GC-MS Analysis of Organic Extracts of *Cymbidium aloifolium* (L.) Sw. (Orchidaceae) Leaves from Eastern Ghats of India

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Abstract

Phytochemical constituents of various leaf extracts of *Cymbidium aloifolium* (L.) Sw. have been studied using gas chromatography–mass spectrometry (GC-MS) data. The dried leaves powder were extracted with methanol at room temperature by using a Soxhlet extractor. The methanol crude extract of *Cymbidium aloifolium* was again taken with hexane, chloroform and ethyl acetate. The analysis was carried out on an Agilent GC-MS equipped with a HP-5MS fused capillary column; the compounds are separated using helium as a carrier gas at a constant flow of 1 ml/min. A qualitative analysis of various organic extracts showed eight different photochemical compounds, namely n-hexadecanoic acid; 9,12-octadecadienoic acid (Z,Z); 9,12,15-octadecatrienoic acid, (Z,Z,Z); octadecanoic acid; phytol; 2-butyne; 2-cyclopenten-1-one; and 1,4-benzenedicarboxylic acid. Most of the identified compounds are biologically important. This study offers a platform of using *Cymbidium aloifolium* leaves as herbal alternatives for various diseases. The compounds reported in this investigation also have some phylogenetic significance.

Keywords

Cymbidium aloifolium · qualitative analysis · Orchidaceae · GC-MS analysis

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26.1 Introduction

Orchids are scientifically a significant and commercially important group of flowering plants under the family Orchidaceae. It includes about 788 genera (Mabberly 1997) and 25,000–35,000 species (Dressler 1993; Hossain 2011). In India, orchids are grown in high altitude areas of the Himalayas, Western Ghats and Eastern Ghats. Nearly 1129 species and 184 genera (Jalal et al. 2008) were found in India, of which 190 species were recorded in Andhra Pradesh (Reddy et al. 2005). Many orchids having cultural value and are used as herbal medicines and food supplements by tribes in different parts of the world (Khasim and Rao 1999). Phyto-constituents are responsible for the medicinal activity of the plants, and these are classified into primary and secondary metabolites. The screening of active compounds from orchids has led to the invention of novel drugs, and they have efficient protection against various diseases (Dandekar et al. 2015). In recent years, there has been tremendous progress in the study of organic compounds from the medicinal plants and orchids (Keerthiga and Anand 2015; Kalaiarasan and Ahmed John 2011). The combination of gas chromatography (GC) and mass spectrum (MS) is an ideal technique for the qualitative and quantitative analysis of volatile and semi-volatile compounds (Nishaa et al. 2013).

26.2 Ethnobotany and Traditional Use of *Cymbidium Aloifolium*

Traditionally, *Cymbidium aloifolium* has been used in various parts of the world as folk medicine. The tribal community of North East India use the seeds for healing wounds (Medhi and Chakrabarti 2009). In Bangladesh, the plant is used as anti-inflammatories and anticancer agents, while in South India, it is used as emetic and purgative. The leaves of the plant are reported to cure earache, cuts and wounds (Sharief Ahmed Makul et al. 2007). It is an epiphytic orchid distributed widely in Eastern Ghats of Andhra Pradesh. The tribal community of East Godavari district, locally called as Pedda vajanika, are using leaf juice to cure earache. The existing literature indicates that tribal communities of different regions use various phyto-preparations of this plant to cure diseases. No investigation on *Cymbidium aloifolium* has so far been undertaken to provide enough scientific data in favour of reported traditional use. Traditional use varies among local practitioners for boils, earache, vomiting, fever, wounds, paralysis, digestive disorders, sores etc., (Nongdam and Chongtham 2011; Medhi and Chakrabarti 2009; Sharief Ahmed Makul et al. 2007). The various organic extracts of this plant has recently been reported to have antimicrobial and antibacterial activity (Radhika et al. 2013). As a part of the endeavor to search for therapeutic properties of *Cymbidium aloifolium*, we herein presented the GC-MS analysis of various extracts from the leaf.

26.3 Sample Collection and Preparation of Crude Extracts

Leaves of *Cymbidium aloifolium* (L.) Sw. were collected from the Pedda konda sacred grove of the East Godavari district, Andhra Pradesh, India, in January 2015. The specimen was identified with the help of regional floras, and the voucher specimen was deposited at Acharya Nagarjuna University Botany Herbarium (ANUBOTH 11123), Guntur, and Andhra Pradesh, India.

