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GC-MS analysis of leaf and Bark Extract of *Moringa concanensis* Nimmo, a siddha medicinal plant of South India

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Abstract
Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. *Moringa concanensis* (*M. concanensis*) is belonging to family of Moringaceae and well known in Indian traditional system for its traditional uses.
Aim of the study: Hence the objective of the present study is to explore the possible bioactive compounds in the ethanolic extracts of leaf and bark of *M. concanensis*, using GC-MS Methods.
Methods: GC-MS analysis of the plant extracts were performed by using Perkin-Elmer GC clauses 500 system and mass spectra of the compounds found in the extract was matched with the data in the library of National Institute of Standards and Technology (NIST).
Results: In this GC-MS analysis, 15 compounds in leaf and 16 compounds in bark were identified in ethanolic extracts of *M. concanensis*.
Conclusions: The presence of various bioactive compounds justifies *M. concanensis* is an excellent phytocomponents and to treat various disease and complications in human beings.
Abbreviations: *M. concanensis, Moringa concanensis*; GC–MS, gas chromatography-mass spectrometry; NIST, National Institute of Standards and Technology; RT, retention time; MV, molecular weight

Keywords: GC-MS analysis; *Moringa concanensis*; leaf; bark; phytocomponents.

1. Introduction

Medicinal plants have been used since thousands of years from the beginning of human civilization for its therapeutic properties, containing inherent active ingredients that have properties to heal sores, relieve pain, cure diseases (Owolabi *et al*., 2007) [12] and maintenance of overall good health of the human beings (Bailey and Day, 1989) [2]. Medicinal properties of plants provide ample opportunity for development and obtaining a wide variety of drugs. Therefore investigated further to better understand their safety and efficacy (Nascimento *et al*., 2000) [10]. The property of herbal medicine is highly dependent upon the composition of chemical phytoconstituents in their extracted final product. There is a current need for the development of method for establishing quality control parameters for ayurvedic formulations owing to variability and complexity of chemical constituents present in herbal plant based drugs (Shailajan and Menon, 2011)]14].

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [4].

The preparation of the drug from *M. concanensis* this plant is easy and simple. The wide structural variability of this compound has attracted the curiosity of chemists and the biological activities protested by various natural products and inspired the pharma industries to reach for new structure.

The plant Kattumurungai is entirely different from the Murungai (*Moringaoleifera*). Leaves and flowers are larger in size than *M. oleifera.* The appearance of bark shows distinct feature in both the species of *Moringa*. Bark is smooth and is hard in both the plants respectively. Twenty types of human ailments may be cured by using *M. concanensis* with simple preparations. The therapeutic values of *M. concanensis* are described with disease cured, part used, mode of drug preparation and method of consumption (Anbazhakan *et al*., 2007) [1] *M. concanensis* is a small tree with thick bark, glabrous, except younger parts and inflorescence. Leaves are bipinnate (very rarely tripinnate), ca. 45 cm long. Pods are linear, 30–45 cm long, sharply three-angled. The horseradish odour of *M. concanensis* is more intense than *M. oleifera. M. concanensis* has a strong central trunk that is covered with an extremely distinctive layer of very furrowed bark that can be more than 15 cm thick. The flowers also have distinctive yellow petals, with red or pink veins (Balamurugan and Balakrishnan, 2013 [3, 4], Manzoor *et al*., 2007 [9]). The leaves and bark are the most potent part of the plant for medicinal use. It is used for treatment of skin tumor, tiredness,to reduce blood pressure, aphrodisiac, jaundice, eyecare, diabetes and bloat (Anbazhakan *et al*., 2007) [1]. The aim of this present work has been made to investigate the chemical composition in the leaf and bark extract of *M. concanensis.*

2. Materials and Methods

2.1. Collection of plant Material

The *Moringa concanensis* Nimmo plant was collected in the month of March 2010 from the Esanai Village of Perambalur District, Tamil Nadu, India, surrounded by South Arcot in the North (Villupuram and Cuddalore), Trichirrappalli on south, and Salem on west and Thanjavur on the east and lies between Lat.11° 14' N; Long. 78° 56' E. *M. concanensis* is widely distributed on dry lands.

2.2. Preparation of Extract

The samples were air-dried and powdered. Required quantity of powder was weighed and transferred to stopper flask and treated with the ethanol until the powder was fully immersed. The flask was shaken on every hour for the first six hours and it was kept a side and again shaken after 24 hours. This process was repeated for three days and the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue was obtained and subjected to GC-MS analysis.

