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Essential oils analysis of pseudobulbs of *Crepidium acuminatum* (D.DON) SZLACH by GC-MS

Mamta Arora[1*], Anupama Mahajan [2], Jaspreet K. Sembi[3]

[1] Research scholar of I.K. Gujral Punjab Technical University, Jalandhar,Faculty of Biotechnology, ASBASJSM College, Bela Rupnagar, Punjab, India
[2] Assistant Professor, Department of Biotechnology, SUSCET Tangori, Mohali Punjab,India
[3] Assistant Professor, Department of Botany, Panjab University, Chandigarh,India

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ABSTRACT

Objective: To analyse Essential oils of *Crepidium acuminatum* (D.Don) Szlach. **Methods**: DCM Extracts were prepared for Essential oil analysis. GC-MS was used for perusal. **Results:** In the present study, 6 phytoconstituents have been identified from the DCM pseudobulbs extract of *Crepidium acuminatum D. Don Szlach* by GC-MS analysis. The results showed that DCM extract contains mainly essential oils which are caryophyllene 74.89% ; Eugenol 5.56%; Humulene 5.48% ; phenol, 2,4 bis (1,1 dimethylethyl) 5.57%; Caryophyllene oxide 5.33%; 2,5 Octadecadiynoic acid, methyl ester 3.16%. Analysis and identification of the presence of the compound in the extract were done by using the database of the National Institute of Standards and Technology (NIST) library. **Conclusions**: Out of various compounds analysed Eugenol has already been reported whereas Caryophyllene, Caryophyllene oxide, Humulene, Phenol, 2,4bis (1, 1dimethylethyl) and 2,5 Octadecadiynoic acid, methyl ester are being reported first time. These compounds have immense medicinal potential. Thus, it found to possess significant phytonutrients, which attribute to its medicinal worth.

KEY WORDS: *Crepidium acuminatum* (D. Don) Szlach, GC-MS, Phytoconstituents, Medicinal uses, Essential oils

Introduction

Crepidium acuminatum (D.Don) Szlach is an important a medicinal plant. [1] The pseudobulbs which are swollen underground stem base of the plant constitute the drug *'Jeevak'* used in Ayurvedic formulation 'Chawyanprash' (Ayurvedic rejuvenating tonic) and in other formulations such as Astavarga churna, Rasayan, Ghrita, Gutika, Taila, Agada.[2] It is considered as refrigerant, aphrodisiac, febrifuge and is useful in haematemesis, fever, seminal weakness, burning sensation, dipsia, emaciation, tuberculosis and general debility [3,4,5]. Realizing the medicinal potential of the plant a no. of reports showcase their in vitro propagation [6]

**Correspondence*
Mamta Arora
Research scholar of I.K. Gujral Punjab Technical University, Jalandhar, Faculty of Biotechnology, ASBASJSM College, Bela Rupnagar, Punjab,India
E Mail: mamtaarora.2007@rediffmail.com

A little phytochemical work has been carried out. One sterol namely β-Sitosterol ,Two sugars glucose and rhamnose, Cetyl alcohol, Choline and diterpenes along with [3,4,5]. Piperitone, Citronellal, Eugenol, Limonene, 1,8-Cineole, p-Cymene, o-Methylbatatasin, have been reported [7] Therefore, the present study was focussed to study essential oil constituents of the plant by using gas chromatography mass spectrometry (GC-MS) .

Materials and methods

Chemicals and Reagents: Hexane, Dichloromethane, Methanol, Sulphuric acid, Sodium bicarbonate, Sodium sulphite of analytical grade.

Plant collection and authentication: Pseudobulbs were purchased from Chandigarh and authenticated by NISCAIR New Delhi.

Preparation of plant extract: Dried hexane extract (2.5 gm) was taken which was prepared by Soxhlet apparatus.10 ml methanol was added. Poured 5-6 drops of sulphuric acid and heated at 57-80 °C for 12

hrs. After Complete removal of methanol, added water and neutralised with Sodium bicarbonate. Then extracted with dichloromethane. After extraction washed with water 3 times and dried with sodium sulphite. Concentrated the extract and proceeded for GC-MS.

