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ORIGINAL ARTICLE

GC – MS ANALYSIS OF ETHANOLIC LEAVES EXTRACT OF *Moringa concanensis* Nimmo

S.Chandasekar and *R.Malathi

Research Department of Biotechnology, Bharathidasan University Constituent College, Kurumbalur, Perambalur – 621107, Tamilnadu, India.

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ABSTRACT

The present investigation was carried out to determine the functional groups of active phytoconstituents of ethanolic leaves extract of *Moringa concanensis* using Gas Chromatography Mass Spectrometry. GC-MS analysis of ethanolic leaves extract of *Moringa concanensis* revealed that the existence of 1,2-15,16-Diepoxyhexadecane (36.25 %), Butanoic Acid, 3-Cyano-3-Hydroxy-, Ethyl Ester (14.29), N-Hexadecanoic Acid (11.48 %), Butanoic Acid, 3-Cyano-3-Hydroxy-, Ethyl Ester (8.15 %), Phytol (6.75 %), Tetratetracontane (6.18 %), Tetratetracontane (5.66 %), 2-[3-(4-Tert-Butyl-Phenoxy)-2-Hydroxy-Propylsulfanyl]-4,6-Dimethyl-NI (3.643 %), Acetamide, N-(6-Acetylaminobenzothiazol-2-YL)-2-(Adamantan-1-YL) (3.56 %), Nonadecane, 2-Methyl (2.54 %), 3,7,11,15-Tetramethyl-2-Hexadecen-1-OL (1.453 %). The result of this study reported that a platform in the leaves of *Moringa concanensis* for the bioactive components identification.

Keywords: GC-MS Analysis, Phytoconstituents, *Moringa concanensis*

1.INTRODUCTION

The *Moringa concanensis* is a multifunction tree. This tree has been cultivated in tropical regions of all over the world for the following characteristics. 1. High protein, vitamins, mineral, and carbohydrate content of entire plants and high value of nutrition for both humans and livestock; 2. high oil content (42%) of the seed which is edible, and with medicinal uses. The coagulant of seed could be used for waste water treatment (Foidl *et al.*, 2001). *Moringa concanensis* leaves have been used to combat malnutrition, especially among infants and nursing mothers and hasten uterine contraction during child birth in pregnant women. This leaves contain the properties of antihypertensive, diuretic, antispasmodic, antiulcer, anticancer, and cholesterol lowering activities have been reported (Caceres, 1992; Dangi *et al.*, 2002; Fahey *et al.*, 2004). In recent years, gas chromatography and mass spectrography (GC–MS) has been applied unambiguously to identify the structures of different phytoconstituents from plant extracts and biological samples (Prasain *et al.*, 2004; De Rijke *et al.*, 2006). Gas chromatography and mass spectrum is a reliable technique to identify the functional group of phytoconstituents of volatile matter, long chain branched hydrocarbons, alcohols, acids and esters (Anjali *et al.*, 2009). *Moringa concanensis* Nimmo (Moringaceae) is one of the imperative medicinal trees which are restricted in its distribution. *M. concanensis* occurs in tropical dry forest from southeastern Pakistan almost to the southern tip of India. It has recently been found in western Bangladesh. Indigenous knowledge of this plant in that region has not been so far studied. The main aim of the present work was to GC-MS analysis of the ethanolic leaves extract of *Moringa concanensis* Nimmo leaf for confirmation of active phytoconstituents.

Corresponding author:* ***Dr. R.Malathi,* *Research Department of Biotechnology, Bharathidasan University Constituent College, Kurumbalur, Perambalur – 621107, Tamilnadu, India*

2.MATERIALS AND METHODS

Collection of Plant Material

The fresh *Moringa concanensis* Nimmo leaves were collected from the Essanai village of Perambalur District in the month of February to March in 2016. Then the sample was washed with running tap water for removing the unwanted contaminants and debris.

Extract preparation

After washing the leaves were shade dried and powdered finely by using electrical grinder. After that grind into

powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with ethanol. The crude extract were collected and dried at room temperature at 30˚C after which yield was weighed and then performed
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GC-MS analysis

Gas chromatography study includes the important optimization process such as i) Introduction of sample extract into the GC column, ii) Separation of its components on an analytical column and iii) Detection of target analysis by using mass spectrometric (MS) detector. 5 ml of ethanolic leaves extract was evaporated to dryness and reconstituted in to 2 ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with instrument GC-MS-QP 2010 [SHIMADZU] instrument with Db 30.0 column (0.25µm diameter × 0.25um thickness). The oven temperature was programmed from 70 °C (isothermal for 5 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 35 min isothermal at 280°C. Mass spectra was taken at 70 eV; a scan interval of 0.5 s and Scan range from 40–1000 m/z. Helium was used as carrier gas at 99.999 % pressure with flow 1.0 ml/min and electronic pressure control on. Samples were dissolved in ethanol and injected automatically.

