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RESEARCH ARTICLE

IDENTIFICATION OF BIOACTIVE CONSTITUENTS IN *MORINGA CONCANENSIS* LEAF USING GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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ABSTRACT

The present investigation was carried out to determine the chemical components of ethanolic extracts of *Moringa concanensis* leaves using Gas Chromatography–Mass Spectrometry. GC-MS analysis of ethanolic extract of *Moringa concanensis* leaves 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 results of this study offer a platform of using *Moringa concanensis* leaves as herbal alternative for various diseases.

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INTRODUCTION

The Moringa tree is a multi-function plant. It has been cultivated in tropical regions all over the world for the following characteristics. 1. High protein, vitamins, mineral, and carbohydrate content of entire plants: 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 leaves have been used to combat malnutrition, especially among infants and nursing mothers and hasten uterine contraction during child birth in pregnant women. It's 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 with great success (Prasain *et al.*, 2004; De Rijke *et al.*, 2006). Gas chromatography and mass spectrum is a reliable technique to identify the 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 extract of *Moringa concanensis* Nimmo leaf for confirmation and quantification of active phytoconstituents.

MATERIALS AND METHODS

Collection of Plant Material

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

Extract preparation

After washing the leaf 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, 30°C after which yield was weighed and then performed.

GC-MS analysis

Gas chromatography study includes the important optimization process such as i) introduction of sample extract onto 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 ethanol 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 × 0.25µm 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

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. Identification based on the Molecular weight, Molecular formula, Retention time and peak area %. It is 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 putative biological and therapeutic relevance.

RESULTS AND DISCUSSION

Gas Chromatography – Mass Spectroscopy

GC-MS is the most excellent technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compound. The present investigation deals with the ethanolic extracts of leaf of *Moringa concanensis* to analysis the Gas Chromatography – Mass Spectroscopy. The extracts are a complex mixture of many constituents totally eleven compounds identified (Table 1 & Fig 1).

3,7,11,15- Tetramethyl-2-Hexadecen-1-OL (1.453 %) were present. The compounds are identified with their retention time (RT), Molecular formula, Molecular weight and concentration (peak area %) of corresponding compounds.

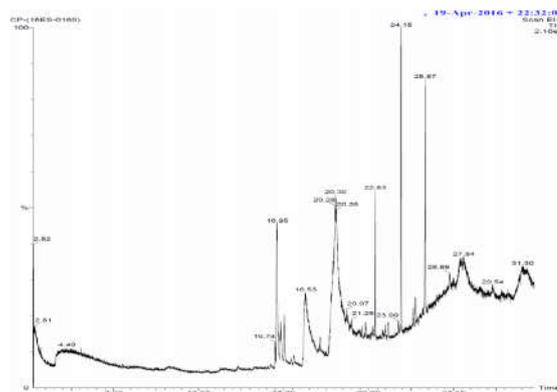


Figure 1 Showing GC-MS analysis of the Ethanolic extract of *Moringa concanensis* Nimmo leaf

The GC-MS chromatogram of the five peaks of the compounds was detected. Chromatogram GC-MS analysis of the ethanolic extract of *Moringa concanensis* showed the presence of 3 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 based on clinically validated scaffolds. Rising concerns about the unwarranted side effects of many allopathic drugs coupled with drug resistance are pushing the re entry of herbal drugs into the clinical R&D

Table 1 Gas Chromatography and Mass Spectrometry analysis of the Ethanolic extract of *Moringa concanensis* Nimmo

S. No	Compound Name	% of Peak Area	Retention time (RT)	Molecular formula (MF)	Molecular weight (MW)
1.	BUTANOIC ACID, 3-CYANO-3-HYDROXY-, ETHYL ESTER	14.29	4.49	C ₇ H ₁₁ O ₃ N	157
2.	BUTANOIC ACID, 3-CYANO-3-HYDROXY-, ETHYL ESTER	8.15	5.32	C ₇ H ₁₁ O ₃ N	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-ACETYLAMINO BENZOTHAZOL-2-YL)-2-(ADAMANTAN-1-YL)-	3.56	27.66	C ₂₁ H ₂₅ O ₂ N ₃ S	383
11.	2-[3-(4-TERT-BUTYL-PHENOXY)-2-HYDROXY-PROPYLSULFANYL]-4,6-DIMETHYL-NI	3.643	27.83	C ₂₁ H ₂₆ O ₂ N ₂ S	370

It may useful to identify the active chemical compounds from the plant species of *Moringa concanensis*. GC-MS analysis of ethanolic extract of *M. 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%), Tetratetra contane (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

Laboratories. In fact, 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).

Therefore, there is an urgent need to systematically research the well established medicinal plants using state of the art tools and methodologies to derive the maximum pharma benefit from them. India is known for a rich tradition of ethnobotanical medicine-based remedial practices primarily

involving medicinal plants and their products. The country accounts for sizeable biodiversity on the planet (Kusari *et al.*, 2014).

Previous researchers reported that the activities of some plant constituents with compound nature of flavonoids, (hexadecanoic acid, ethyl ester and n-hexadecaonic acid), unsaturated fatty acid as antimicrobial, antiinflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary (Kumar *et al.*, 2010). Phytol is one of the active compound present in the *M.concanensis* are said to be cancer preventive. The presence of phytol compounds attributes to the antimicrobial, anti-inflammatory and anticancer property of the plant leaves (Cho *et al.*, 2010; Munakata, 1983). It is believed that crude extracts from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and Shekhawat, 2010). Hexadecanoic acid and stigmasterol compounds have the property of antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory (Jagadheeswari *et al.*, 2012).

CONCLUSION

It was concluded that ethanol extract of *Moringa concanensis* leaves possess various potent bioactive compounds and anti microbial, anti inflammatory and anticancer properties it is recommended as drug formation to pharmaceutical industries. Further studies are needed to explore the potential bioactive compounds responsible for the biological activities of *Moringa concanensis*.

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