GC-MS method: Analysis was performed on a GC-MS TSQ 8000 by Thermo Fisher Scientific Inc., Waltham, USA equipped with a split/split less injector. Separations were achieved using a TG 5MS (30m X 0.25mm, 0.25µm) column. Helium was used as the carrier gas at flow rates of 1 ml/min. The injector temperature was 250°C. The oven temperature was programmed at 80°C for a hold of 2 min and increased to 260°C at a rate of 10°C/min and hold at the final temperature for 10 min. GCMS is equipped with NIST Library . MS spectra were obtained at range width m/z 50-700, Run time 29.09, Injection Volume 1ul , Scans 3463. Peaks were identified by comparing data with NIST Library.

Results and Discussion

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time [RT]. In chromatogram height of the peak shows the relative concentrations of the components present in the sample. The mass spectrometry MS analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data Library. Gas Chromatogram for DCM extract has been presented in figure 1 compounds were identified in DCM extract of pseudobulbs of *C. acuminatum*. The active compounds with their retention time (RT), molecular formula (MF), concentration (Area %) and Medicinal uses are presented in Table 1. The prevailing compounds are presented separately. Figs.2, 3, 4, 5, 6 and 7show the mass spectrum and structures of important constituents of DCM extract. Table 1 also illustrates the medicinal benefits of these phytoconstituents. Therefore GC-MS analysis is the great tool towards understanding the nature of active volatile compounds. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

