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RESEARCH ARTICLE

LONG CHAIN ALIPHATIC HYDROCARBONS FROM *Momordica dioica* (ROXB) EX WILD. (CUCURBITACEAE) FRUITS

***Kale, M. S. and Laddha, K. S.**

Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology (ICT), Nathalal Parekh Marg, Matunga (E), Mumbai, India-400 019

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ABSTRACT

Long chain saturated hydrocarbons were isolated from petroleum ether extract of *Momordica dioica* Roxb. Ex Wild (Cucurbitaceae) fruits. The petroleum ether extract of fruits was subjected to silica gel column chromatography using petroleum ether (100%) as mobile phase. The isolated hydrocarbons fraction was characterized by gas chromatography mass spectroscopy (GCMS), along with IR, ^{1}HNMR and ^{13}CNMR spectroscopic techniques. Major compounds present in hydrocarbon fraction were tridecane ($C_{13}H_{28}$) at a retention time (RT) of 10.8 min, tetradecane ($C_{14}H_{30}$) at RT of 12.5 min, pentadecane ($C_{15}H_{32}$) at RT of 14.1 min, hexadecane ($C_{16}H_{34}$) at RT of 15.6 min, octacosane ($C_{28}H_{58}$) at RT of 26.2 min, nonacosane ($C_{29}H_{60}$) at RT of 26.5 min and tetratriacontane ($C_{34}H_{70}$) at RT of 26.7 min.

INTRODUCTION

In last decade, there is an increasing interest in researches for production of biologically active compound from natural sources. Bioactive compounds are remarkable because of their role in prevention and control of certain diseases such as diabetes, cardiovascular diseases and cancer. *Momordica dioica* Roxb. Ex Wild (Cucurbitaceae) is a perennial, dioecious climber found throughout India. It is often cultivated for its fruits which are also used as vegetable (CSIR India 1962). *M. dioica* Roxb. Ex Wild is claimed to be anti-inflammatory, hepatoprotective, antimicrobial, antifungal, anti-diabetic (Shreedhara *et al*., 2006, Reddy *et al*., 2006 and Shrinivas *et al*., 2009). Phytochemical investigation of fruits have revealed the presence of the traces of alkaloids, Lectin, B-sitosterol, saponin glycosides, triterpenoids, long chain aliphatic hydrocarbons, tannins, and fixed oil (Ghosh *et al*., 1981, Luo *et al*., 1991 and Ali *et al*., 1998). The plant is relatively virgin and phytochemical and pharmacological profiles are inadequate.

Hydrocarbons are biologically stable common components of many species of plants, n-alkanes (C_nH_{2n+2}) are thought to be endogenous to a plants, they are formed as a result of decarboxylation of long chain fatty acids (Iyer *et al*., 1998). Hydrocarbon may function to prevent desiccation, affect the adsorption of agricultural chemicals, provides a barrier to penetration by microorganism, and serve as a chemotaxonomic character (Kolattukudy, 1976). The durability of hydrocarbons makes them a candidate for marking individual plant species. GC is certainly the most efficient method used for analyzing hydrocarbons, enabling separation of the individual members of the homologous series. A very powerful aid in the identification of hydrocarbons is the coupling of GC and MS, since GC can separate all hydrocarbons and their mass spectra can be easily characterized (Lanzon *et al*., 1994). From the literature it appears that no such remarkable work has been carried out on fruits of *Momordica dioica* which is needed in order to explore its pharmacological importance. In the present work we are reporting the isolation and characterization of aliphatic straight chain saturated hydrocarbons by column chromatography followed by GCMS from the fruits of *Momordica dioica.*

MATERIAL AND METHODS

Plant materials

The fresh fruits of *Momordica dioica* were collected from local market of Mumbai, Maharashtra, India during the month of August 2011 and authenticated. Freshly collected fruits were dried in tray dryer at 55 ^{o}C for 24 h. The dried fruits were cut and powdered by mechanical grinder. The powder was used for further study.

Chemicals and reagents

All chemicals and solvents used were of analytical and HPLC grade.

Extraction and isolation

The dried powder of fruits of *Momordica dioica* (500 kg) was extracted with petroleum ether in soxhlet extractor for 72 h.

