GC-MS Profile of the Unsaponifiable and Saponifiable Matters of *Coldenia procumbens* Linn. Leaves

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Abstract

A lipoid is an oily organic compound soluble in organic solvents which are essential structural components of all living cells. The lipoidal matter, i.e., unsaponifiable and saponifiable fractions of hexane extract of *Coldenia procumbens* Linn. leaves, was characterized by gas chromatography and mass spectrometric (GC-MS) analysis. The unsaponifiable matter was found to contain sterols (61.06%) and hydrocarbons (34.4%), while the saponifiable matter was found to contain about 20 compounds composed of 49.43% saturated fatty acid methyl esters and 50.57% unsaturated fatty acids.

Keywords

Coldenia procumbens Linn. · GC-MS · Steroids · Hydrocarbons

30.1 Introduction

Coldenia procumbens Linn. (Pullaiah and Ali Moulali 1998) grows like an annual herb, and is a common weed in India (Nadkarni and Nadkarni 1955) that belongs to Boraginaceae (Ge-Ling et al. 1995) family, which has around 150 genera and almost 2500 species across the globe. It is found widely in South India on wastelands and common in dry paddy field grounds. The genus has 24 species of plants, and this plant is reported to be widely used in traditional medicines in India, Africa, and Malaysia (The Wealth of India 1950). *Coldenia procumbens* Linn. is the only

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species of its genus which has a place both in the Hortus engalensi's and Moon's Catalogue of Ceylon plants (WhiteLaw Anisile 1826).

The plant is known to be efficacious in treating fever, piles, and scorpion stings. In the traditional system of medicine, the plant was reported to be used as anti-inflammatory (Arul et al. 2005), antimicrobial (Beena 2005), analgesic (Senthamarai et al. 2001), antidiabetic (Patel et al. 2007), and CNS depressant (Naga Rani et al. 1991). Fresh leaves of *Coldenia procumbens* Linn. are reported to be powdered and applied to rheumatic swellings, and equal parts of dried powder mixed with seeds of fenugreek is known to cause suppurations of boils (Chopra et al. 1958). Acetone, water, and methanolic extract of dried aerial parts showed weak angiotensin-converting enzyme inhibition in vitro (Schmelzer et al. 2008; Aleemuddin et al. 2011).

The preliminary investigation of this plant has shown the presence of flavonoids, carbohydrates and glycosides, steroids, and alkaloids (Senthamari et al. 2002). The active constituents like coumestan derivative wedelolactone (Beena et al. 2011) and rare cyano glucosides (Niranjan Kumar et al. 2013) were also extracted from this plant. A GC-MS analysis of volatile components of leaves of *Coldenia procumbens* Linn. has shown 20 compounds with 9,12-octadecadienoic acid and hexadecanoic acid as major compounds (Usha Rani and Kesava Rao 2016). Whereas, the hexane extract of leaves of *Coldenia procumbens* Linn. was not explored for the chemical composition of lipoidal matter till now. The present study completely describes the chemical composition of lipoidal matter and their concentrations in hexane extract of *Coldenia procumbens* Linn. for the first time.

30.1.1 Experimental

The aerial parts of the *Coldenia procumbens* Linn. were collected at Nunna near Vijayawada, Andhra Pradesh, India, from moist place of agricultural land. The plant was authenticated by Prof. VS Raju, and voucher specimen was kept in the Department of Botany, Kakatiya University, and Warangal, India, with accession number 1877.

Hexane, ethyl acetate, and methanol were purchased from Avra Synthesis Pvt. Ltd., (Hyderabad), and potassium hydroxide, sodium sulfate, diethyl ether, and sulfuric acid were supplied by Merck Life Science Private Limited (Mumbai). Silica gel G for thin-layer chromatography was supplied by Acme Synthetic Chemicals (Mumbai).