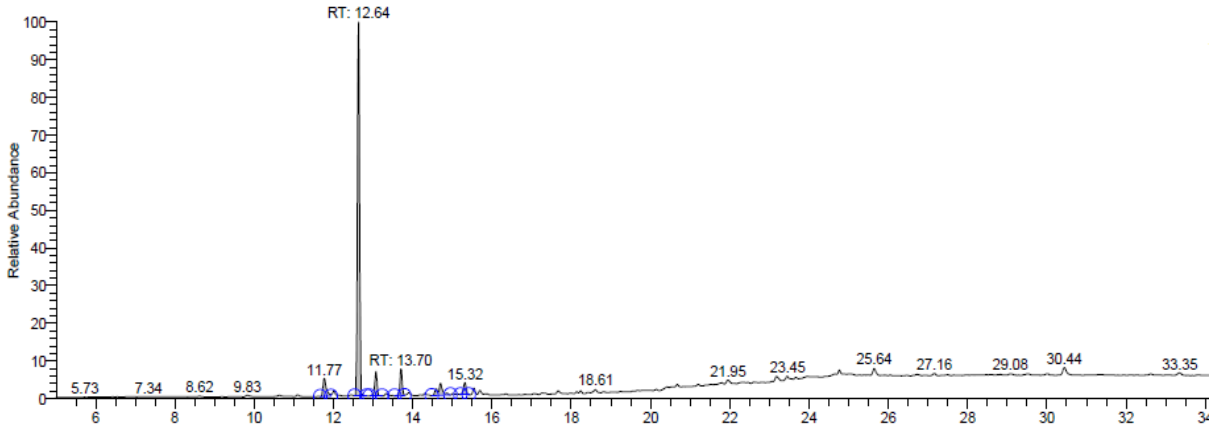

Fig 1: GC-MS Chromatogram of DCM extract of pseudobulbs *of C. acuminatum*

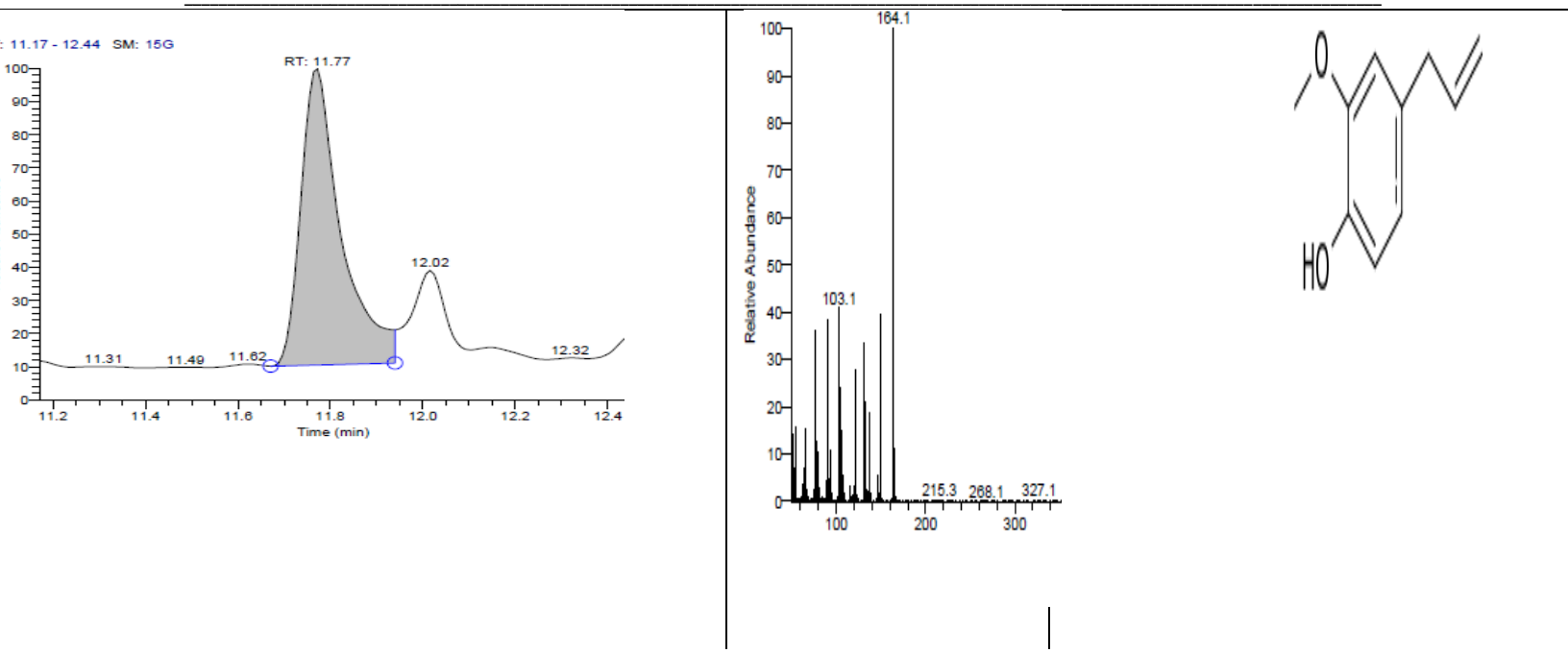

Fig 2:Gas chromatogram of eugenol along with