INNOVARE JOURNAL OF SCIENCES

Vol 5, Issue 1, 2017

ISSN - 2321-5496

Research Article

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSIS OF *CANTHIUM PARVIFLORUM* LEAVES

RADHAKRISHNAN K[1], JAMES F[1], MOHAN A[1], CHANDRA MOHAN S[2]*

[1]Department of Chemistry, Saraswathi Narayanan College, Madurai, Tamil Nadu, India. [2]Division of Phytochemistry, Shanmuga Centre for Medicinal Plants Research, Thanjavur, Tamil Nadu, India. Email: cm_123ss@yahoo.co.in

Received: 04 October 2016, Revised and Accepted: 11 October 2016

ABSTRACT

Objective: In the present study, the leaves of *Canthium parviflorum* were subjected to preliminary phytochemical and gas chromatography and mass spectrometry (GC-MS) analysis.

Methods: GC-MS analysis was carried out on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier.

Results: GC-MS results revealed the presence of 8 phytoconstituents including 4',5,7-trihydroxyisoflavone, phytol, etc.

Conclusions: Results of this study show that the leaves of *C. parviflorum,* the rich source of phytocompounds, which can play an important role in preventing the progression of many diseases.

Keywords: *Canthium parviflorum,* GC-MS, Phytochemical screening, 4',5,7-trihydroxyisoflavone.

INTRODUCTION

Canthium parviflorum (Syn.: *Canthium coromandelicum*) (Rubiaceae family) is a bushy thorny herb, found throughout the Western Ghats, and coast of the coromandel region of India. Canthium herbal medicine is used for the treatment of diabetes among major tribal groups in South India [1,2]. The leaves were used for wound healing and diuretic activity in animals [3] and gastrointestinal disorders such as diarrhea and constipation [4]. The root and leaves were used as diuretic, diarrhea, strangury, fever, leucorrhea, and intestinal worm in children [5]. The bark is made into paste along with turmeric and lime and applied on the forehead to cure headache. The whole plant is used against diabetes, controls high blood pressure, reduce unwanted fats in the body, and as a blood purifier. *C. parviflorum* leaves have been reported to exhibit significant antimicrobial, anti-HIV activity [6], hypocholesterolemic activity [7], oral hypoglycemic activity, wound healing and diuretic activities, antioxidant properties, and antibacterial activity [8]. However, complete information on the phytochemical composition and its antioxidant stability is not very well studied. In the present experiment, the phytochemical composition by gas chromatography and mass spectrometry (GC-MS) of the *C. parviflorum* was reported.

METHODS

Plant materials

Leaves of *C. parviflorum* were collected from Siluvinippatti village, Sivagangai, Tamil Nadu, during the month of June - August 2016.

Preparation of extract

The leaves were washed thoroughly with tap water and in distilled water and then dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 20 g of powdered plant extracted with 200 ml of different solvents such as methanol, ethanol, and hexane. The extracts were filtered through Whatmann No.1 filter paper, and the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.

Phytochemical screening

Phytochemical screening chemical tests were carried out on the various extracts using standard procedures to identify the phytoconstituents [9,10].

GC-MS

The ethanolic extract was subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCmate II GC-MS with data system is a high resolution, double-focusing instrument. The column (HP5) was fused silica 50 m×0.25 mm i.d. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature and 240°C for injector temperature, helium was the carrier gas, and split ratio was 5:4. The sample (1 µl) was evaporated in a splitless injector at 300°C. Run time was 22 minutes. The components were identified by GC coupled with MS.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08 and Wiley08 library. Identification of components was based on comparison of their mass spectra. As the compounds separated, on elution through the column, were detected in electronic signals. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of *C. parviflorum* leaves containing bioactive chemicals such as alkaloids, flavonoids, steroids, terpenoids, and phenolic compounds (Table 1).

GC-MS analysis

GC-MS analysis was carried out on the ethanolic extract of *C. parviflorum,* and 8 compounds were identified. The chromatogram shows 8 prominent peaks and given in Fig. 1.