30.1.2 Extraction of Lipoidal Matter

The air-dried and powdered leaves (3.5 kg) of *Coldenia procumbens* Linn. were exhaustively extracted by hexane by Soxhlet extraction technique. The solvent was concentrated under reduced pressure using a rotary evaporator to get the crude extract (76 g).

30.1.3 Saponification of Lipoidal Matter

30.1.3.1 Preparation of Unsaponifiable Matter

Hexane extract weighing 25 g was saponified with 75 ml of 3% alcoholic KOH by refluxing it for 6 h. After cooling, alcohol was completely removed and the residue was diluted with distilled water. Then, the unsaponifiable matter was extracted with diethyl ether and dried over anhydrous sodium sulfate. Evaporation of ether completely afforded the unsaponifiable matter of about 10 g (Johnson and Davenport 1971).

30.1.3.2 Separation of Unsaponifiable Matter

The crude unsaponifiable matter was applied on TLC silica plates and developed with hexane and ethyl acetate in 8:2 (v/v) ratios. The plates were taken out from the chamber and visualized under UV light to identify and mark the bands. Eight bands were identified, marked, and scraped from the plates with a spatula. The bands were dissolved in ethyl acetate and filtered through vacuum to get the pure compounds separately. All the eight bands were concentrated and then analyzed by GC-MS, while the seventh band was fluorescent and phytosterols were separated as white needlelike crystals.

30.1.3.3 Preparation of Saponifiable Matter

The aqueous alkaline layer remained after the removal of unsaponifiable matter was acidified with sulfuric acid (10%) and the liberated fatty acids were extracted with ether, and then washed with water until neutral and dried over anhydrous sodium sulfate followed by evaporation of ether to afford total fatty acids (TFA) residue (6 g) (Vogel 1966). The remaining 9 g of extract was left in water part when fractionated with diethyl ether.

30.1.4 Preparation of Fatty Acid Methyl Esters

The free fatty acids were converted to their methyl esters by refluxing them with 100 ml absolute methanol and 5 ml sulfuric acid for 1 h; the alcohol was distilled, and the residue was dissolved in water and then extracted several times with ether. The combined ethereal extracts were washed with water till free from acidity. The ethereal extract was concentrated, and the residue was dried over anhydrous sodium sulfate to get fatty acid methyl esters (FAME) (Elsaid and Amer 1965). The experimental procedure was shown in Fig. 30.1.

30.1.5 Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

The GC-MS analysis was performed on an Agilent 6890N Gas Chromatograph equipped with an Agilent HP-1 ms capillary column (30 m × 25 mm id; 0.25 μm) and connected to Agilent Mass Spectrometer operating in EI mode (70 eV; *m*/*z* 50–650; source temperature, 230 °C; quadruple temperature, 150 °C). The column