fragmentation pattern and structure

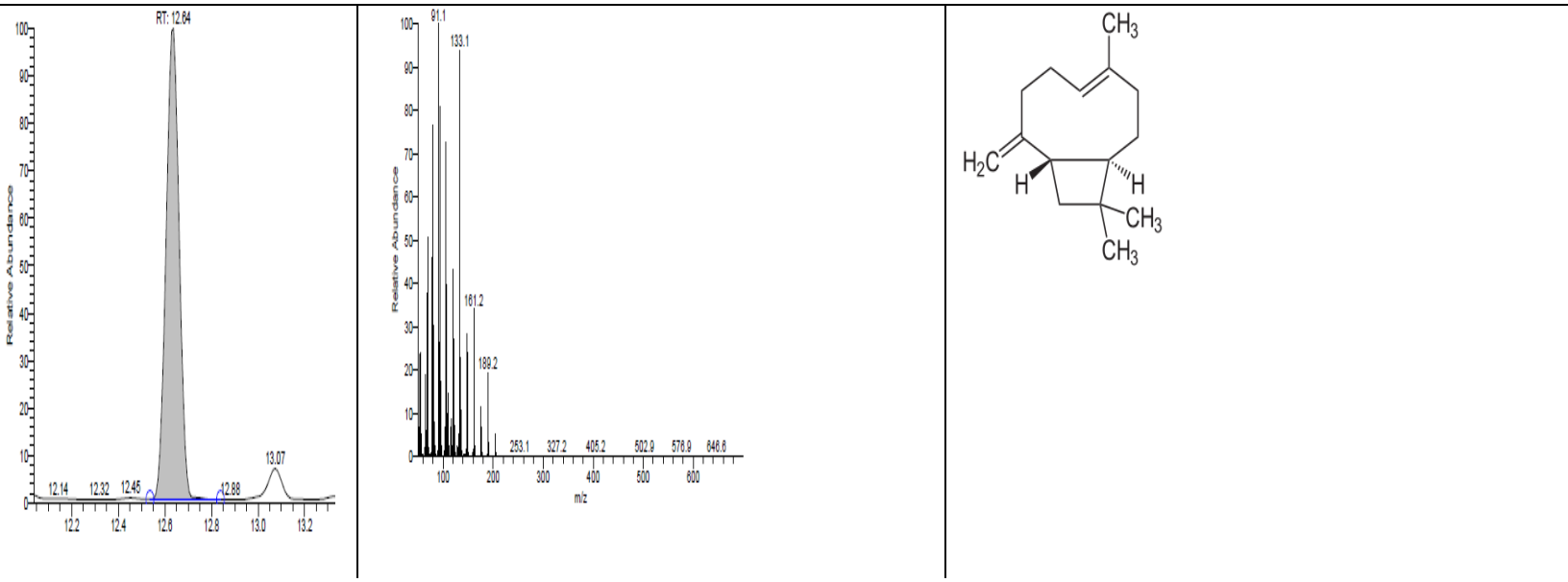

Fig 3: gas chromatogram, fragmentation pattern and structure of caryophyllene

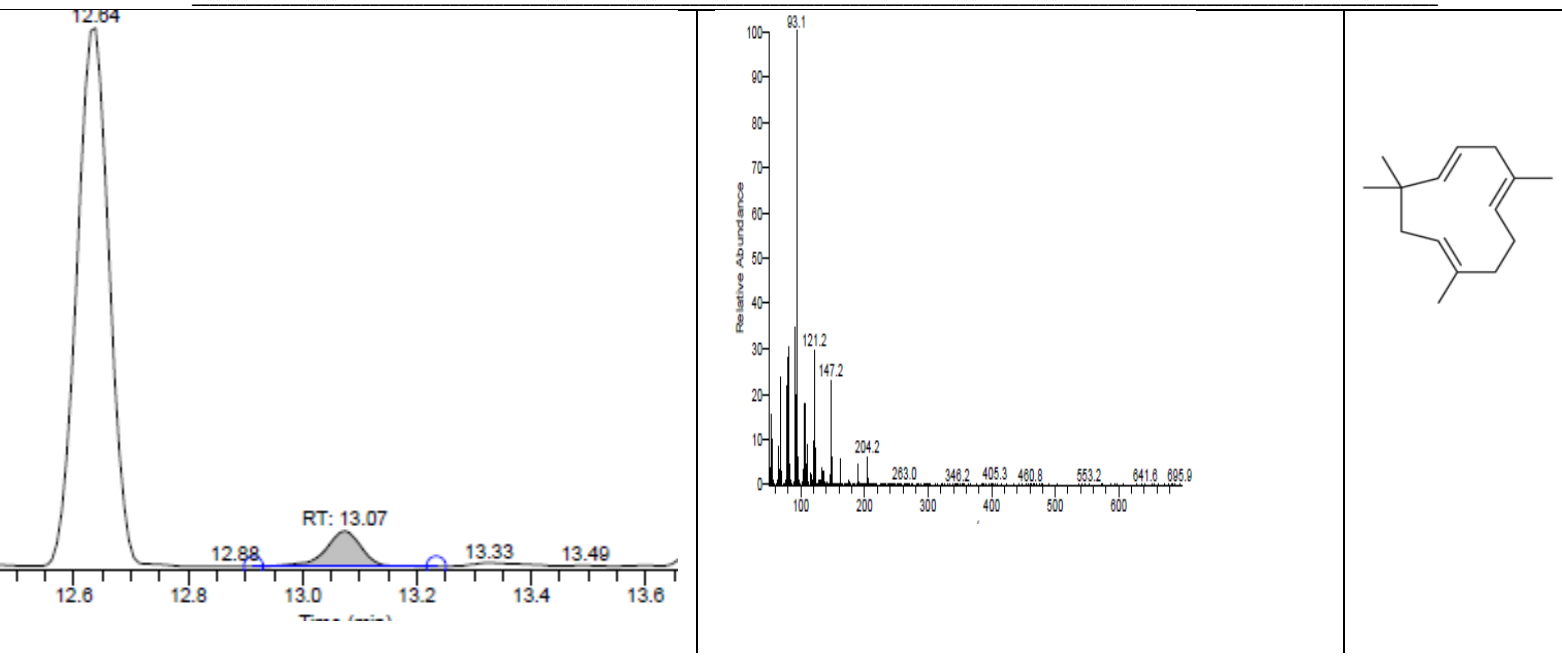

