



European Journal of Biotechnology and Bioscience

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European
Journal of
Biotechnology
and
Bioscience

ISSN: 2321-9122

www.biosciencejournals.com

EJBB 2014; 2 (5): 46-51

Received: 13-11-2014

Accepted: 29-11-2014

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Phytochemical Analysis and GC-MS Analysis of Leaves of *Macrotyloma uniflorum*

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Abstract

Horse gram (*Macrotyloma uniflorum* Lam.) is a popular pulse, locally known as Gaheth belongs to the family Fabaceae that still remain an under exploited legume. The present study was carried out to investigate the phytochemical profile and analyze the chemical composition of leaves of *Macrotyloma uniflorum*. The leaves powder was successively extracted with petroleum ether, n-hexane, ethanol, methanol, and ethyl acetate. Phytochemical analysis shows the presence of flavonoids, tannins, terpenoids, saponins, anthocyanins, phenols, proteins, alkaloids and carbohydrates. To isolate and analyze the chemical composition in ethanol crude extracts from leaves of *Macrotyloma uniflorum* by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis provided different peaks determining the presence of seven different phytochemical compounds namely hexadecanoic acid, methyl ester (8.81%), 1,2,4-trioxolane-2-octanoic acid, 5-octyl-, methyl ester (8.53%), 10-octadecenoic acid, methyl ester (21.04%), n-hexadecanoic acid (20.59%), oleic acid (22.10%), [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (12.64%), and 2-hexadecanol (6.25%). This study helps to predict the formula and structure of biomolecules which can be used as drugs and further investigation may lead to the development of drug formulation.

Keywords: Phytochemical analysis, GC-MS analysis, *Macrotyloma uniflorum*, and oleic acid.

1. Introduction

Medicinal plants have been used for centuries as remedies for human disease [1,2&3]. Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to search for biologically active principles in plant [4]. In India plenty of plants are being used as drug due to their medicinal properties. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics have lead researchers to scrutinize the antimicrobial compounds [5]. Natural products have played an important role in the development of drugs and drug leads for various diseases including cancer [6]. Among bioactive natural compounds, several plant essential oils and plant extracts considered to have an activity because of the presence of several chemicals that can exert their activities both as fumigants and by direct contact, these active insecticide, repellent, antifeedant, and insect growth regulatory properties [7]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc [8]. Horse Gram is scientifically known as *M. uniflorum*. It also goes by the name *Dolichos uniflorus* due to a lot of confusion in the *Dolichos* category the right name for the horse gram scientifically is *Macrotyloma uniflorum*. According to USDA (United States Department of Agriculture) database both the name's *Macrotyloma uniflorum* and *Dolichos uniflorus* mean the same.

Horse gram (*Macrotyloma uniflorum* Lam.) is a popular pulse, locally known as Gaheth belongs to the family Fabaceae that still remain an under exploited legume crop. It is usually grown up to the area at 1800msl. Horse gram seeds are rich in protein and consumed in majority by poorest section of the society. *Macrotyloma uniflorum* has been used in traditional system of medicine for treating haemorrhoids, tumours, bronchitis, cardiopathy, nephrolithiasis, urolithiasis, splenomegaly, strangury, hiccup, ophthalmopathy, verminosis, and vitiated condition of *vata*, remove kidney stone, inflammation, liver trouble^[9]. Although the plant is used in Ayurvedic medicine for the treatment of ailments there are no reports on the constituents that are responsible for the therapeutic effect. With this background the present studies was aimed to phytochemical profile and identify the phytoconstituents present in *Macrotyloma uniflorum* leaves using GC-MS analysis.

2. Materials and Methods

2.1. Collection and Preparation Plant Material

The fresh plants *Macrotyloma uniflorum* leaves were collected from Agraharam Village in Namakkal district of Tamil Nadu, India during January to December 2013 and authenticated by director of the Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamil Nadu, and India. The sample were washed thoroughly in running up tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were preserved in airtight containers until use.