2.3. GC-MS Analysis

GC-MS analysis of the leaf and bark extracts was carried out by following the method of Hema *et al.,* (2010) [5]. GC- MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-I fused silica capillary column (30m×0.25mm ID × 1µdf) and composed of 100% Dimethyl polysiloxane. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed split ratio of 10:1 injector temperature 2500 °C; ion-source temperature 2800 °C. The oven temperature was programmed from 110ºC (isothermal for 2 min) with an increase of 100 °C/min to 2000 °C, then 50 °C/min to 2800 °C, ending with a 9 min isothermal at 2800°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

2.4. Identification of phytocomponents

Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute of Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Then, molecular weight and structure of the components of the test materials were ascertained.

3. Result

3.1. Phytocomponents identified in the plant *M.concanensis* leaf by GC-MS

GC-MS method was used for the analysis of the leaf and bark extracts can be an interesting tool for testing the amount of active compounds in herbs used in cosmetic, drugs, pharmaceutical or food industries. The acidic fractions were silylated and subjected to GC-MS investigation. It is evident from the (Figure 1) all fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of authentic samples and library data of corresponding compound. The components present in the ethanol extract of whole plant of *M. concanensis* leaf were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of whole plant of *M. concanensis* leaf is presented in the (Table 1). Fifteen compounds were detected in ethanol extract of the whole leaf. The results revealed that, Pantolactone (22.72%) was found as major component followed by DL-3-4 Dimethyl-3,4- hexanediol (14.05%), 3,4 dimethyl 5 hexen 3 – ol (14.05%), 1,5-Hepatadiene, 3,3, Dimethyl-(E) (11.71%), Pentane, 1, 3-epoxy – 4 methyl (11.12%), Butanic acid, 2, hydroxyl-2-methyl, methyl ester (10.54%), 3-Dodecen-1-ol (6.44%), 1,3-Dioxolane, 2 (1.76%), 2,2'- Bioxirane (1.76%), Allylipo nitrite (1.17%), 3- Butyn – 2- ol (1.17%), 3 buten -2 – ol (1.17%), 3,4 dimethyl 5 hexen 3 – ol (1.17%), 2- propanoic acid, 2 propanyl ester (0.59%), 3-Pentanol,2, 4 Dimethyl (0.59%). The GC-MS chromatogram with peak area is given in figure 1. The important phyto-components and structure identified in *M.concanensis* leaf extract is shown in Table 3.

Table 1. Phytocomponents identified in the plant *M.concanensis* leaf by GC-MS

S.No	Phyto Components	Molecular Formula	% of peak area	Retention Time	Molecular weight
01.	3-Dodecen-1-ol	$C_{12}H_{24}O$	6.44	11.38	184
02.	Allylipo nitrite	$C_6H_{10}N_2O_2$	1.17	13.20	142
03.	3- Butyn – 2- ol	C_4H_6O	1.17	14.52	70
04.	Pantolactone	$C_6H_{10}O_3$	22.72	14.65	130
05.	2- propanoic acid, 2 propanyl ester	$C_6H_8O_2$	0.59	15.39	112
06.	3 buten -2 – ol	C_4H_8O	1.17	19.92	72
07.	2,2'- Bioxirane,	$C_4H_6O_2$	1.76	20.45	86
08.	1,5-Hepatadiene, 3,3, Dimethyl-(E)	C_9H_{16}	11.71	24.22	124
09.	Pentane, 1, 3-epoxy – 4 methyl	$C_6H_{12}O$	11.12	25.40	100
10.	3,4 dimethyl 5 hexen 3 – ol	$C_8H_{16}O$	1.17	27.06	128
11.	Butanic acid, 2, hydroxyl-2-methyl, methyl ester	$C_6H_{12}O_3$	10.54	27.30	132
12	1, 3 – Dioxolane, 2 –(3 – bromo -5,5,5 – tricloro -2,2 – dimethylpentyl)	$C_{10}H_{16}Brcl_3O_2$	1.76	29.30	352
13.	DL-3-4 Dimethyl-3,4- hexanediol	$C_8H_{18}O_2$	14.05	30.51	146
14.	3,4 dimethyl 5 hexen 3 – ol	$C_8H_{16}O$	14.05	31.66	128
15.	3-Pentanol,2,4Dimethyl	$C_7H_{16}O$	0.59	33.53	116