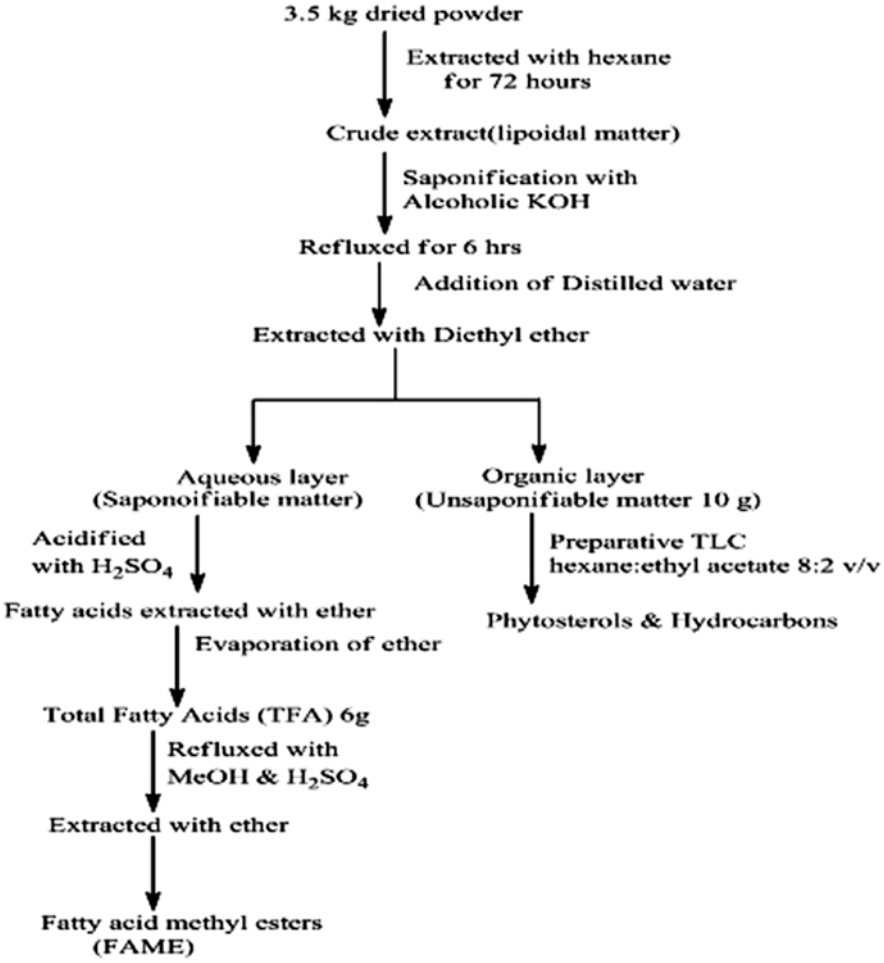


Fig. 30.1 Saponification of hexane crude extract of *Coldenia procumbens* Linn

temperature used was 150 °C for 2 min, increased to 300 °C at 10 °C/min, and maintained for 20 min at 300 °C. The carrier gas was helium at a flow rate of 1.0 ml/min. The inlet temperature was maintained at 280 °C and the split ratio was 50:1. Structural assignments were based on interpretation of mass spectrometric fragmentation and confirmed by comparison of retention times as well as fragmentation pattern of authentic compounds like sterols and alcohols and the spectral data obtained from the Wiley and NIST libraries.

30.2 Results and Discussion

The investigation of lipoidal matter of hexane extract of leaves of *Coldenia procumbens* Linn. was carried out by GC-MS which has shown higher percentage of phytosterols, like β-sitosterol, stigmasterol, and campesterol, unsaturated fatty acids, and saturated fatty acids, respectively.

Hexane extract of leaves of *Coldenia procumbens* Linn. was found to contain unsaponifiable matter and saponifiable matter in 40% and 24%, respectively. The results of GC-MS analysis of unsaponifiable matter of leaves of *Coldenia procumbens* Linn. are shown in Fig. 30.2, and the composition is given in Table 30.1. The analysis revealed the presence of total 19 compounds with identified compounds 94.81% and unidentified compounds with 5.19%. The identified compounds were classified as hydrocarbons (33.45%) and sterols (61.06%). Among all the compounds, β-sitosterol, stigmasterol, gamma tocopherol, and vitamin E were found maximum in unsaponifiable matter of hexane extract. The saponifiable matter of hexane extract of leaves of *Coldenia procumbens* Linn. is shown in Fig. 30.3. It was observed that the saponifiable matter was composed with saturated fatty acids with 49.43% and unsaturated fatty acids with 50.57%. Among the fatty acids identified,