2.2. Extraction procedure

The powdered sample of *Macrotyloma uniflorum* leaves (100g) were extracted with petroleum ether, n-hexane, ethanol, methanol, and ethyl acetate (500ml, 46 h) at temperature between 50-55°C by using Soxhlet extractor. The solvent was evaporated by rotavapor (Yamato Rotary Evaporator, Model RE-801) to obtained viscous semi solid masses. The semi dry crude extract was suspended in water and it analyzed by phytochemical analysis and GC-MS, it had led to the identification and characterization of seven different organic compounds. The crude extracts were filtered separately through Whatman No. 41 filter paper to obtained dust free plant crude extract. The residue was re-extracted twice follow the same and filtered. The combined extracts were concentrated and dried by using rotary evaporator under vacuum.

2.3 Phytochemical Analysis

The preliminary phytochemical evaluation of *Macrotyloma uniflorum* was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered leaves of plant (100g) were extracted in a Soxhlet apparatus with petroleum ether, n-hexane, ethanol, methanol, and ethyl acetate successively. The resultant extracts were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids and steroids etc.^[10&11]

2.4. GC-MS analysis

The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 mm ID × 1 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The µL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes holding. The injector temperature was set at 250°C (mass analyzer). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da.

2.5. Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology having more than 62,000 patterns. The spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Result and Discussion

3.1. Phytochemical Analysis

The phytochemical screening in the present study has revealed the presence of presence of flavonoids, tannins, terpenoids, saponins, anthocyanins, phenols, proteins, alkaloids and carbohydrates. Most of the phytochemical constituents were present in the extracts of n-hexane, ethanol and methanol. Table 1 showed the results of phytochemical screening of various extracts of leaves of *Macrotyloma uniflorum*.

Table 1: Phytochemical Analysis of *Macrotyloma uniflorum* Leaves

S.No	Name of the Test	Ethanol Extract	Ethyl acetate Extract	n-Hexane Extract	Petroleum ether Extract	Methanol Extract
1.	Alkaloids	+	+	+	+	+
2.	Carbohydrates	-	+	+	+	+
3.	Phenols	+	-	+	-	+
4.	Terpenoids	+	-	+	-	+
5.	Tannins	+	-	+	+	+
6.	Flavonoids	-	-	+	+	+
7.	Sponins	+	-	+	+	-
8.	Protiens	+	-	+	+	-
9.	Anthocyanins	+	+	+	+	+

+: Indicates the presence and -: Indicates the absence of phytoconstituents

The phytochemical analysis showed the presence of presence of flavonoids, tannins, terpenoids, saponins, anthocyanins, phenols, proteins, alkaloids and carbohydrates. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against various diseases. Cardiac glycosides are a class of medications used to treat heart failure [12]. Phenols and tannins composition of medicinal plants is mainly responsible for antioxidant activity and contributes to their biofunctionalities such as reduction of chronic and degenerative diseases including cancer, cardiac, and infectious diseases etc. [13]. With reference to alkaloids which act as cardiac or respiratory stimulants show maximum quantity in leaves of plant [14].

3.2. GC-MS Analysis

The compounds present in the ethanol extract of leaves of *Macrotyloma uniflorum* plant were identified by GC-MS analysis (Fig.1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration % in the ethanol extract of leaves of *Macrotyloma uniflorum* are presented in Table 2. The prevailing compounds in ethanol extract of leaves of *Macrotyloma uniflorum* were hexadecanoic acid, methyl ester (8.81%), 1,2,4-trioxolane-2-octanoic acid, 5-octyl-, methyl (8.81%), 1,2,4-trioxolane-2-octanoic acid, 5-octyl-, methyl

ester (8.53%), 10-octadecenoic acid, methyl ester(21.04%), n-hexadecanoic acid (20.59%), oleic acid (22.10%), [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (12.64%), and 2-hexadecanol (6.25%). The spectrum profile of GC-MS confirmed the presence of seven major components with retention time 17.15, 15.72, 18.90, 17.87, 19.65, 20.08and 29.88 respectively (Fig.1). The individual fragmentations of the components were illustrated in (Fig. 2A-2G).