Fig 4:gas chromatogram, fragmentation pattern and structure of humulene

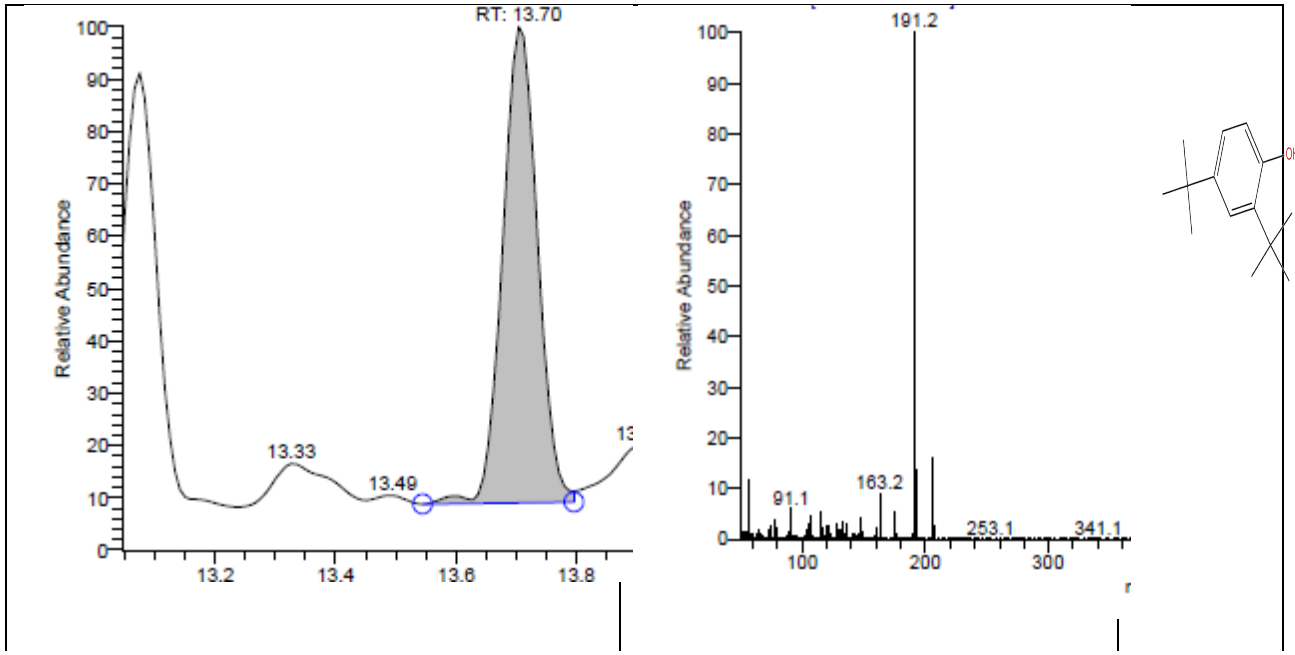

Fig 5:Gas chromatogram, fragmentation pattern and structure of phenol, 2,4bis(1,1dimethylethyl)