The fresh green leaves of *Cymbidium aloifolium* growing on *Borasus flabellifer* host plant were collected from their natural habitat and packed in polyethylene bags. The leaf samples were washed thoroughly in running tap water to remove soil particles. The plant samples were shade dried and ground into fine powder and stored in air-tight polythene bags until use. The dried leaves powder (150 g) was extracted in methanol at room temperature by using a Soxhlet extractor for 12–18 h. Crude extracts were prepared according (Amzad Hossain 2011). The crude methanol extracts were evaporated by a vacuum rotary evaporator (Buchi Labortech Ag, model l, R-215) under reduced pressure. The crude extract was diluted with water and extracted successively with n-hexane, ethyl acetate and chloroform. The extracts were filtered using Whatman no. 41 filter paper to obtain particle-free extract. The residue was re-extracted twice with solvents (E-Merk) used to obtain extracts. The 2 µl of each sample was injected into the GC-MS instrument for phytochemical analysis.

26.4 GC-MS Equipment

The GC-MS analysis of various crude extracts from leaves was performed using an Agilent GC-MS (Model-5975c inert MSD with Triple-Axis Detector, USA) equipped with an HP-5MS fused capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Inert helium gas was used as a carrier gas at a constant flow rate of 1ml/1min. An aliquot of 2 µl of various extract solution of the sample was injected into the column with injector temperature of 250 °C. Mass transfer line and injector temperature were set at 220 °C and 300 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. In gas chromatography–mass spectroscopic detection, an electron ionisation system with ionisation energy of 70 ev was used, and the detector was operated in scan mode from 40 to 500 amu (atomic mass unit). The total running time was 55.3 min.

Interpretation of GC-MS data was carried out using the National Institute of Standard and Technology (NIST) database library 2.0 version, which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

26.5 GC-MS Analysis of Organic Extracts

Leaves of *C. aloifolium* are linear-oblong, obtuse, 50 cm long and 3 cm broad. Inflorescence arises from the base of the stem tuber, and raceme is 50 cm long. The various plant extracts analysed using GC-MS had led to the identification of eight different organic compounds, and their retention time (RT) and concentration (peak area %) were shown in Table 26.1. The typical gas chromatograms of chemical constituents of ethyl acetate extract (Fig. 26.1), chloroform extract (Fig. 26.2) and hexane extracts (Fig. 26.3) have been shown in repective figures. Molecular formula, molecular weight, the nature of the compounds, and biological activity are presented in Table 26.2. A total of six chemical compounds were identified in ethyl acetate and chloroform extract each; five were traced in hexane extract. The compounds like n-hexadecanoic acid (Fig. 26.4c); octadecanoic acid (Fig. 26.5b); 9,12-octadecadienoic acid (Z,Z) (Fig. 26.4d), 9,12,15-octadecatrienoic acid, (Z,Z,Z) (Fig. 26.5a) and phytol (Fig. 26.5c) were found in all extracts. However, phytol was not detected in ethyl acetate extract. 2-butyne (Fig. 26.4a) and 2-cyclopenten-1-one (Fig. 26.4b) are found in ethyl acetate extract only, while 1,4-benzenedicarboxylic acid, bis(2-hydroxyethyl) ester (Fig. 26.5d) is detected in chloroform extract only.

Table 26.1 Chemical composition of different extracts of *Cymbidium aloifolium*

Extracts	Name of the compound	Retention time (min)	Peak area%
	n-hexadecanoic acid	50.409	22.6956
	Phytol	52.538	4.5813
n-hexane extract	9,12-octadecadienoic acid (Z,Z)	52.803	28.5815
	9,12,15-octadecatrienoic acid, (Z,Z,Z)	52.872	38.3769
	Octadecanoic acid	53.11	5.7647
	2-butyne	13.623	4.5256
	2-cyclopenten-1-one	17.907	6.1662
Ethyl-acetate extract	n-hexadecanoic acid	50.399	20.4864
	9,12-octadecadienoic acid (Z,Z)	52.792	24.1308
	9,12,15-octadecatrienoic acid, (Z,Z,Z)	52.861	37.3557
	Octadecanoic acid	53.105	7.3353
	n-hexadecanoic acid	50.388	21.0246
	Phytol	52.533	3.5791
	9,12-octadecadienoic acid (Z,Z)	52.776	24.754
Chloroform extract	9,12,15-octadecatrien-1-ol, (Z,Z,Z)	52.845	34.2954
	Octadecanoic acid	53.099	6.6246
	1,4-benzenedicarboxylic acid, bis(2-hydroxyethyl) ester	53.428	9.7223