Analytical condition

The injection temperature at 2400°C, interface temperature at 2400°C and ion source temperature at 700°C were determined. Injection was performed in split less mode.

Identification of compounds (Data analysis)

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operator in scan mode from 40 to 1000 m/z atomic mass units. The identification based on the molecular weight, molecular formula, retention time and peak area in percentage. This work done in order to determine whether this plant species contains any individual compound or group of compounds which may substantiate its current commercial and traditional use as herbal medicine, in addition to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their assumed biological and therapeutic relevance.

3. RESULT AND DISCUSSION

Gas Chromatography – Mass Spectroscopy

GC-MS is the most excellent technique to identify the functional groups of bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compound. The present investication deals with the ethanolic leaves extract of *Moringa concanensis* to analysis the Gas Chromatography – Mass Spectroscopy. The extract is a complex mixture of many constituents totally eleven compounds identified (Table 1 & Fig 1). It may useful to identify the active phytochemical compounds from the plant species *Moringa concanensis*. GC-MS analysis of ethanolic leaves extract of *M.concanensis* revealed that the existence of the active compounds like 1,2-15,16-Diepoxyhexadecane (36.25 %), Butanoic Acid, 3-Cyano-3-Hydroxy-, Ethyl Ester (14.29), N-Hexadecanoic Acid (11.48 %), Butanoic Acid, 3-Cyano-3-Hydroxy-, Ethyl Ester (8.15 %), Phytol (6.75 %), Tetratetracontane (6.18 %), Tetratetracontane (5.66 %), 2-[3-(4-Tert-Butyl-Phenoxy)-2-Hydroxy-Propylsulfanyl]-4,6-Dimethyl-NI (3.643 %), Acetamide, N-(6-Acetylaminobenzothiazol-2-YL)-2-(Adamantan-1-YL) (3.56 %), Nonadecane, 2-Methyl (2.54 %), 3,7,11,15-Tetramethyl-2-Hexadecen-1-OL (1.453 %) were present. The compounds are identified with their retention time, Molecular formula, Molecular weight and concentration (percentage of peak area). The GC-MS chromatogram of the five peaks of the compounds was detected. Chromatogram GC-MS analysis of the ethanolic leaves extract of *Moringa concanensis* leaf showed the presence of 11 major peaks and the components corresponding to the peaks were determined.

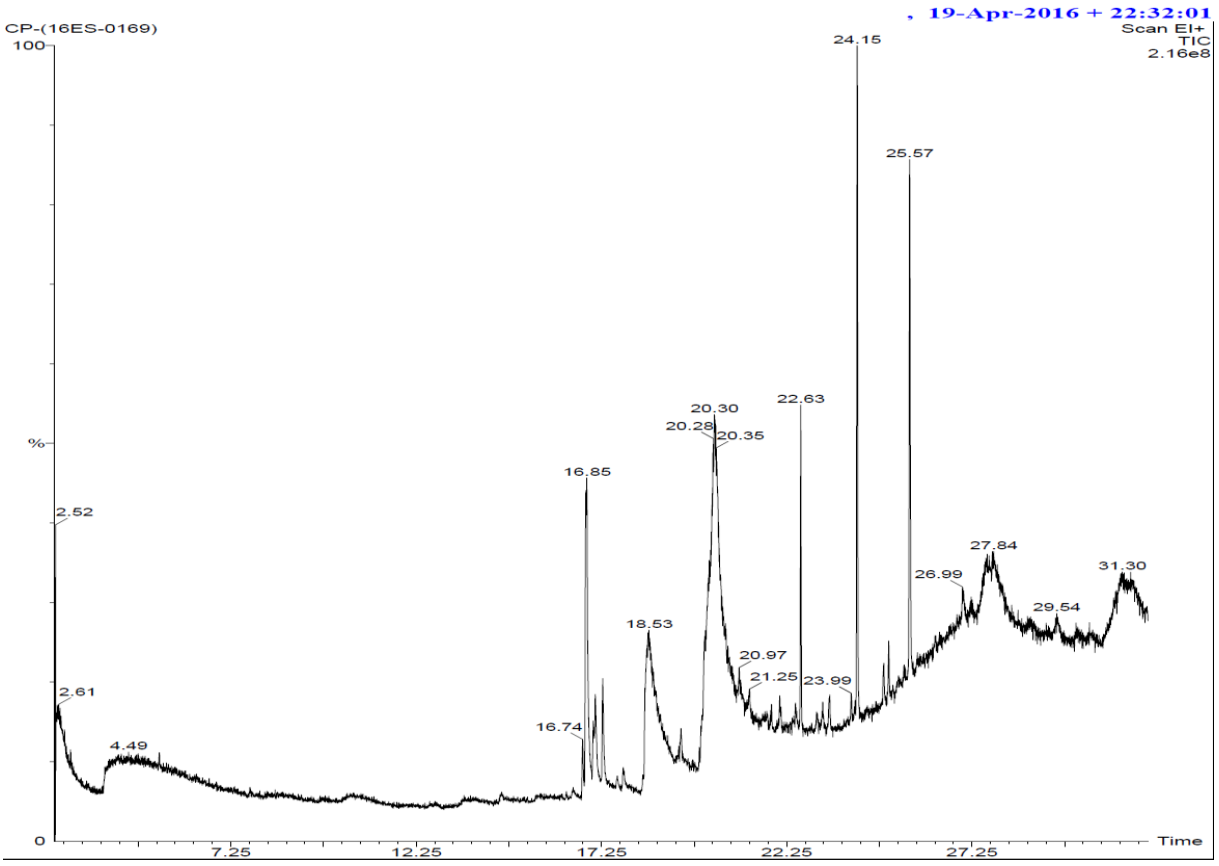
Secondary metabolites such as alkaloids, terpenoids, polyketides, steroids, flavonoids, phenolics, glycosides, etc. have remained the major contributors in addressing the traditional and modern pharma needs of mankind. Not only are they used directly as therapeutic entities, but also as raw materials for developing novel structural derivatives. In the past decade the international trade in herbal medicines alone was estimated at nearly USD 60 billion and is estimated to reach several hundred billions US dollars in near future (Koehn and Carter, 2005; Kusari *et al.*, 2014).