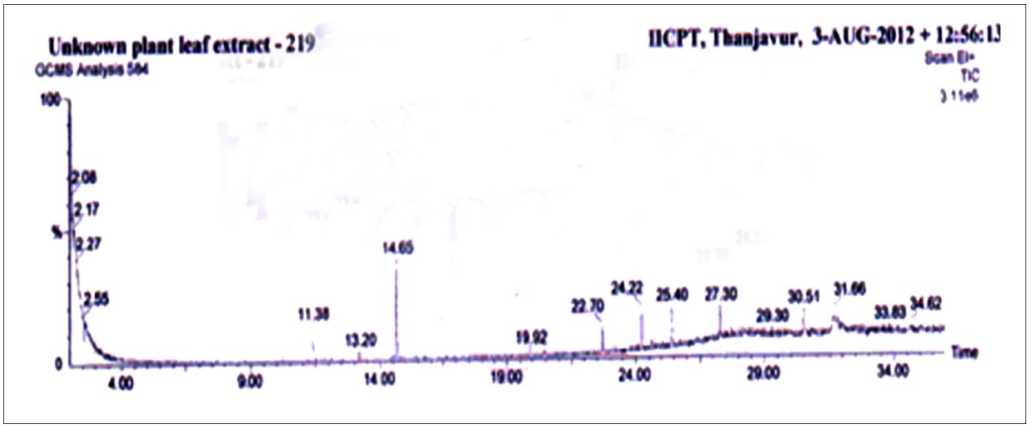


Fig 1. GC-MS Chromatogram of *M. concanensis* leaf

3.2. Phytocomponents identified in the plant *M.concanensis* bark by GC-MS

The components present in the bark extract of whole plant of *M.concanensis* bark were identified by GC-MS analysis (Figure 2). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of whole plant of *M.concanensis* bark are presented (Table 2). Sixteen compounds were detected in ethanol extract of the whole bark. The results revealed that, Squalene (13.34%), 1-Nonene, 4,6,8- trimethyl-(13%), Trimethyl (4-tert-butyphenoxy silane) (11.51%), 1-Hexanol, 2-ethyl-2-propyl-(11.04%), 2,4,6-cycloheptatrien-1-one,3,5-bis-trimethylsilyl-(9.68%) 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (9.34%), 2-Bromonane(7.52%), Hexanedioic acid, bis (2-ethylhexyl)(6.36%), Heptane, 2,2,3,3,5,6,,6-Heptamethyl-(3.86%), Dibutyl phthalate (3.66%), Heptanoic acid, 2 – ethyl-(2.71%), 4- Dodecanol (2.37%), 1,14-Tetradecanediol (2.23%), 1-Hepten-4-ol (2.17%), 4-Nonene, 3-methyl- (0.68%), Isooctanol (0.54%).The GC-MS chromatogram with peak area was given in figure 2. The important phytocomponents and structure identified in *M.*concanensis bark extract is shown in Table 4.

Table 2. Phytocomponents identified in the plant *M.concanensis* bark by GC-MS

S.No	Phyto Components	Molecular Formula	% of peak area	Retention Time	Molecular weight
01.	Isooctanol	$C_8H_{18}O$	0.54	8.68	130
02.	4-Nonene, 3-methyl-,	$C_{10}H_{2O}$	0.68	10.91	140
03.	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	2.23	11.38	230
04.	Dibutyl phthalate	$C_{16}H_{22}O_4$	3.66	12.93	278
05.	Heptanoic acid, 2 – ethyl-	$C_9H_{18}O_2$	2.71	13.22	158
06.	1-Hepten-4-ol	$C_7H_{14}O$	2.17	14.73	114
07.	4- Dodecanol	$C_{12}H_{26}O$	2.37	15.74	186
08.	Hexanedioic acid, bis(2-ethylhexyl)	$C_{22}H_{42}O_4$	6.36	18.65	370
09.	Heptane, 2,2,3,3,5,6,,6-Heptamethyl-	$C_{14}H_{30}$	3.86	20.23	198
10.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	9.34	20.75	278
11.	2-Bromonane	$C_9H_{19}Br$	7.52	21.72	206
12	1-Nonene, 4,6,8- trimethyl-	$C_{12}H_{24}$	13.0	23.22	168
13.	1-Hexanol, 2-ethyl-2-propyl-	$C_{11}H_{24}O$	11.04	24.70	172
14.	Squalene	$C_{30}H_50$	13.34	24.80	410
15.	Trimethyl(4-tert-butyphenoxy) silane)	$C_{13}H_{22}OSi$	11.51	26.19	222
16.	2,4,6- cycloheptatrien-1-one,3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi$	9.68	34.71	250