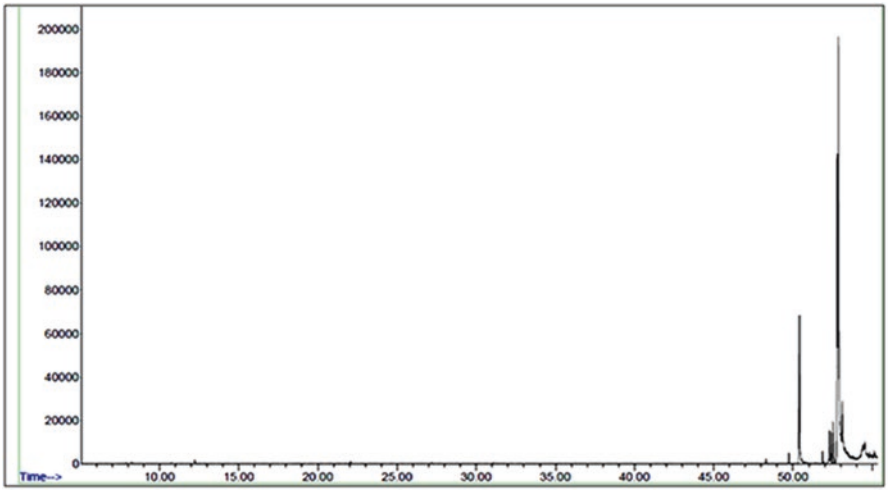

Fig. 26.1 A typical gas chromatogram of the chemical constituents of ethyl acetate extract

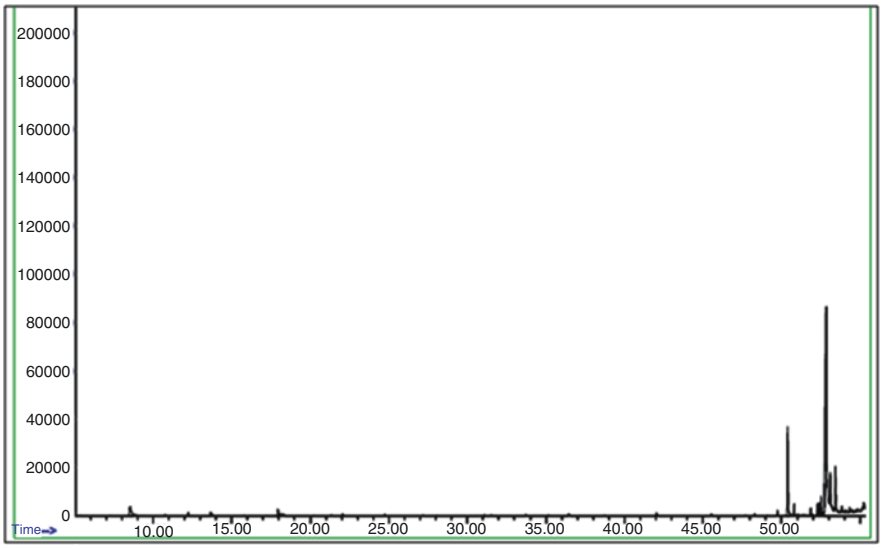

Fig. 26.2 A typical gas chromatogram of the chemical constituents of chloroform extract

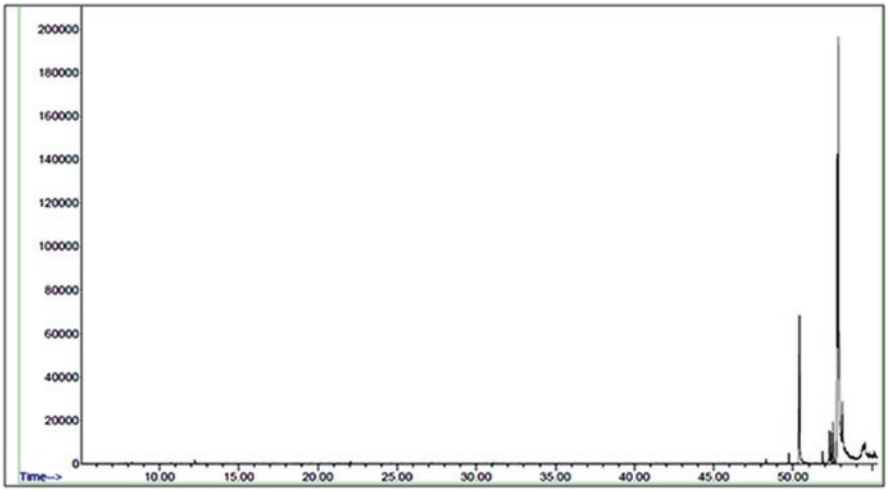

Fig. 26.3 A typical gas chromatogram of the chemical constituents of hexane extract