Corresponding author: mahesh01kale@yahoo.co.in

The petroleum ether extract was concentrated (50 g) by rotary evaporator. The dried extract was subjected to column chromatography on silica gel G (Mesh size 60-80) as a stationary phase and petroleum ether (100%) as eluent. The fractions were collected in bulk (250 ml each) and monitored by TLC. Combined initial fractions (Fraction 4-15) afforded a crystalline residue, which was further purified by crystallizing from petroleum ether.

Identification and characterization

Melting point was determined using in open capillary tubes in a Veego (India) melting point apparatus and is uncorrected. The residue gave an appearance of single charred band on TLC with petroleum ether (100%) as a mobile phase and 5% sulphuric acid as a detecting reagent. IR spectrum was taken on a Perkin Elmer FT-IR spectrophotometer. ^{1}HNMR and ^{13}C NMR data were measured on a JOEL 400 MHz spectrometer in $CDCL_3$. Gas chromatography mass spectroscopy (GCMS) study was carried on GC-V gas chromatograph (The Accutof GC-V) fused with HP5 gas chromatograph capillary column coupled with JMS-T100 quadruple mass selective detector. Helium was used as carrier gas. Other GCMS conditions are Column: Capillary column HP- 5(Length 30 m, id 0.25 mm), carrier gas: Helium, Flow Rate: 1mL/min, Inlet Temp: 60 ^{o}C, Detector temp: 280 ^{o}C. Injector was operated in a split mode with a split ratio 1:20. The column temperature program started at 60 °C and changed to 200 °C at the rate of 8 °C/min and hold for 3 min. The temperature was raised to 250 °C at the rate of 10 °C/min and hold for 3 min. Then the temperature was increased to 280 °C at the rate of 30 °C/min and kept constant for 3 min. Total elution time was 30 min. Identification of individual compounds was carried out with library search by HPCHEM Software from Indian Institute of Technology (IIT), Bombay and published mass spectra.

RESULTS AND DISCUSSION

The Long chain aliphatic hydrocarbons from *Momordica dioica* (Cucurbitaceae) fruits were isolated by silica gel G (Mesh size 60-80) column chromatography using petroleum ether as mobile phase. The structures of the individual components were determined by spectroscopic methods. Identification was based on melting point, IR, ^{13}CNMR, ^{1}HNMR spectroscopy and GCMS (gas chromatography equipped with mass spectrophotometer). The hydrocarbon fraction has melting point ranging from 69-74 ^{o}C, showed IR absorption bands at 2920, 2860, 1460, 730 cm^{-1} indicating it's saturated and aliphatic nature. ^{1}H NMR showed a triplet at δ value 0.88 for terminal methyl group and a multiple peaks at δ value 1.26 for $(CH_2)_n$, indicating the absence of branching in either of the hydrocarbon components (Fig. 1). ^{13}C NMR spectrum exhibited signals between δ 14.08 - 37.08 indicating the presence of methyl and methylene proton (Fig.2). The absence of any signal beyond δ 2.26 in the ^{1}H NMR spectrum and δ 31.90 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. Gas chromatograph of hydrocarbon fraction showed the presence of seven components (Fig 3). Mass spectrum of fraction defined each member more specifically (Fig.4). The members of the homologous series of n-alkanes elutes in the increasing order of the carbon chain length. Based on the retention time (RT) and fragmentation pattern, the GCMS analysis indicates the presence of tridecane ($C_{13}H_{28}$) at a retention time (RT) of 10.8 min, tetradecane ($C_{14}H_{30}$) at RT of 12.5 min, pentadecane ($C_{15}H_{32}$) at RT of 14.1 min, hexadecane ($C_{16}H_{34}$) at RT of 15.6 min, octacosane ($C_{28}H_{58}$) at RT of 26.2 min, nonacosane ($C_{29}H_{60}$) at RT of 26.5 min and tetratriacontane ($C_{34}H_{70}$) at RT of 26.7 min as major components of hydrocarbon fraction, while some of the minor peaks could not be identified.