The identified chemical constituents and biological activities of identified chemical constituents are shown in Table 2.

The largest peak (RT 19.13) is due to the presence of phytol. The molecular formula and molecular weight of this compound are $C_{20}H_{40}O$ and 296, respectively. Phytol is known to be antimicrobial, anticancer, anti-inflammatory and hepatoprotective [11]. Mass spectrum of phytol was shown in Fig. 2.

The second less prominent peak (RT 19.35) is due to the presence of octadecanoic acid, methyl ester. Mass spectrum of octadecanoic acid, methyl ester was shown in Fig. 3.

Table 1: Phytochemical screening of *Canthium parviflorum* leaves

Phytochemicals	Methanol extract	Ethanol extract	Hexane extract
Alkaloids	Present	Present	Present
Flavonoids	Present	Present	Absent
Phenols	Present	Present	Absent
Steroids	Present	Present	Absent
Terpenoids	Present	Present	Present

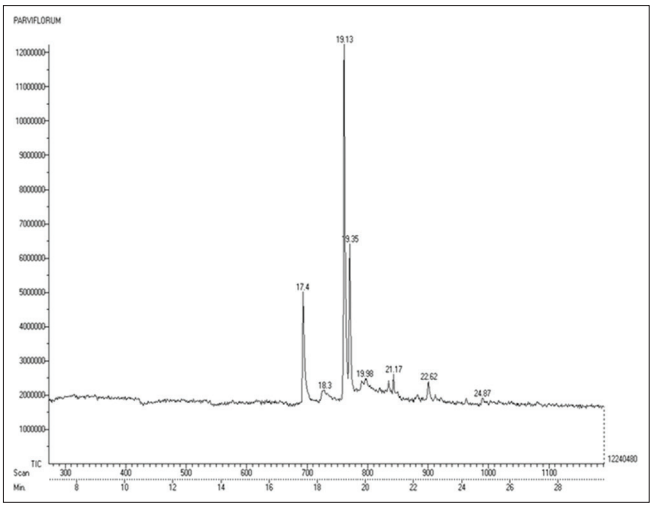

Fig. 1: Gas chromatography and mass spectrometry chromatogram of *Canthium parviflorum* leaves

The third less significant peak (RT 17.3) is due to 4',5,7-trihydroxyisoflavone, known as genistein. Genistein is an angiogenesis inhibitor and a phytoestrogen and belongs to the category of isoflavones. Genistein was first isolated in 1899 from the dyer's broom, *Genista tinctoria*; hence, the chemical name. The compound structure was established in 1926 when it was found to be identical with prunetol. It was chemically synthesized in 1928. Besides functioning as antioxidant and anthelmintic, genistein was, among other flavonoids, found to be a strong topoisomerase inhibitor, similarly to some chemotherapeutic anticancer drugs, for example, etoposide and doxorubicin [12,13]. In high doses, it was found to be strongly toxic to normal cells [14]. This effect may be responsible for both anticarcinogenic and carcinogenic potential of the substance [15,16]. Mass spectrum of 4',5,7-trihydroxyisoflavone was shown in Fig. 4.

The steroidal compound estra-1,3,5(10)-trien-17a'-ol is found to be responsible for the peak at RT 18.3. The mass spectrum of the compound showed the molecular ion peak at m/z 256 (Fig. 5). Molecular formula is $C_{19}H_{26}O_2$, and molecular weight is 256.4085. This compound is found to show medicinal activity such as androgenic alopecia (hair loss).

Peak height 19.98 is responsible for (E)-9-octadecenoic acid, ethyl ester. Its molecular formula is $C_{20}H_{38}O_2$, and molecular weight is 310. The mass spectrum of the compound showed the molecular ion peak at m/z 310 (Fig. 6).

Peak height 21.17 is responsible for eicosanoic acid, methyl ester. Molecular formula is $C_{21}H_{42}O_2$, and molecular weight is 326. 9-octadecenoic acid (Z)-methyl ester found to be effective against fungi *Aspergillus flavus* [17]. The mass spectrum of the compound showed the molecular ion peak at m/z 326 (Fig. 7).

