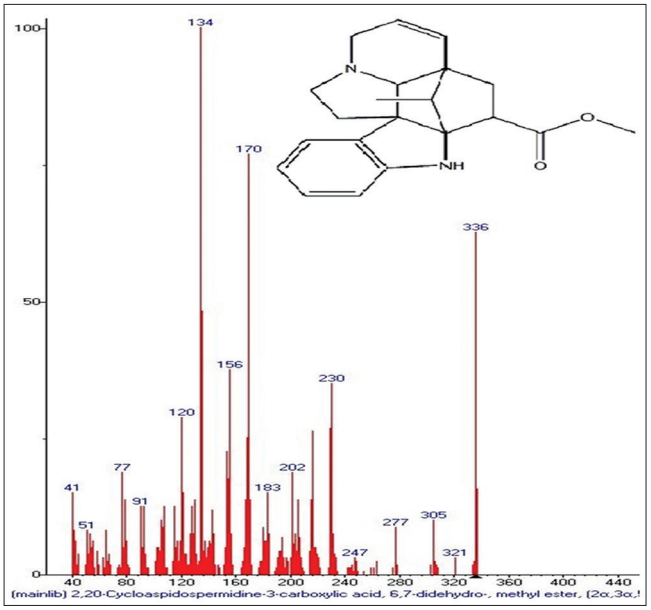

Fig. 12: Mass spectrum showing presence of 2,20-cycloaspidospermid-3-carboxylic acid,6,7-didehydro-methyl ester(2α,3α,5α,12β,19α, 20R)

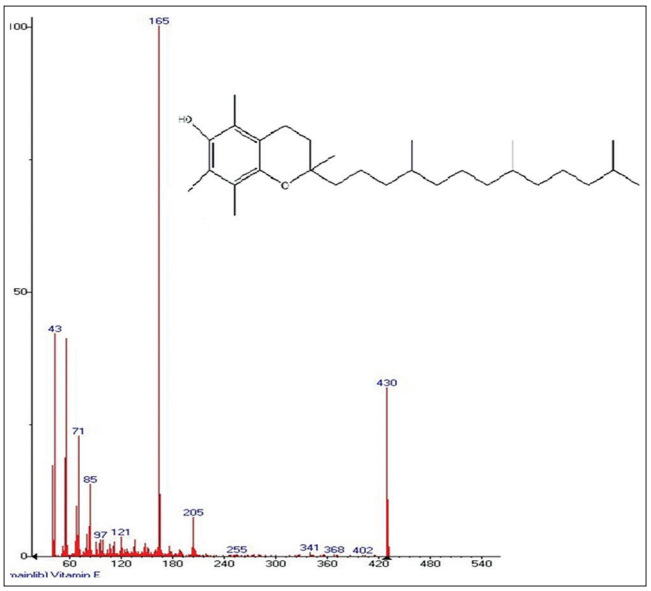

Fig. 13: Mass spectrum showing the presence of Vitamin E

with diameter of 0.25 mm. The mobile phase used was chloroform. The carrier used was helium with heat rate of 2k/min to 3k/min.

- The libraries used were NIST 2.0 f and Fine, NIH, EINECS, TSCA, RTECS, HODOC, IRDB, and LIB for identification and interpretation of compounds.

RESULTS AND DISCUSSION

In the present GC-MS study, The term is 17-Octadecynoic acid. hexadecenol derivatives, palmitic acid, phytol, oleic acid, Isovindolinine, and tocopherols were eluted. The principal compounds found to be present in the extract were predominantly saturated and unsaturated fatty acids and their esters, diterpenes and methylated phenols, all of which possess a significant pharmacological activity. In the present research study, 15 compounds were identified by GC-MS technique from *C. roseus* ethanolic extract (Fig. 1). 10-Methyl-8-tetradecen-1-ol acetate (Fig. 2) with *m/z* 268 and fragment ions 43, 55,

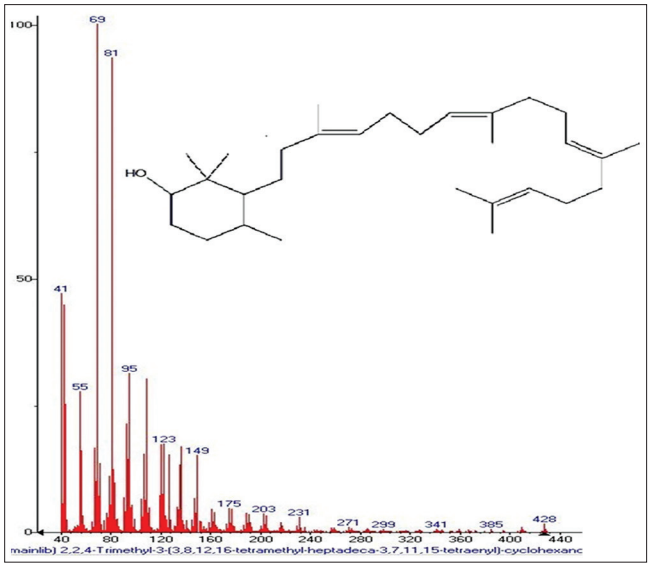

Fig. 14: Mass spectrum showing presence of 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol

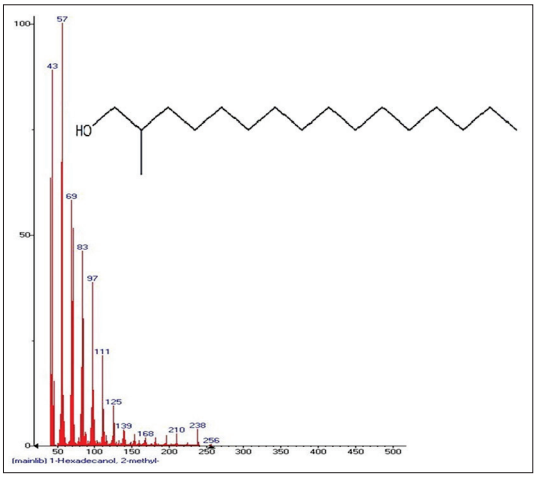

Fig. 15: Mass spectrum showing the presence of 2-methyl-1-hexadecanol

67, 111, 151, 211, 268 and 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2*H*-chromene (Fig. 3) with *m/z* 180 and fragment ions 41, 55, 69, 83, 96, 111, 124, 138, 180 and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Fig. 4) with *m/z* 296 and fragment ions 43, 55, 81,95, 123, 179, 278 and 17-octadecynoic acid (Fig. 5) with *m/z* 280 and fragment ions 41, 55, 67, 81, 95, 109, 123, 137, 177, 195, 223, 247, 277 and hexadecanoic acid, ethyl ester (Fig. 6) with *m/z* 284 and fragment ions 43, 55, 73, 88, 157, 199, 239, 284 and n-hexadecanoic acid (Fig. 7) with *m/z* 256 and fragment ions 43, 60, 73, 97, 129, 157, 213, 256 are characterized. Phytol (Fig. 8) with *m/z* 296 and fragment ions 43, 57, 71, 81, 95, 111, 123, 137, 152, 179,197, 250, 279 and oleic acid (Fig. 9) with *m/z* 282 and fragment ions 41, 55, 69, 83, 97, 111, 125, 137, 180, 222, 264, 282 and 9-octadecenoic acid(Z),2-hydroxy-1-(hydroxymethyl) ethyl ester (Fig. 10) with *m/z* 356 and fragment ions 41, 55, 69, 81, 98, 123, 137, 151, 180, 221, 264, 280, 325 and hexadecanoic acid-1-(hydroxymethyl)-1,2-ethanediyl ester (Fig. 11) with *m/z* 568 and fragment ions 43, 57, 73, 83, 98, 116, 129, 157, 185, 213, 239, 256, 299, 313, 331, 367, 423, 451, 507 and the term is 2,20-cycloaspidospermid-3-carboxylic acid-6,7-didehydro methyl ester-(2α,3α,5α,12β,19α, 20R). (Fig. 12) with *m/z*336 and fragment ions 41, 51, 77, 91, 120, 134, 156, 170, 183, 202, 230, 247, 277, 305, 321, 336 and Vitamin E

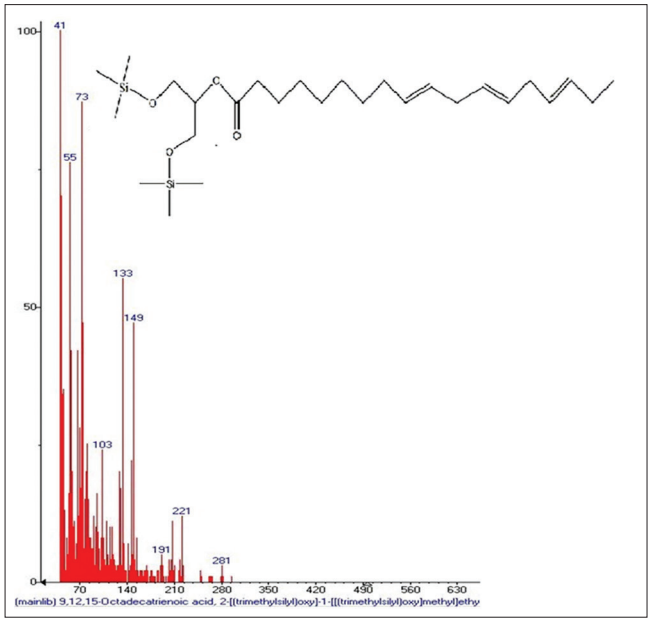

Fig. 16: Mass spectrum showing the presence of 9,12,15-octadecatrienoic acid,2-[(trimethylsilyl) oxy]-1-[{(trimethylsilyl)oxy}methyl]ethyl ester, (Z,Z,Z)

(Fig. 13) with *m/z* 430 and fragment ions 43, 71, 85, 97, 121, 165, 205, 255, 341, 368, 402, 430 and 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol (Fig. 14) with *m/z* 428 and fragment ions 41, 55, 69, 81, 95, 123, 149, 175, 203, 231, 271, 299, 341, 385, 428 and 2-methyl-1-hexadecanol (Fig. 15) with *m/z* 256 and fragment ions 43, 57, 69, 83, 97, 111, 125, 139, 168, 210, 238, 256 and 9,12,15-octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1-[{(trimethylsilyl)oxy}methyl]ethyl ester, (Z,Z,Z) (Fig. 16) with *m/z* 496 and fragment ions 41, 55, 73, 103, 133, 149, 191, 221, 281, respectively, are seen prominently.

CONCLUSION

This research article will edify a researcher and the reader toward 15 compounds that have been screened from the ethanolic extract of *C. roseus* by a hyphenated technique of GC-MS. It will also help to build upon future research endeavors in related fields by further elaboration focusing on different extraction procedures and elucidation and comparison of various phytoconstituents and their ethnopharmacological activities by application of various chromatographic hyphenated techniques.

ACKNOWLDGMENT

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