26.6 Orchid Chemicals and Their Importance

In the present study, the GC-MS analysis of the various organic extracts of *Cymbidium aloifolium* showed the presence of eight compounds. In terms of percentage amounts 9, 12, 15-octadecatrienoic acid (Z, Z, Z); 9, 12-octadecadienoic acid (Z, Z); and n-hexadecanoic acid were predominant in all three extracts. Compounds such as 9,12-octadecadienoic acid (Z,Z) and 9,12,15-octadecatrienoic acid, (Z,Z,Z) are polyunsaturated fatty acid (PUFA) compounds. PUFAs play a key role in cellular and tissue metabolism and electron and oxygen transport and also reduce the risk for coronary heart disease (Funk 2001; Mozaffarian et al. 2005). The 2-cyclopenten-1 was only ketone identified in *C. aloifolium*. It acts as an inducer for heat shock protein that has antiviral activity (Rossi et al. 1996a, b).

In recent times, there is a growing awareness in correlating photochemical components and their biological activities (Fernie et al. 2004; Summer et al. 2003). The 9,12,15-octadecatrienoic acid, (Z,Z,Z) has anti-inflammatory, hypercholesterolemia, cancer preventive, hepatoprotective, nematicide and antiarthritic activities (http://www.ars- http://www.ars-grin.gov/duke/chem-activities.html). Octadecanoic acid is a saturated fatty acid, and it might act as a cholesterol-reducing agent (Hunter et al. 2009). Phytol is an acyclic diterpene, and it is also a precursor for vitamins E and K1. Phytol is a promising novel class of pharmaceuticals used for the treatment of antiarthritis and other chronic inflammatory diseases (Ogunlesi et al. 2009).

C. aloifolium belongs to the subtribe Crytopodiinae, tribe Cymbidieae, subfamily Epidenchoideae of Orchidaceae (Dressler 1993). The compound n-hexandecanoic acid reported in the present study was also recorded in *Bulbophyllum kaitense* (Kalaiarasan and Ahmed john 2011). This chemical data would indicate that the tribe Cymbidieae has close affinity with genus *Bulbophyllum*.

Table 26.2 GC-MS analysis showed the phytochemical compounds, their nature, molecular formula, molecular weight and biological activities of *Cymbidium aloifolium*

Sl. no	Name of the compound	Nature of the compound	Molecular formula	M.W	Biological activity	Reference No.
1	n-hexadecanoic acid	Palmitic acid(saturated fatty acid)	$C_{16}H_{32}O_2$	256	Antioxidant, hypocholesteromic, nematicide, hemolytic, 5-alpha reductase inhibitor, antipsychotic	Vijisaral and Subramanian (2014), Sermakkani and Thangapandian (2012), Akpuaka et al. (2013)
2	Phytol	Acyclic, diterpene	$C_{20}H_{40}O$	296	Antimicrobial, anti-cancer, anti-inflammatory, hypocholesteromic, nematicide, anti-arthritic, anticoronary, anti-androgenic, diuretic	http://www.ars- http://www.ars-grin.gov/duke/chem-activities.html, Ogunlesi et al. (2009)
3	9,12-octadecadienoicacid(Z,Z)-	Linoleic acid	$C_{18}H_{32}O_2$	280	Hypercholesterolemic, nematicide, 5-alpha reductase inhibitor, antihistaminic, insectifuge, antieczemic	http://www.ars- http://www.ars-grin.gov/duke/chem-activities.html
4	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid ester	$C_{18}H_{30}O_2$	278	Anti-inflammatory, hypercholesterolemic, cancer preventive, hepatoprotective, nematicide, antiarthritic,	http://www.ars- http://www.ars-grin.gov/duke/chem-activities.html
5	Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	284	Antifungal, antitumour, antibacterial, cholesterol-reducing agent	Vijisaral and Subramanian (2014), Sermakkani and Thangapandian (2012), Akpuaka et al. (2013), Hunter et al. (2009)
6	2-butyne	Alkyne	C_4H_6	54	A simple asphyxiant	CRC Handbook of Chemistry and Physics (2013)
7	2-cyclopenten-1-one	Ketone	C_5H_6O	82	Inducer of Hock protect Shock 70 with antiviral activity	Antonio Rossi et al. (1996a, b)
8	1,4-benzenedicarboxylic acid, bis(2-hydroxyethyl) ester	Ester	$C_{12}H_{14}O_6$	254	Antitumour	Da Hong Wang and Wen Yi Tao (2009)