The peak value, i.e., RT 22.62 corresponds to (Z)-9-octadecenoic acid, butyl ester other name oleic acid, butyl ester. The mass spectrum of the compound showed the molecular ion peak at m/z 338.3965 (Fig. 8). Molecular formula is $C_{22}H_{42}O_2$, and molecular weight is 338.5677.

The peak value, i.e., RT 24.87 corresponds to corynan-17-ol, 18,19-didehydro-10-methoxy-acetate. The mass spectrum of the compound showed the molecular ion peak at m/z 367.7373 (Fig. 9); molecular formula is $C_{22}H_{28}N_2O_3$, and molecular weight is 368.47.

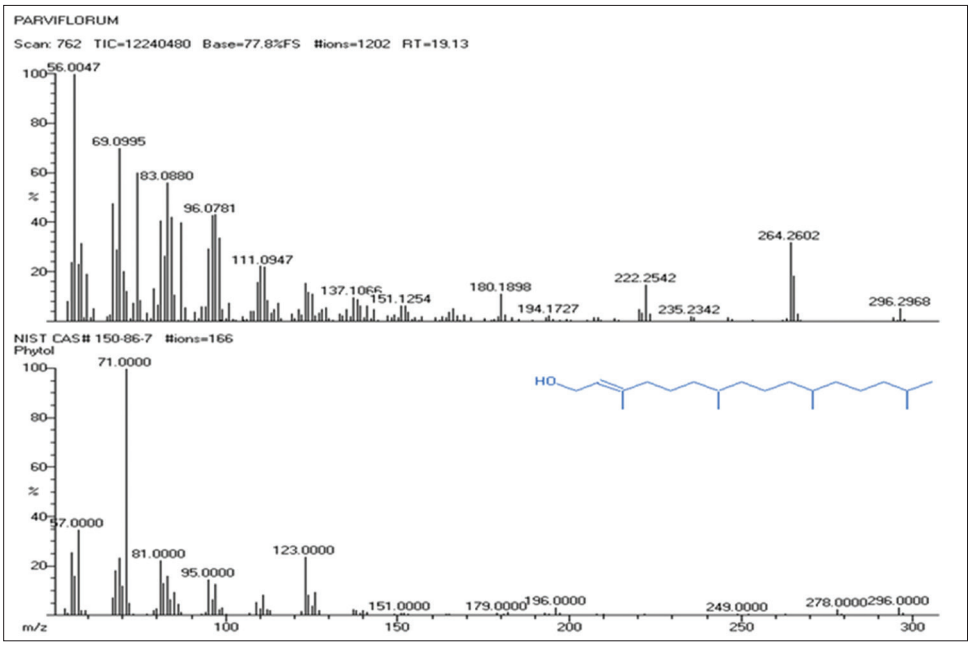

Fig. 2: Mass spectrum of phytol

Table 2: Phytocomponents identified in the ethanolic extract of *C. parviflorum* by GC-MS

S. No.	RT	Name of the phytocompounds	Biological activity*
1	17.4	4',5,7-trihydroxyisoflavone	Antioxidant, anthelmintic, anticancer, atherosclerosis
2	18.3	Estra-1,3,5 (10)-trien-17a'-ol	Androgenic alopecia (hair loss)
3	19.13	Phytol	Antimicrobial, anticancer, anti-inflammatory, hepatoprotective
4	19.35	Octadecanoic acid, methyl ester	Antioxidant, Antimicrobial.
5	19.98	(E)-9-octadecenoic acid, ethyl ester	Antioxidant, anti-inflammatory
6	21.17	Eicosanoic acid, methyl ester	Alpha-glucosidase inhibitors
7	22.62	(Z)-9-octadecenoic acid, butyl ester	Antimicrobial
8	24.87	Corynan-17-ol, 18,19-didehydro-10-methoxy-acetate (ester)	Antacids, anti-inflammatory

*Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database

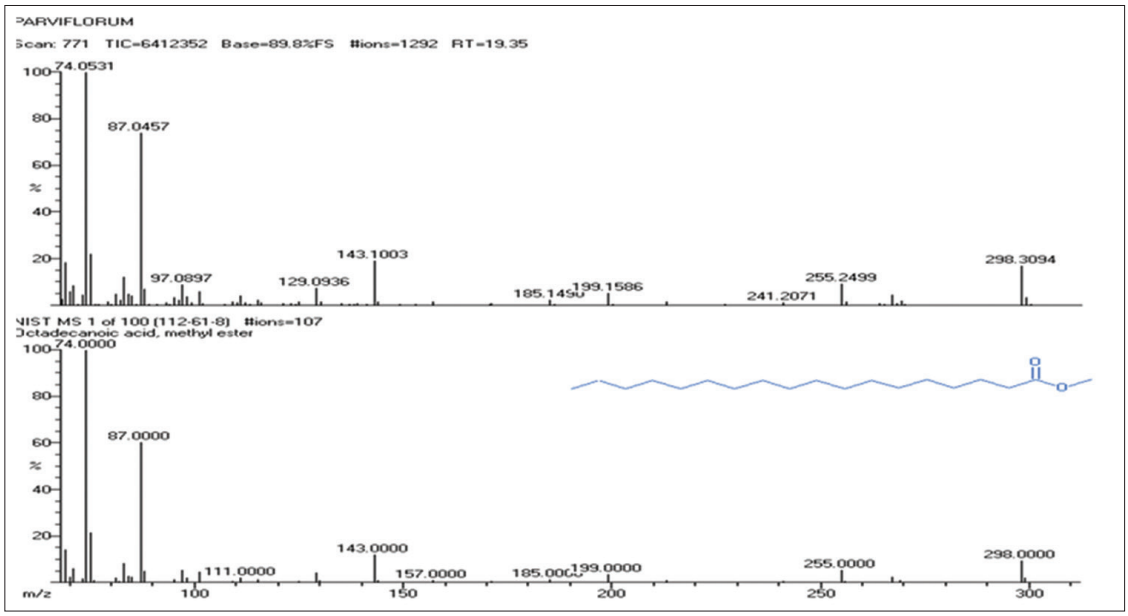

Fig. 3: Mass spectrum of octadecanoic acid, methyl ester

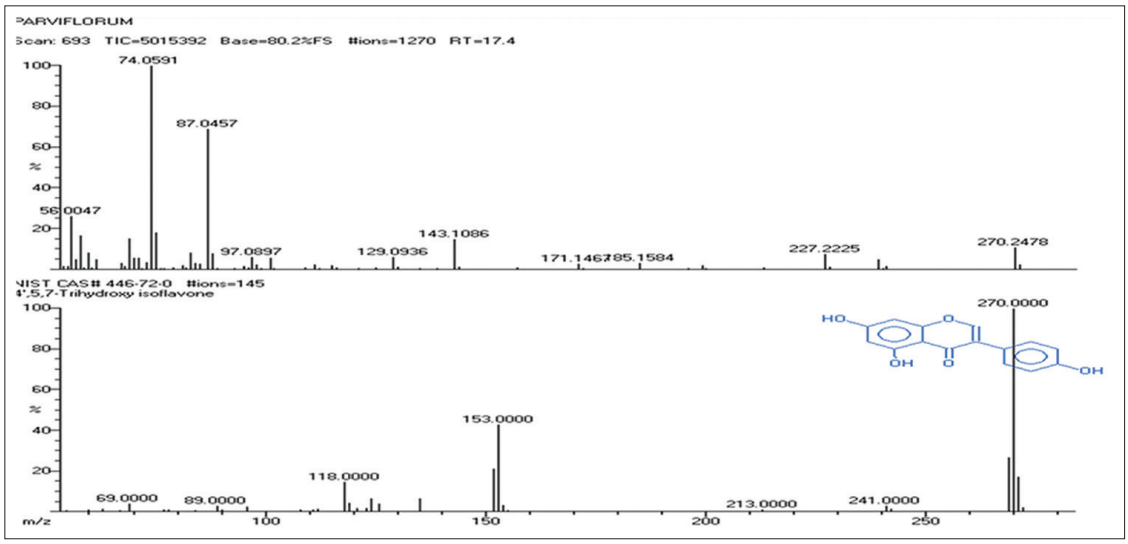

Fig. 4: Mass spectrum of 4',5,7-trihydroxyisoflavone

CONCLUSION

In the present study, 8 phytoconstituents were identified from ethanol extract of the *C. parviflorum* by GC-MS analysis. Phytochemical analysis of *C. parviflorum* leaves extracts revealed the presence of various biochemical compounds such as flavonoids, alkaloids, terpenoids, steroids, and phenolic compounds. This plant-derived bioactive compounds used as source of antibiotic, antioxidant, anti-inflammatory, anticancer properties, and pharmaceutical industries used for drug formulation [18]. This plant is widely used in traditional medicinal system of India. Due to medicinal properties, there is enormous scope for future research on *C. parviflorum,* and further clinical and pharmacological investigation should be conducted to investigate unexploited potential of this plant.