Authentication of medicinal plants as genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations. In recent years, various scientists have accelerated research on the drug and dietary supplements from the plants [15] and used them as herbal medicines for the treatment of infectious diseases [16]. Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable for tool for reliable identification of phytocompounds [17&18]. The GC-MS profiles were used and identified seven constituents in leaves of *Macrotyloma uniflorum*. The major constituents were found to be oleic acid (22.10%), 10-octadecenoic acid, methyl ester (21.04%), n-hexadecanoic acid (20.59%), 2'-hexyl-, methyl ester (12.64%) respectively. We report the presence some of the important components resolved by GC-MS analysis.

Table 2: Components detected in the plant of ethanol extract of *Macrotyloma uniflorum* leaves

S.No	Compound Name	Molecular Formula	MW	RT	Peak Area	%Peak Area
1.	hexadecanoic acid, methyl ester	C17H34O2	270	17.15	16562896	8.81
2.	1,2,4-trioxolane-2-octanoic acid, 5-octyl-, methyl ester	C19H35 O5	344	15.72	16042608	8.53
3.	10-octadecenoic acid, methyl ester	C19H36 O2	296	18.90	39552048	21.04
4.	n-hexadecanoic acid	C16H32 O2	256	17.87	38712432	20.59
5.	oleic acid	C18H34O2	282	19.65	41548544	22.10
6.	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C21H38O2	322	20.08	23769200	12.64
7.	2-hexadecanol	C16H34O	242	29.88	11754064	6.25