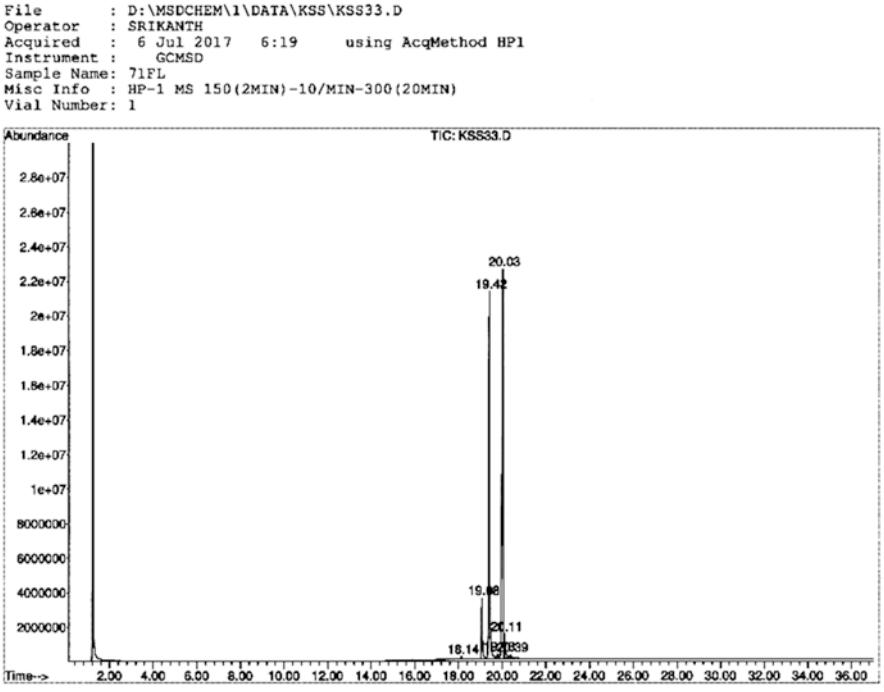
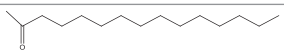
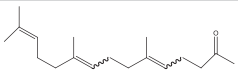
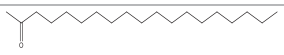
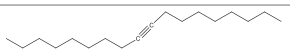
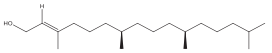
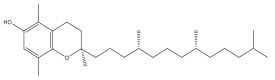
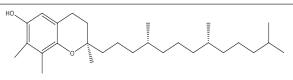
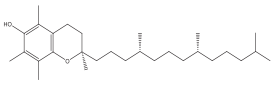
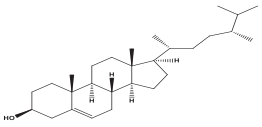
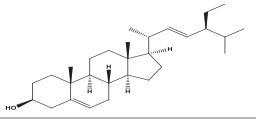


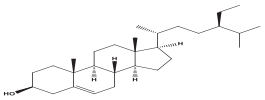
Fig. 30.2 GC chromatogram of phytosterols isolated from unsaponifiable matter of hexane extract of *Coldenia procumbens* Linn

Table 30.1 Chemical composition of unsaponifiable matter of hexane extract of leaves of *Coldenia procumbens* Linn

S. no.	Compounds	Structure	MF	MW	RT	Relative area %
1	9-Octadecene		$C_{18}H_{36}$	252	5.32	0.30
2	Hexadecane		$C_{16}H_{34}$	226	5.42	0.95
3	1-Octadecene		$C_{18}H_{36}$	252	7.40	0.38
4	Octadecane		$C_{18}H_{38}$	254	7.50	2.74
5	2-Pentadecanone		$C_{15}H_{30}O$	226	7.82	12.89
6	2-Heptadecanone		$C_{17}H_{34}O_2$	270	8.34	0.89
7	5,9,13-Pentadecatrien-2-one		$C_{15}H_{24}O$	220	8.46	3.68
8	3-Eicosene		$C_{20}H_{40}$	280	9.41	0.31
9	13-Octadecenal		$C_{18}H_{34}O$	266	9.66	0.74
10	2-Nonadecanone		$C_{19}H_{38}O$	282	10.31	3.01
11	9-Octadecyne		$C_{18}H_{34}$	250	10.48	8.16
12	9-Eicosyne		$C_{20}H_{38}$	278	10.49	3.23
13	Phytol		$C_{20}H_{40}O$	296	10.53	6.46
14	β-Tocopherol		$C_{28}H_{48}O_2$	416	17.60	2.97
15	γ-Tocopherol		$C_{28}H_{48}O_2$	416	17.61	27.10
16	Vitamin E		$C_{29}H_{50}O_2$	430	18.21	20.66
17	Campesterol		$C_{28}H_{48}O$	400	19.36	11.72
18	Stigmasterol		$C_{29}H_{48}O$	412	19.46	65.46