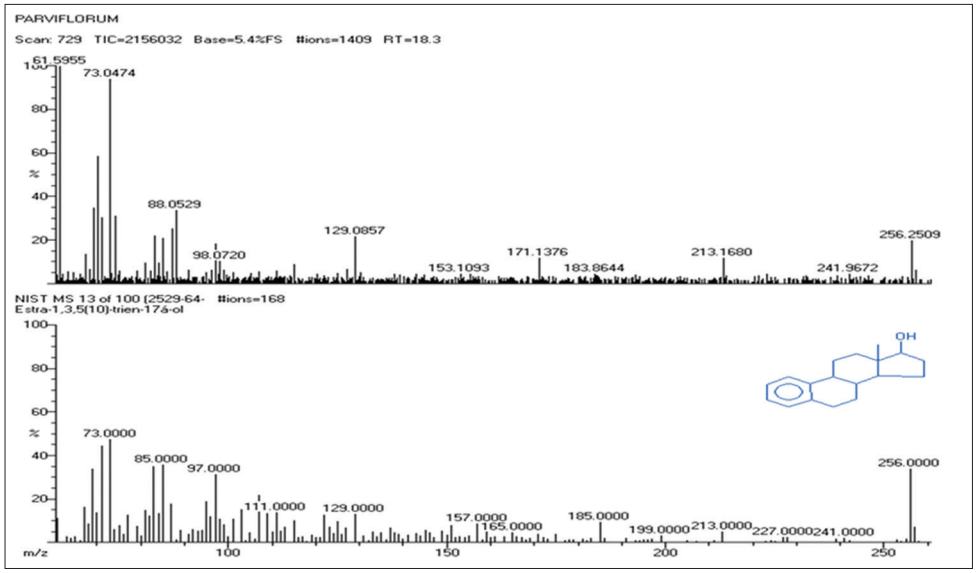

Fig. 5: Mass spectrum of estra-1,3,5(10)-trien-17a'-ol

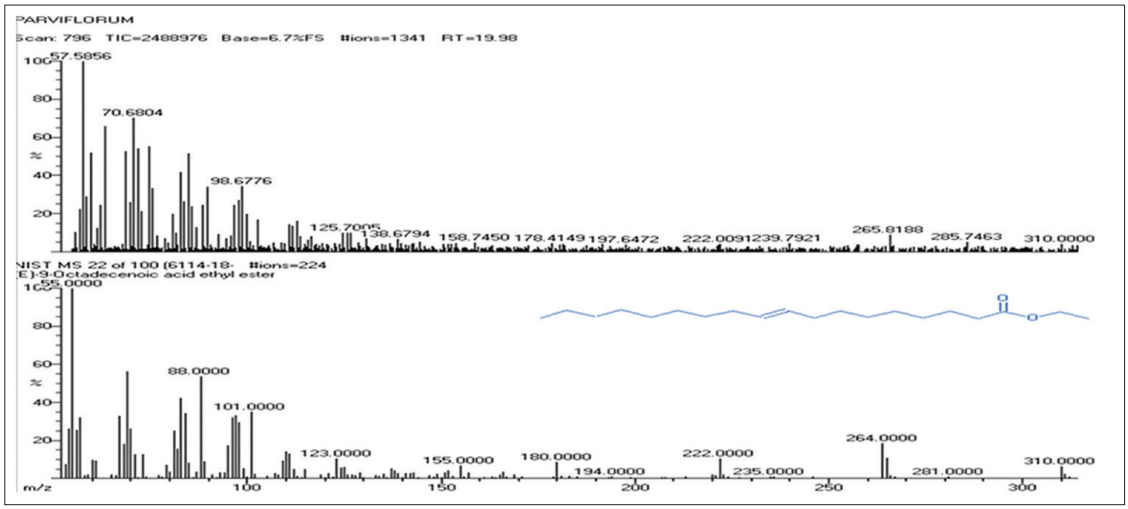

Fig. 6: Mass spectrum of (E)-9-octadecenoic acid, ethyl ester