MW: Molecular Weight, RT: Retention Time

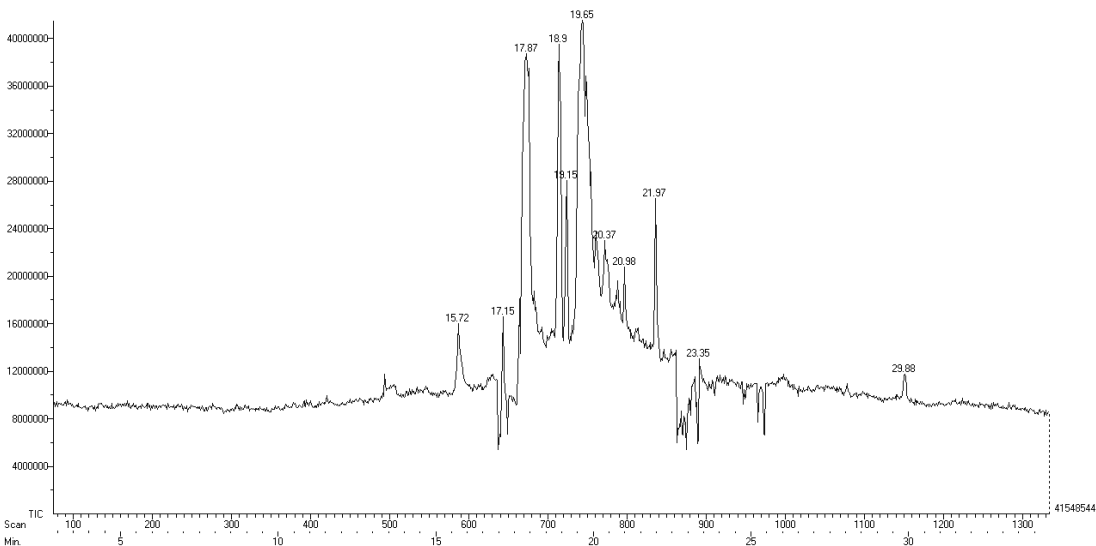


Fig 1: GC-MS spectrum of ethanol extract of *Macrotyloma uniflorum* leaves

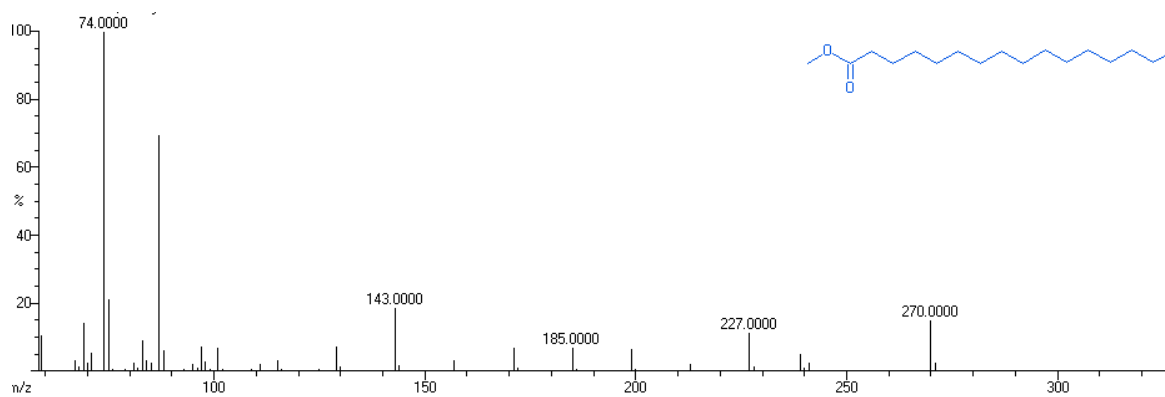


Fig 2A: hexadecanoic acid, methyl ester (RT: 17.15)

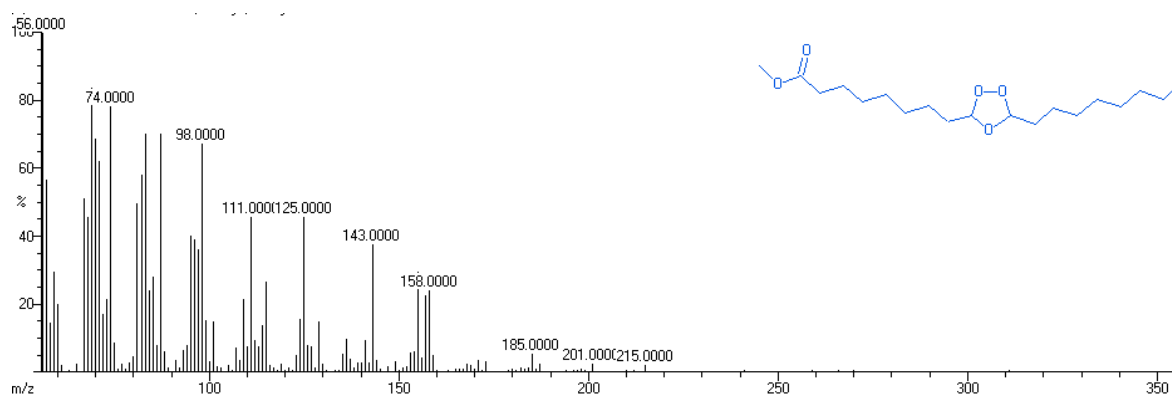


Fig 2B: 1, 2, 4-trioxolane-2-octanoic acid, 5-octyl-, methyl ester (RT: 15.72)