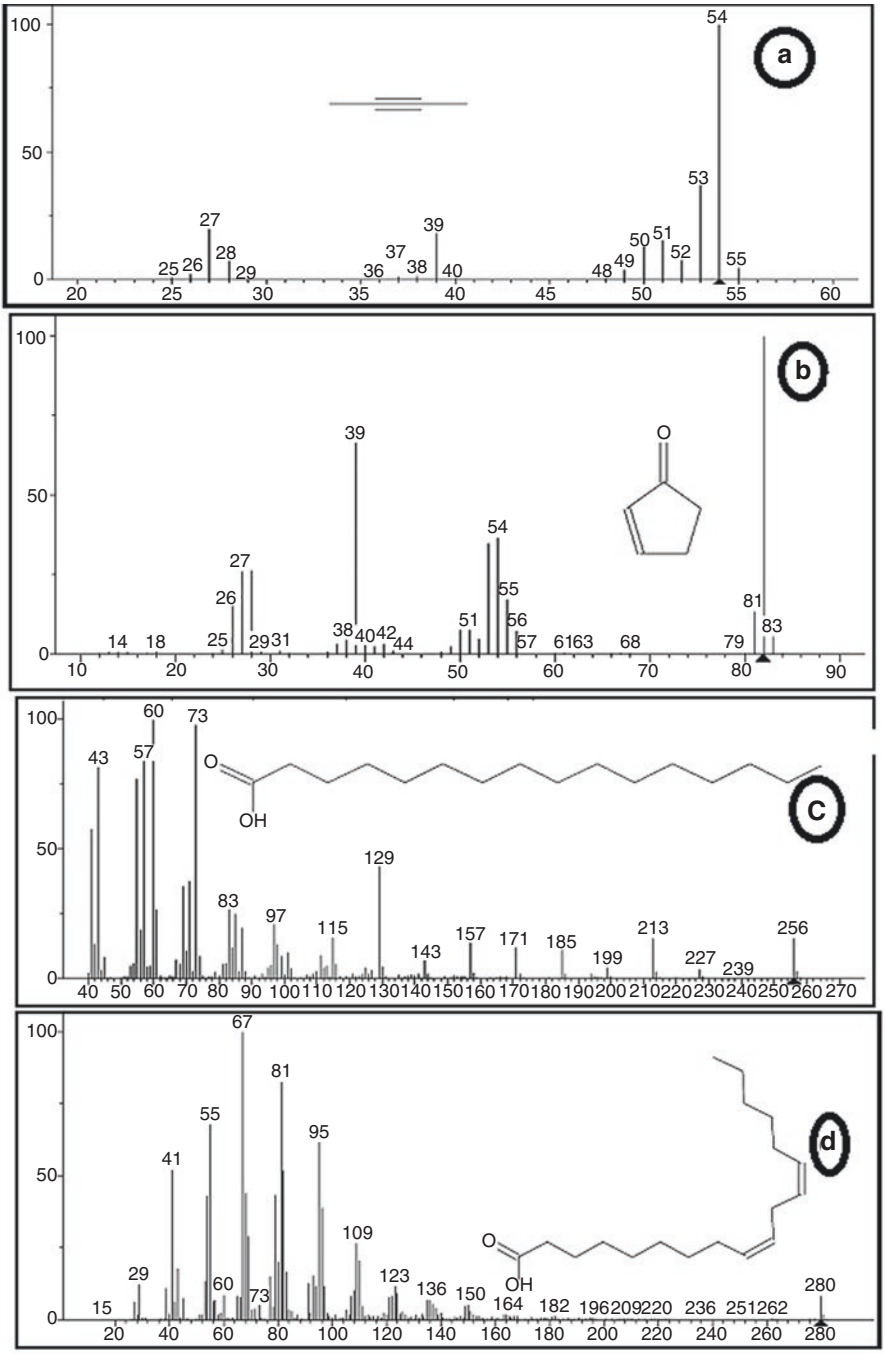

Fig. 26.4 **a**. 2–Butyne, **b**. 2-Cyclopenten-1-one, **c**. n-Hexadecanoic acid, **d**. 9, 12-Octadecadienoic acid (Z,Z)