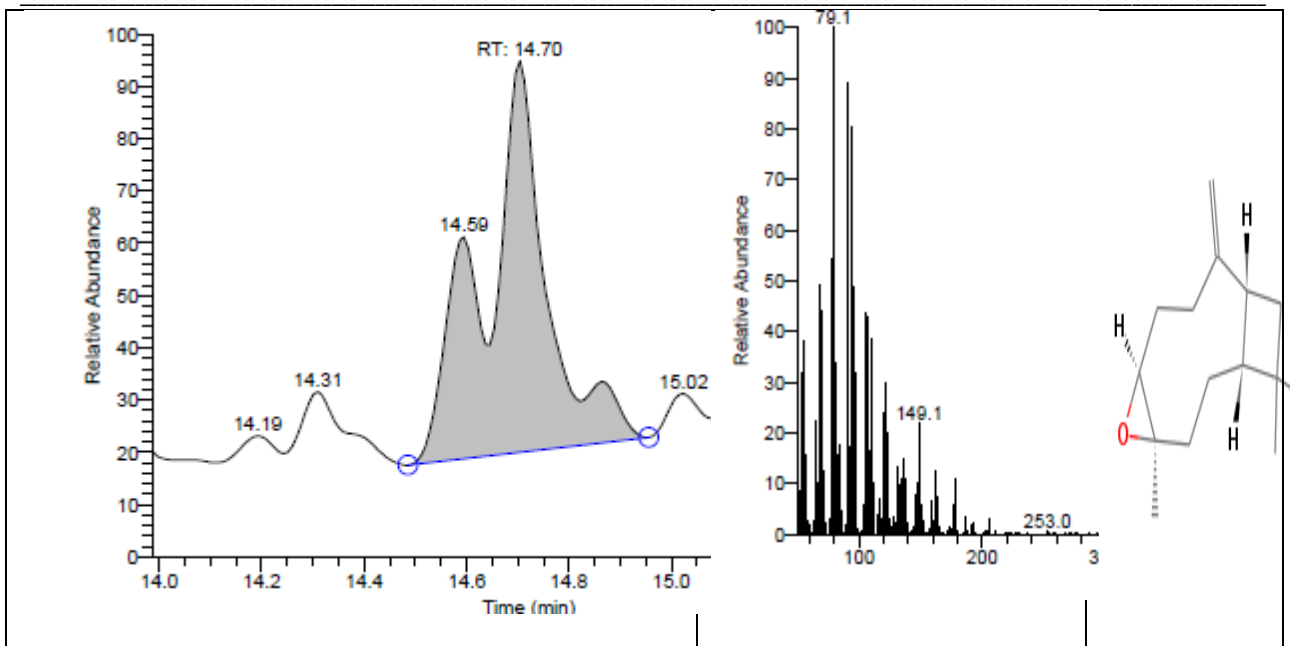

Fig 6: Gas chromatogram, fragmentation pattern and structure of caryophyllene oxide