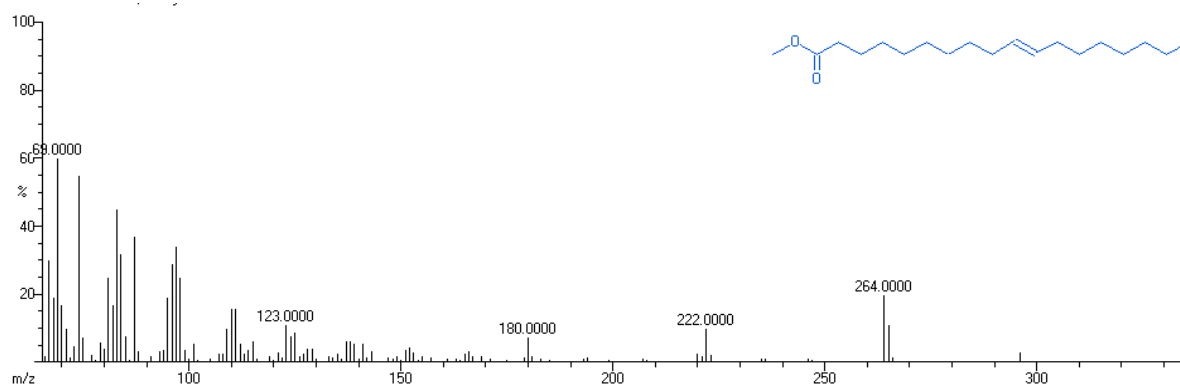


Fig 2C: 10-octadecenoic acid, methyl ester (RT: 18.90)

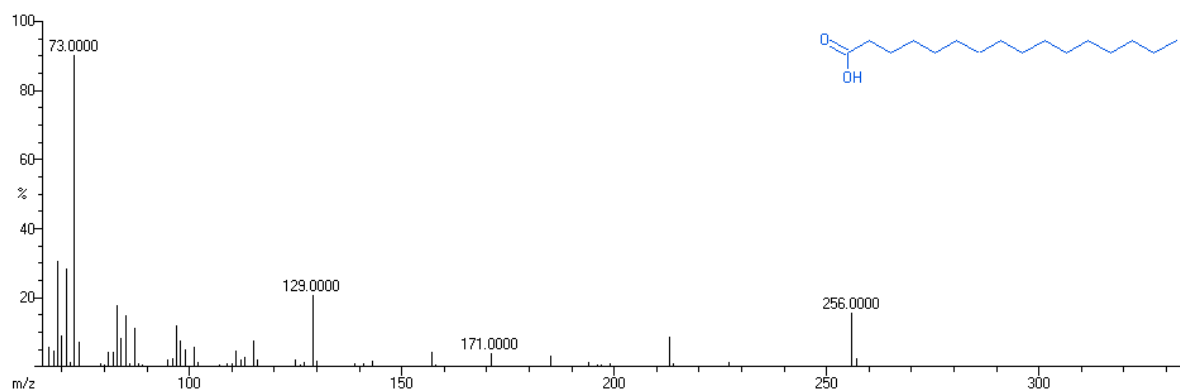


Fig 2D: n-hexadecanoic acid (RT: 17.97)

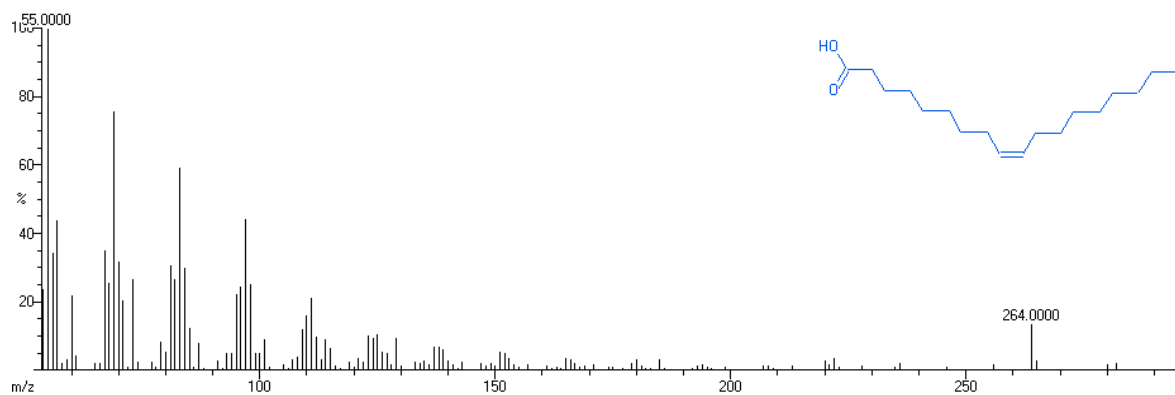


Fig 2E: oleic acid (RT: 19.65)