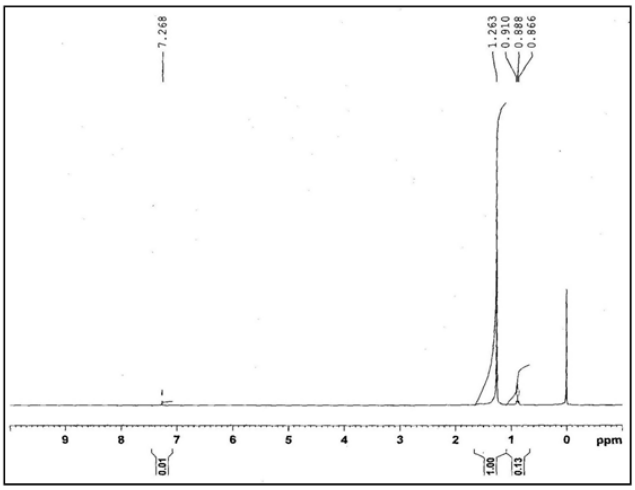


Figure 1: ^{1}HNMR of hydrocarbon fraction from *Momordica dioica* fruits in $CDCl_3$

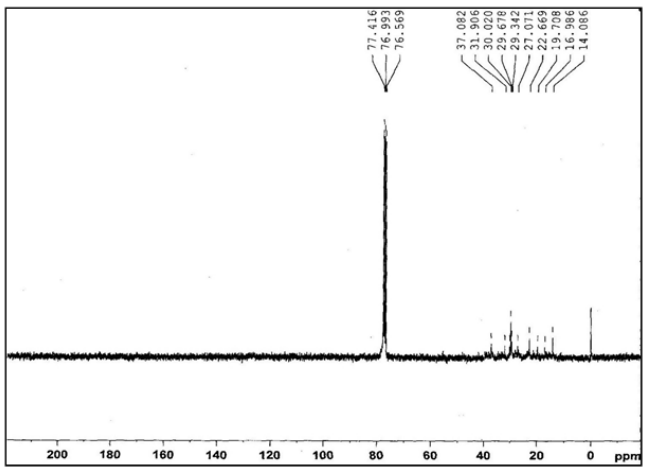


Figure 2: ^{13}CNMR of hydrocarbon fraction from *Momordica dioica* fruits in $CDCl_3$

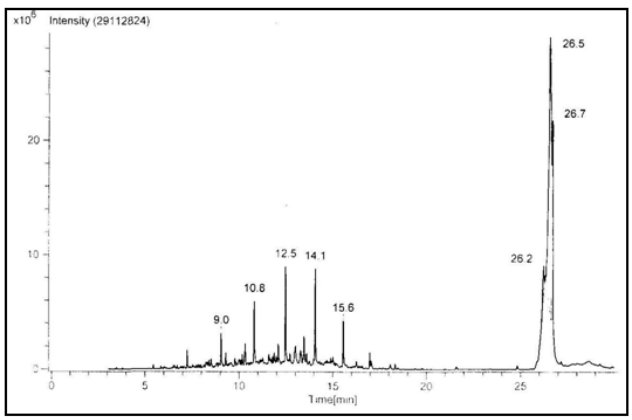
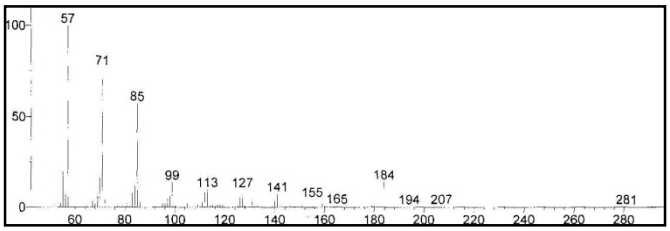
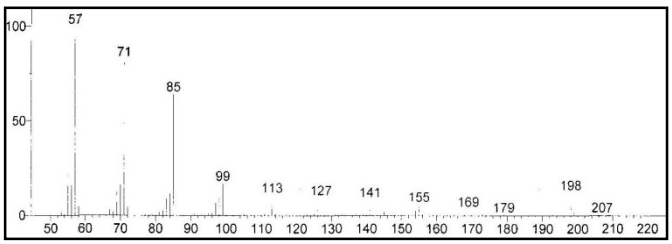