(continued)

Table 30.1 (continued)

S. no.	Compounds	Structure	MF	MW	RT	Relative area %
19	β-Sitosterol		$C_{29}H_{50}O$	414	20.14	95.24
20	Unidentified				23.67	15.48
Total identified hydrocarbons						33.45
Total identified sterols						61.06
Total unidentified compounds						5.48

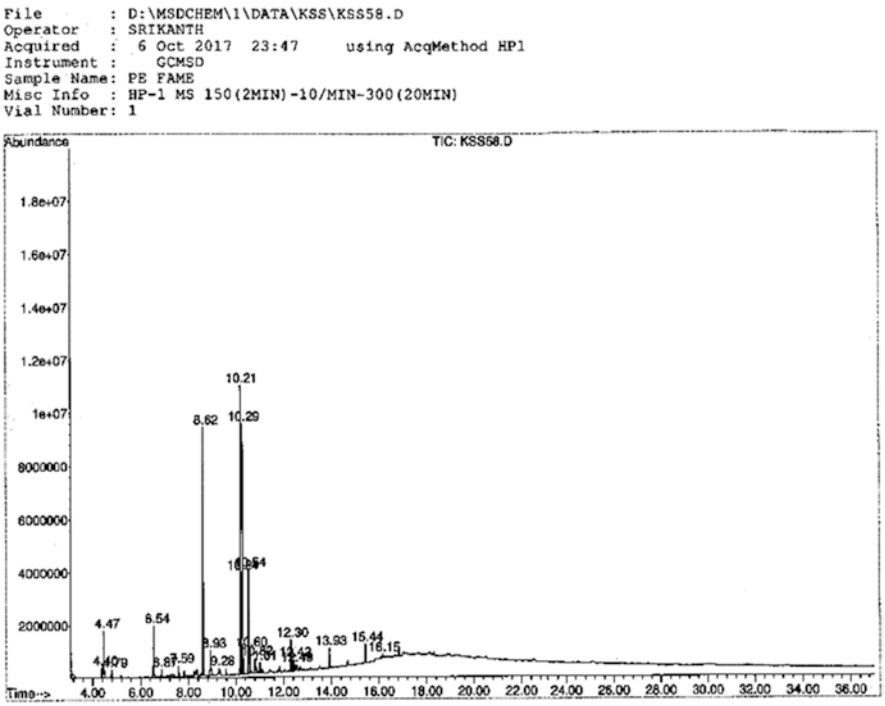
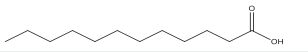
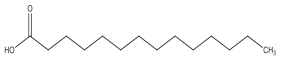
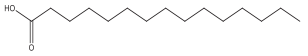
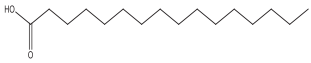
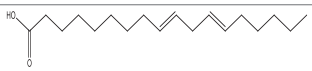
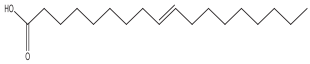
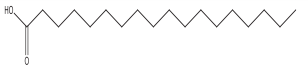
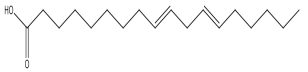
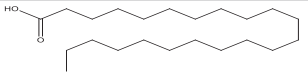


Fig. 30.3 Gas chromatogram of saponifiable matter of hexane extract of leaves of *Coldenia procumbens* Linn

palmitic acid and linoleic acid were found to be maximum with 17.65% and 25.28%, respectively, followed by other fatty acids.