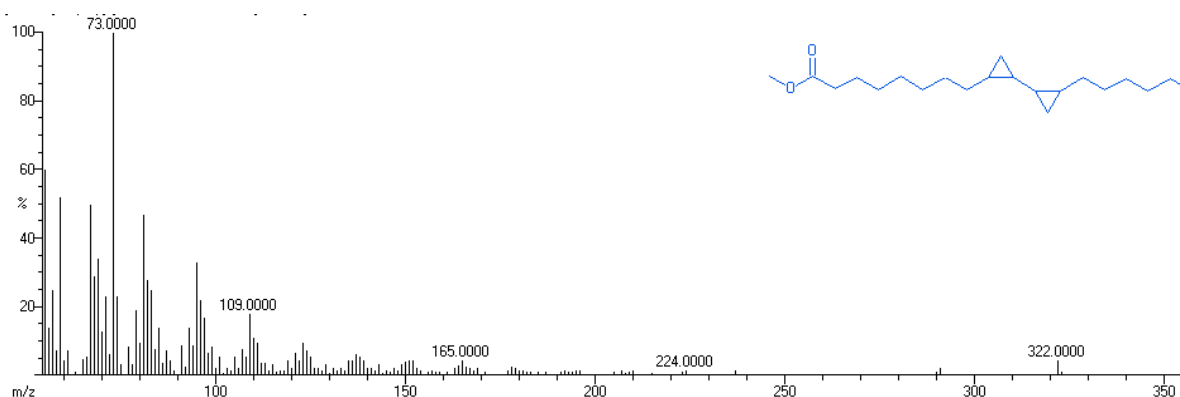


Fig 2F: [1, 1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (RT: 20.08)

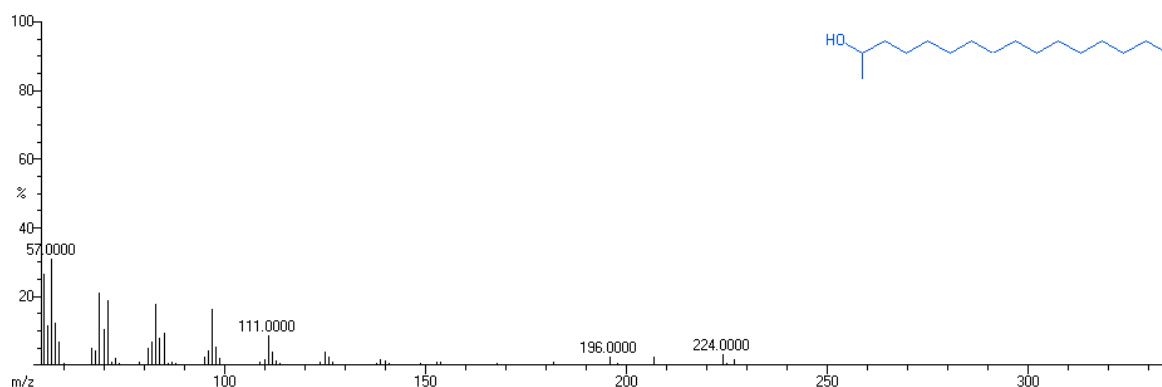


Fig 2G: 2-hexadecanol (RT: 29.88)

4. Conclusion

In this study, revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive and seven chemical constituents have been identified from ethanol extract of the leaf of *Macrotyloma uniflorum* by GC-MS analysis. The presence of various compounds justifies the use of the whole plant for various ailments by traditional practitioners. Thus this type of GC-MS analyses is the first step towards understanding the nature of active principles in this medicinal plant and this type of study

will be helpful for further detailed study. Further investigation into the pharmacological of *Macrotyloma uniflorum* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.

5. Acknowledgement

The authors would wish to acknowledge the management and Principal of Nehru Memorial College for providing research facilities and encouragements.

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