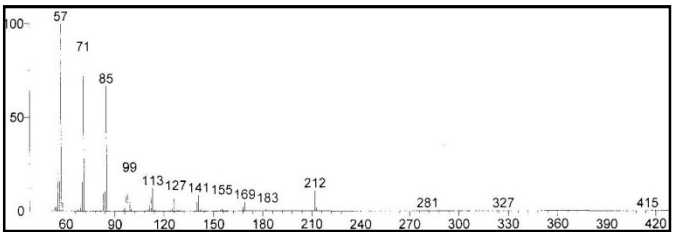
Figure 3: Gas chromatograph (GC) of hydrocarbon fraction from *Momordica dioica* fruits



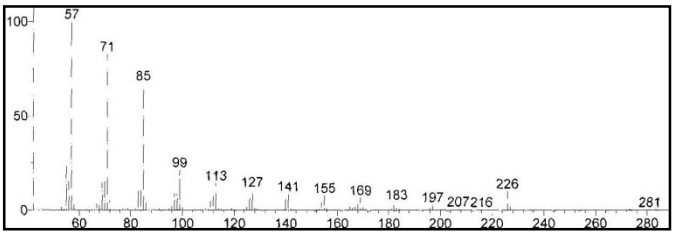
(A)



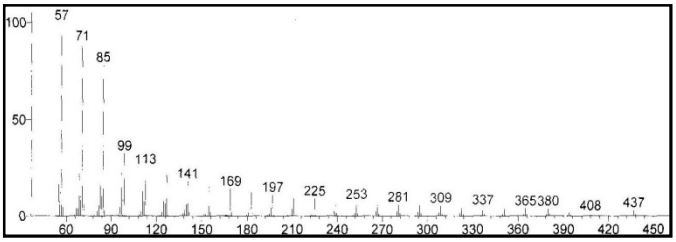
(B)



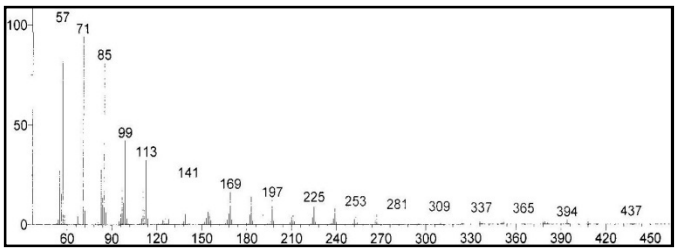
(C)



(D)



(E)



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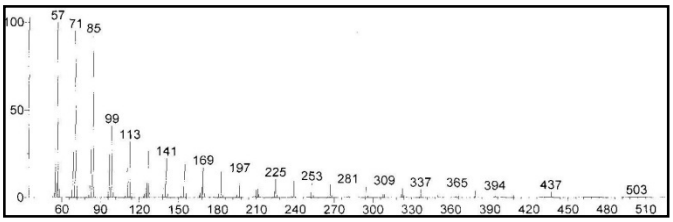

(G)

Figure 4: MS spectra of hydrocarbon fraction from *Momordica dioica* fruits

A-Tridecane ($C_{13}H_{28}$) at a retention time (RT) of 10.8 min, **B**-Tetradecane ($C_{14}H_{30}$) at RT of 12.5 min, **C**- Pentadecane ($C_{15}H_{32}$) at RT of 14.1 min, **D**- Hexadecane ($C_{16}H_{34}$) at RT of 15.6 min, **E**- Octacosane ($C_{28}H_{58}$) at RT of 26.2 min, **F**- Nonacosane ($C_{29}H_{60}$) at RT of 26.5 min, **G**- Tetratriacontane ($C_{34}H_{70}$) at RT of 26.7 min.

Conclusion

This study provides a simple method for isolation of hydrocarbons and in large quantity unlike conventional preparative TLC. The newly reported saturated aliphatic hydrocarbons from the *Momordica dioica* (Cucurbitaceae) fruits, may serve as marker constituent for further characterization and standardization of crude drug and marketed formulations.

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