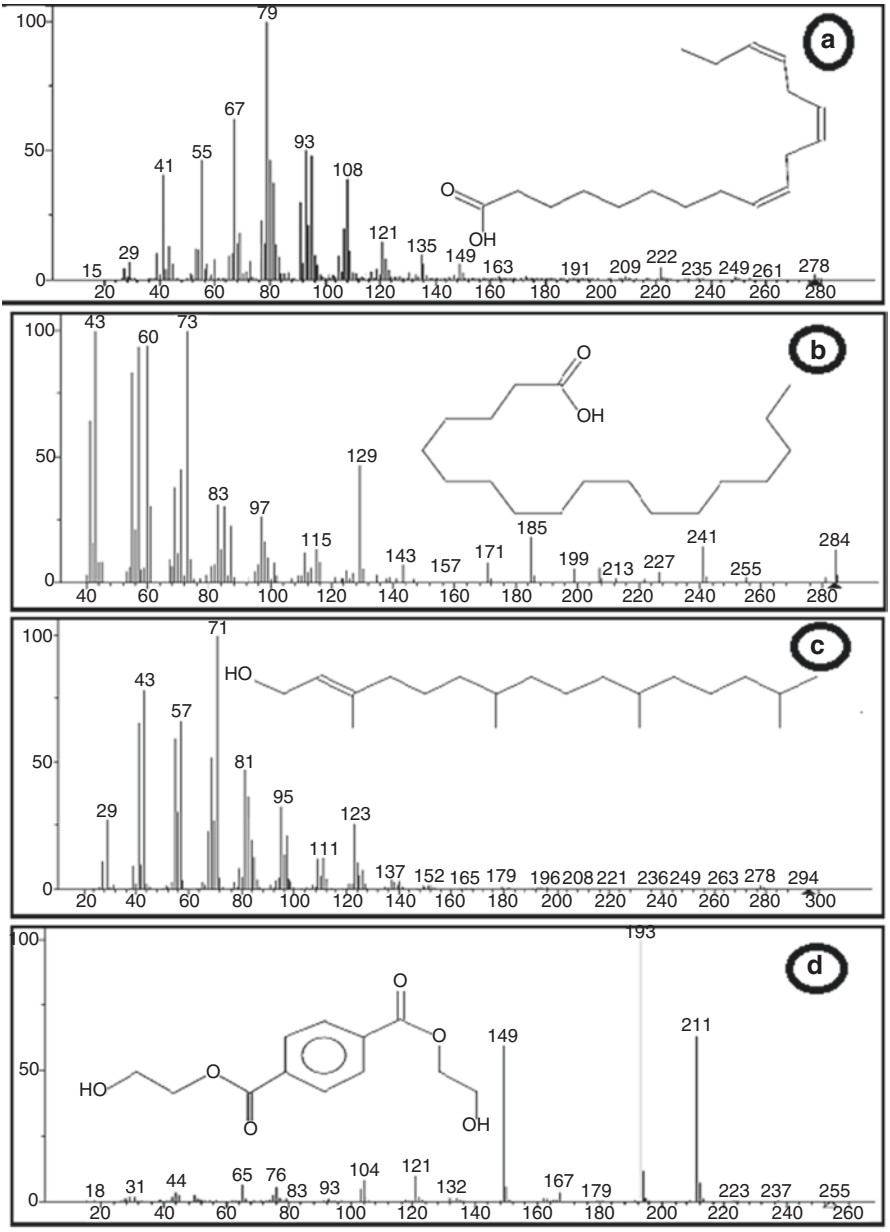

Fig. 26.5 **a.** 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z), **b**. Octadecanoic acid, **c.** Phytol, **d.** 1, 4-Benzenedicarboxylic acid, bis (2-hydroxyethyl) ester

On the other hand, Veerraju et al. (1989) opined that Cymbidieae would have some affinity with the Bletiinae based on chemical data. However, based on recent studies on seed and embryo, it did not share any character with any other genera of Bletiinae.

26.7 Conclusion

The present study characterised the phytochemical profile of the various organic extracts of the *Cymbidium aloifolium* leaves. The compound 1,4-benzenedicarboxylic acid bis(2-hydroxy ethyl) ester was reported from chloroform extract only. Similarly, 2-butyne and 2-cyclopentene-1-one were identified from ethyl acetate extract only. The identified various bioactive compounds have therapeutic properties that can be useful for the treatment of various diseases. These compounds reported from this investigation have some phylogenetic significance.

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