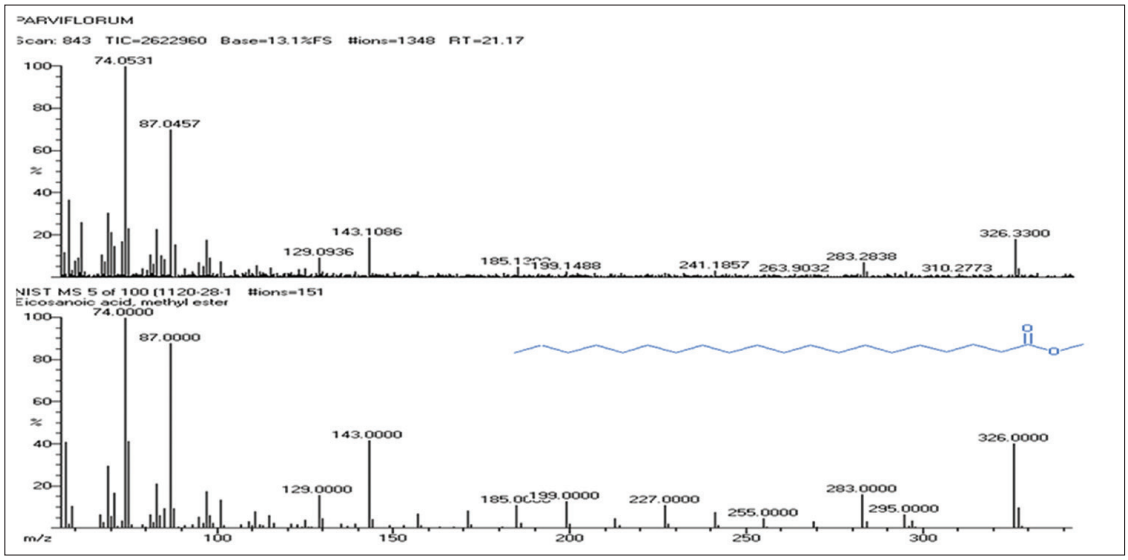

Fig. 7: Mass spectrum of eicosanoic acid, methyl ester

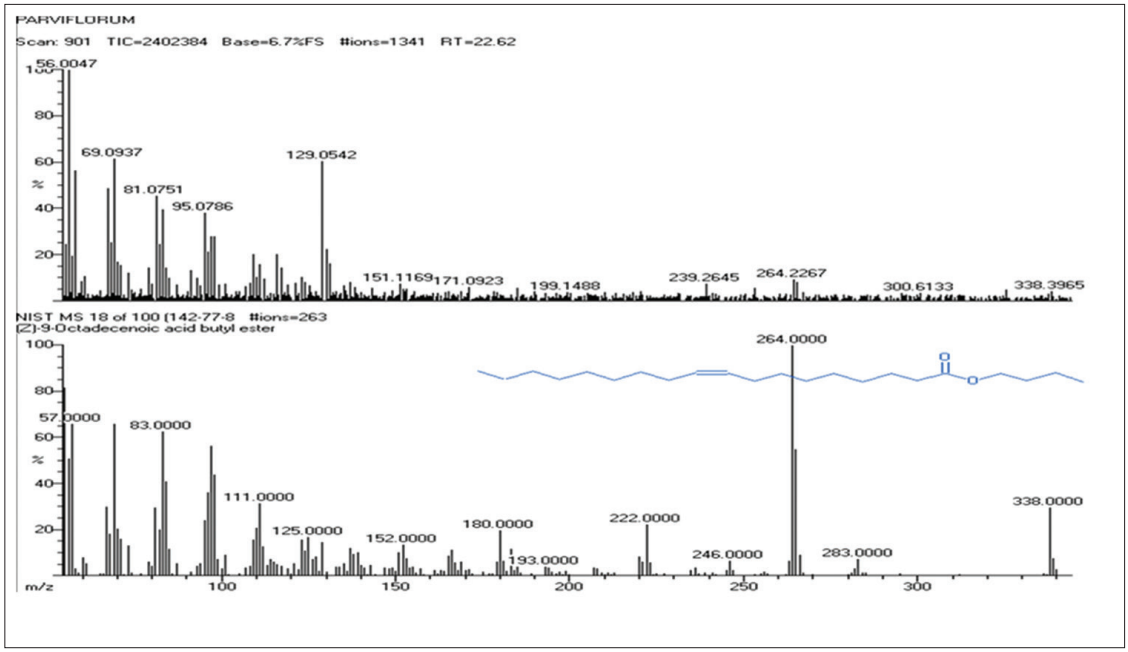

Fig. 8: Mass spectrum of (Z)-9-octadecenoic acid, butyl ester

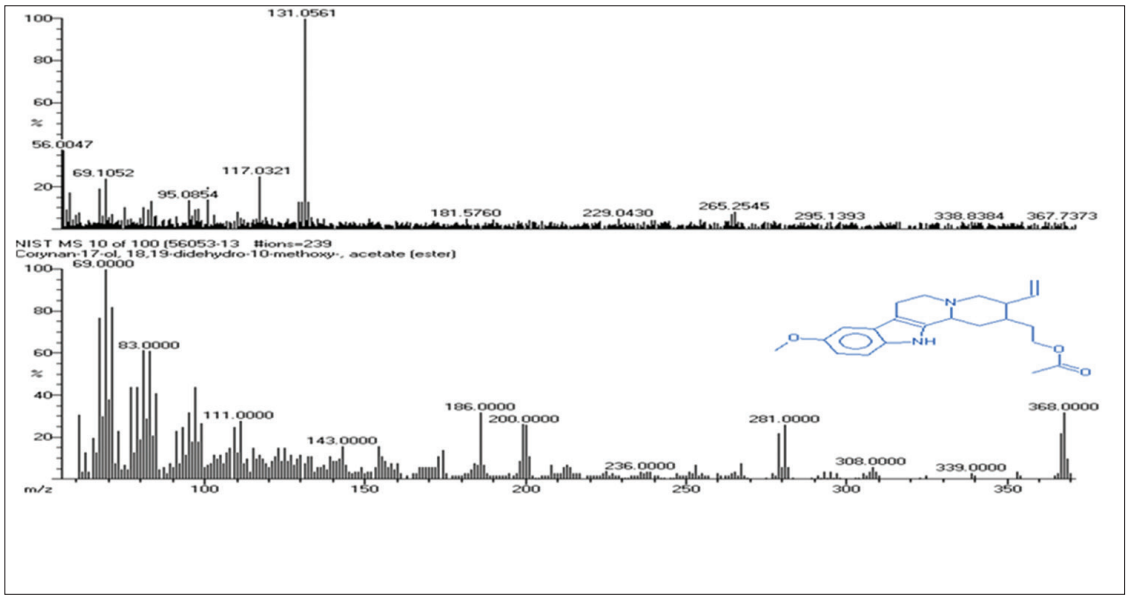

Fig. 9: Mass spectrum of corynan-17-ol, 18,19-didehydro-10-methoxy-acetate

ACKNOWLEDGMENT

The authors wish to thank Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Madras (IITM), Chennai, for GC-MS analysis.