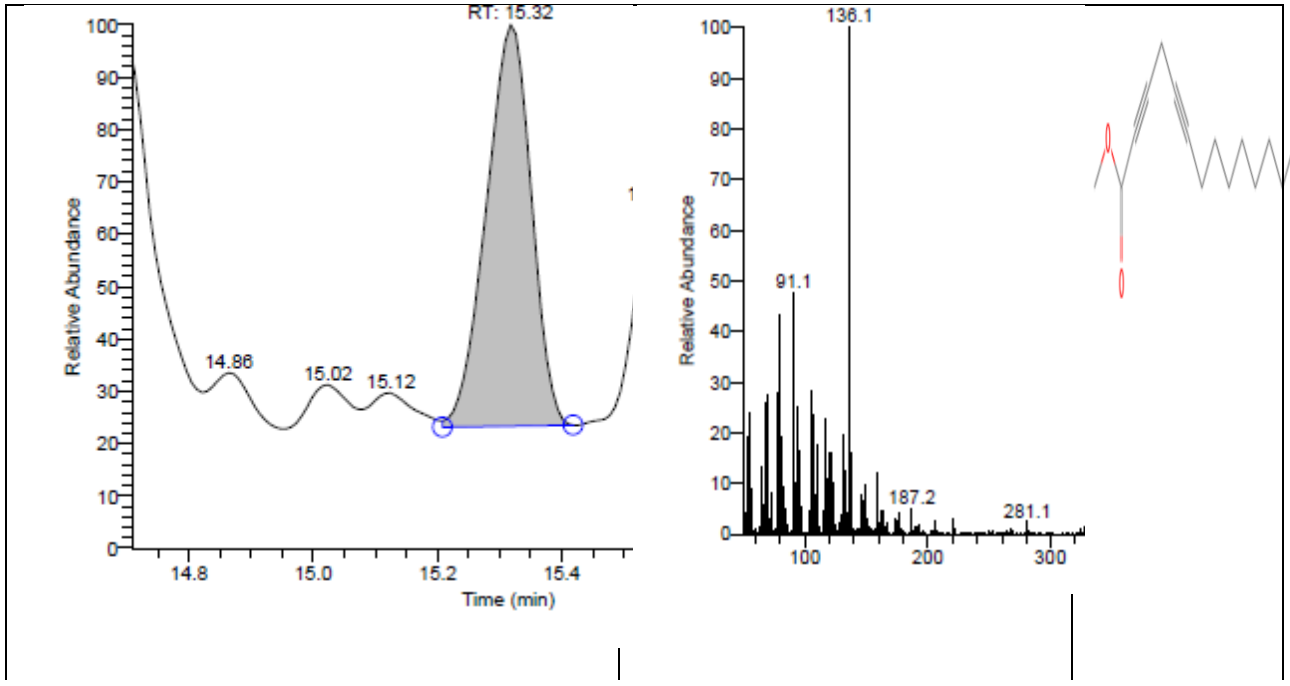

Fig 7:Gas chromatogram, fragmentation pattern and structure of 2,5octadecadiynoic acid, methyl ester

Table 1: Essential oils identified from DCM Extract of pseudobulbs of *C. acuminatum* along with medicinal uses

S.No.	Name of Compound	Molecular formula(MF)	CAS	RT	Area %	Medicinal use
1.	Eugenol	$C_{10}H_{12}O_2$	97-53-0	11.77	5.56	anesthetic and analgesic, antioxidant, anti-inflammatory and cardiovascular properties,[8]
2.	Caryophyllene	$C_{15}H_{24}$	87-44-5	12.64	74.89	antiinflammatory , Antinociceptive, neuroprotective, anxiolytic ,antidepressant and anti-alcoholism activity[9-13]
3.	Humulene	$C_{15}H_{24}$	6753-98-6	13.07	5.48	Anti inflammation [14,15]
4.	Phenol, 2,4bis(1,1dimethylethyl)	$C_{14}H_{22}O$	96764	13.70	5.57	Antimicrobial[16]
5.	Caryophyllene oxide	$C_{15}H_{24}O$	1139-30-6	14.70	5.33	Antifungal, anti inflammatory[17,18]
6.	2,5Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	57156-91-9	15.32	3.16	Anti inflammatory[19]

Conclusion

The present phytochemical study of *Pseudobulbs of C.acuminatum* was studied for the first time using DCM extract. In fact, this is the first available information about the study of essential oils of *C. acuminatum by GC-MS.* As is clear from results this plant possess various potent pharmaceutical compounds and is recommended as a plant of phyto pharmaceutical importance. The presence of various pharmacological compounds has justified the use of *C. acuminatum* pseudobulbs for various Ayurvedic formulations by traditional practitioners. Hence, further studies on isolation and identification of individual constituent are greatly needed. It is also strongly recommended to explore its pharmacological values at the molecular level with the help of various advanced biotechnological techniques.

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Abbreviations

DCM: Dichloromethane, RT: Retention time, CAS: Chemical Abstracts Service Registry Number

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