Previous research reported that the activities of phytochemical constituents like flavonoids and their derivatives (hexadecanoic acid, ethyl ester and n-hexadecaonoic acid), unsaturated fatty acid showed antimicrobial, antiinflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary (Kumar *et al.*, 2010). The etanolic leaves extract of *Moringa concanensis* Nimmo already reported that it has a very good antioxidant activity. It possesses the significant free radical scavenging activity. This result may leads to the development of novel anticancer drugs from the *Moringa concanensis* plant (Malathi and Chandrasekar, 2016). Phytol is one of the active compound are used to be cancer preventive. The presence of phytol compounds attributes to the antimicrobial, anti-inflammatory and anticancer properties (Cho *et al*., 2010; Munakata, 1983). This research work supported that crude extracts from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and Shekhawat, 2010). This report also supports our GC-MS result of ethanolic leaves extract of *M.concanensis* contains hexadecanoic acid and stigmasterol compounds that have the property of antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory (Jagadheeswari *et al.*, 2012). This result confirms that the ethanolic leaves extract of *M.concanensis* has wide variety of biologically active compounds.

Table 1: Gas Chromatography and Mass Spectrometry analysis of the ethanolic leaves extract of *Moringa concanensis* Nimmo

S. No	Compound Name	Percentage of Peak Area	Retention time	Molecular formula	Molecular weight (m.wt)
1.	Butanoic acid, 3-Cyano-3-hydroxy- Ethyl ester	14.29	4.49	$C_7H_{11}O_3N$	157
2.	Butanoic acid, 3-Cyano-3-hydroxy- Ethyl ester	8.15	5.32	$C_7H_{11}O_3N$	157
3.	Phytol	6.75	16.85	$C_{20}H_{40}O$	296
4.	3,7,11,15-Tetramethyl-2-Hexadecen-1-OL	1.453	17.28	$C_{20}H_{40}O$	296
5.	N-Hexadecanoic acid	11.48	18.53	$C_{16}H_{32}O_2$	256
6.	1,2-15,16-Diepoxyhexadecane	36.25	20.30	$C_{16}H_{30}O_2$	254
7.	Nonadecane, 2-Methyl	2.54	22.63	$C_{20}H_{42}$	282
8.	Tetratetracontane	6.18	24.15	$C_{44}H_{90}$	618
9.	Tetratetracontane	5.66	25.56	$C_{44}H_{90}$	618
10.	Acetamide, N-(6-Acetylaminobenzothiazol-2-YL)-2-(Adamantan-1-YL)-	3.56	27.66	$C_{21}H_{25}O_2N_3S$	383
11.	2-[3-(4-Tert-Butyl-Phenoxy)-2-Hydroxy-Propylsulfanyl]-4,6-Dimethyl-NI	3.643	27.83	$C_{21}H_{26}O_2N_2S$	370

Figure 1: Showing GC-MS analysis of the ethanolic extract of *Moringa concanensis* Nimmo leaves



4. CONCLUSION

The work was concluded that ethanolic leaves extract of *Moringa concanensis* possess various potent bioactive compounds. Further studies are needed to explore the potential bioactive compounds responsible for the biological activities of *Moringa concanensis*. It may leads to the development of novel drugs.

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