The crude hexane extract of *Coldenia procumbens* Linn. analyzed by GC-MS summarizes the % composition of hydrocarbons with 8.9%, fatty acids with 67.90%, steroids with 13.31%, terpenoids with 8.585%, and fatty alcohols with 1.325% (Table 30.2). The higher concentration of sterols was found to be more in lipoidal matter than in crude hexane extract of *Coldenia procumbens* Linn., whereas the linoleic acid concentration was high in both the crude extract and the lipoidal matter.

Table 30.2 Chemical composition of saponifiable matter of hexane extract of leaves *Coldenia procumbens* Linn

S. no.	Compounds	Structure		Relative area %
1	Dodecanoic acid (lauric acid)		C(12:0)	3.39
2	Myristic acid methyl ester		C(14:0)	3.85
3	Pentadecanoic acid methyl ester		C(15:0)	0.78
4	Hexadecanoic acid (palmitic acid) methyl ester		C(16:0)	17.65
5	Hexadecanoic acid ethyl ester		C(16:0)	0.13
6	9,12-Octadecadienoic acid methyl ester		C(18:2)	25.28
7	9-Octadecenoic acid methyl ester		C(18:1)	17.85
8	Octadecanoic acid (stearic acid) methyl ester		C(18:0)	9.56
9	Linoleic acid ethyl ester		C(18:2)	1.46
10	Eicosanoic acid (arachidic acid) methyl ester		C(20:0)	2.29
11	Docosanoic acid (behenic acid) methyl ester		C(23:0)	1.54
12	Tetracosanoic acid (lignoceric acid) methyl ester		C(18:0)	1.47
Saturated fatty acids				49.43%
Unsaturated fatty acids				50.57%

This proves that the saponification of lipoidal matter will give better results in the separation of bioactive sterols from other compounds like hydrocarbons, fatty acids, and fatty alcohols. Some of the species of Boraginaceae family were also studied for the chemical composition of lipid matter like *Cordia* and *Echium* species, but the qualitative and quantitative determination of lipoidal matter was described in detail for the first time in the species *Coldenia procumbens* Linn.

The GC-MS analysis of *Echium wildpretii* revealed that the esters of hexadecanoic acid, octadecanoic acid, and hydrocarbons like pentacosane and hexacosane were the main compounds (Santana et al. 2012). Forty five phytochemicals were identified in GC-MS studies of *Cordia rothii* roots (Khan et al. 2016). Phytosterols and their derivatives are important products applied in pharmaceutical, food, and

cosmetic industry due to their anti-inflammatory, antibacterial, antifungal, anti-ulcerative, and antitumor activities (Ling and Jones 1995).

The palmitic acid is reported to have antibacterial, antifungal, antioxidant, anti-inflammatory, and hypocholesterolemic effects (Agoramoorthy et al. 2007; Elagbar et al. 2016; Abubakar and Majinda 2016). The linoleic acid, i.e., an omega-6 fatty acid, which the body is not able to produce, can be consumed through diet. It is needed for growth and repair and possesses pharmacological activities like antioxidant and anti-inflammatory activities (Henry et al. 2002). Hence, the linoleic acid is responsible for anti-inflammatory activity of this species *Coldenia procumbens* Linn. This species is available as common weed and not edible as the leaves are covered with coarse hairs, i.e., trichomes and produce irritation, provoke sneezing when pulverized (Quisumbing et al. 1951). The pharmacological activities of these bioactive compounds from the lipoidal matter of the hexane extract of the *Coldenia procumbens* Linn. support the medicinal application of the plant leading to develop further biological and pharmacological studies.

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