REFERENCES

1. Ayyanar M, Sankarasivaraman K, Ignacimuthu S. Traditional herbal medicines used in the treatment of diabetes among two major tribal groups in South Tamil Nadu, India. Ethnobot Leaf 2008;12:276-80.
2. Kala CC, Mallikarjuna K, Arun P. Qualitative phytochemical analysis of seed and leaf callus extracts of *Canthium parviflorum* Lam. Guntur district, Andhra Pradesh. Int J Pharm Bio Sci 2012;3(4):177-82.
3. Mohideen S, Ilavarasan R, Hemalatha S, Anitha N, Sasikala E. Wound healing and diuretic activities of *Canthium parviflorum* Lam. Nat Prod Sci 2003;9:102-4.
4. Kumar TS, Shanmugam S, Palvannan T, Bharathikumar VM. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. Leaves. Nat Prod Rad 2008;7:122-6.
5. Suresh K, Kotaimuthu R, Norman TS, Kumuthakalavalli R, Simon SM. Ethnomedicinal study of medicinal plants used by Malayali tribals in Kolli hills of Tamil Nadu, India. Int J Res Ayurveda Pharm 2011;2(2):502-8.
6. Kumar CS, Raj SM, Kumar SV, Chandu AN, Karthikeyan D. Antimicrobial and anti-HIV activity of extracts of *Canthium coromandelicum* (Burm.f.) Alston leaves. J Pharm Res 2013;6:1-7.
7. Wvrtdg B, Ekanayake ER, Balasubramanium K. The hypocholesterolaemic effect of *Canthium coromandelicum* leaf on wistar rats. Vidyodaya J Sci 2009;14:105-9.
8. Mohan SC, Sasikala K, Anand T. Antimicrobialand wound healing potential of *Canthium coromandelicum* leaf extract – A preliminary study. Res J Phytochem 2014;8:35-41.
9. Sofowora AE. Medicinal Plants and Traditional Medicines in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books; 1993. p. 289.
10. Trease GE, Evans WC. Pharmacognosy. London: Brailliar Tiridel Canadian MacMillan Publishers; 1989. p. 35-8.

11. Anand T, Gokulakrishnan K. Phytochemical analysis of *Hybanthus enneaspermus* using UV, FTIR and GC-MS. IOSR J Pharm 2012;2(3):520-4.
12. Bandele OJ, Osheroff N. Bioflavonoids as poisons of human topoisomerase II alpha and II beta. Biochemistry 2007;46(20):6097-108.
13. Lutz WK, Tiedge O, Lutz RW, Stopper H. Different types of combination effects for the induction of micronuclei in mouse lymphoma cells by binary mixtures of the genotoxic agents MMS, MNU, and genistein. Toxicol Sci 2005;86(2):318-23.
14. Jin Y, Wu H, Cohen EM, Wei J, Jin H, Prentice H, *et al.* Genistein and daidzein induce neurotoxicity at high concentrations in primary rat neuronal cultures. J Biomed Sci 2007;14(2):275-84.
15. López-Lazaro M, Willmore E, Austin CA. Cells lacking DNA topoisomerase II beta are resistant to genistein. J Nat Prod 2007;70(5):763-7.
16. Schmidt F, Knobbe CB, Frank B, Wolburg H, Weller M. The topoisomerase II inhibitor, genistein, induces G2/M arrest and apoptosis in human malignant glioma cell lines. Oncol Rep 2008;19(4):1061-6.
17. Krishnaveni M, Nandhini N, Dhanalakshmi R. GC-MS analysis of phytochemicals, fatty acids and antimicrobial potency of dry Christmas lima beans. Int J Pharm Sci Rev Res 2014;27(2):63-6.
18. Prabhu TP, Panneerselvam P, Selvakumari SI, Sivaraman D. *In vitro* and *in vivo* anticancer activity of ethanolic extract of *Canthium parviflorum* Lam on DLA and hela cell lines. Int J Drug Dev Res 2011;3:280-5.