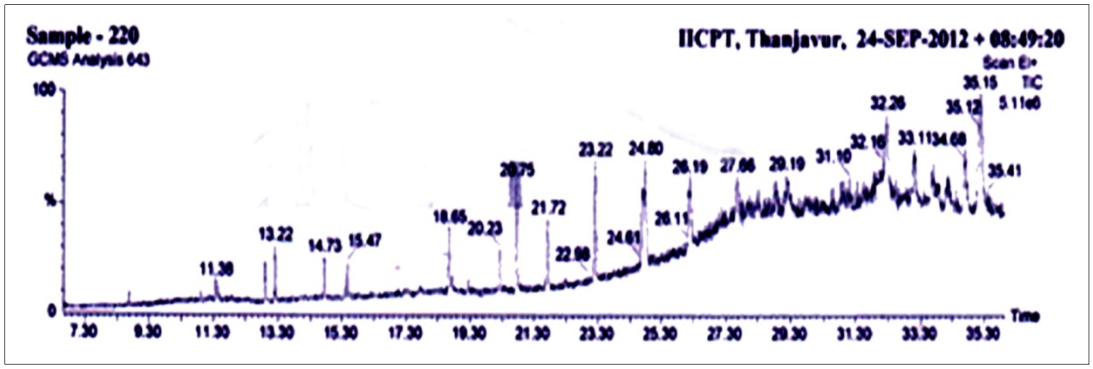


Fig 2. GC-MS Chromatogram of *M. concanensis* bark

Table 3. Structure of phytocomponents from *M. concanensis* leaf by GC-MS analysis

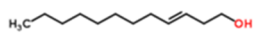
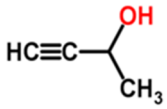
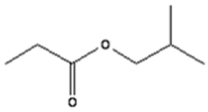
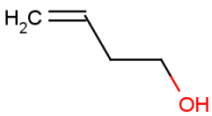
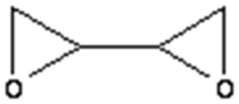
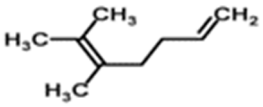
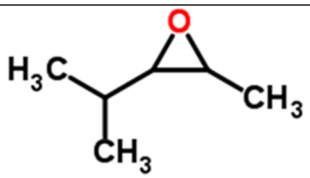
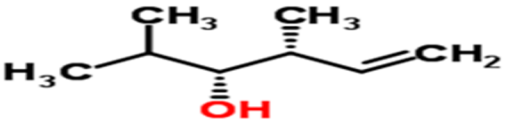
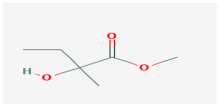
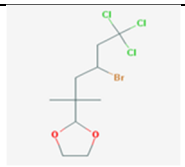
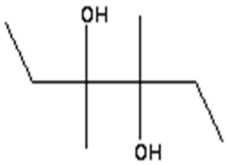
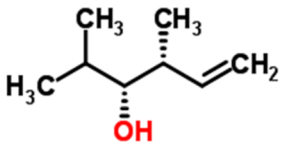
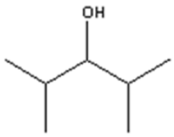
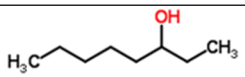
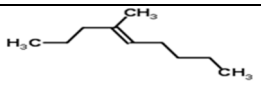
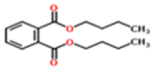
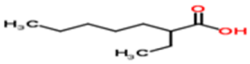
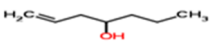
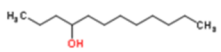
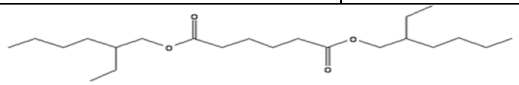
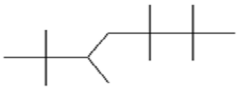
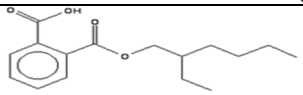
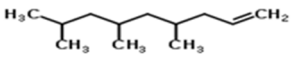
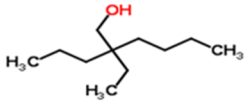
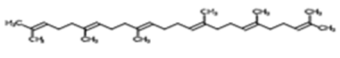
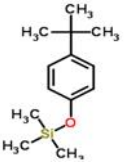
 3-Dodecen-1-ol	 3- Butyn – 2- ol	 Pantolactone
	 2,2'- Bioxirane	 1,5-Hepatadiene, 3,3, Dimethyl-(E)
 Pentane, 1, 3-epoxy – 4 methyl	 3,4 dimethyl 5 hexen 3 – ol	
 Butanic acid, 2, hydroxyl-2-methyl, methyl ester	 1, 3 – Dioxolane, 2 –(3 – bromo -5,5,5 – tricloro -2,2 – dimethylpentyl	 DL-3-4 Dimethyl-3,4- hexanediol
	 3-Pentanol,2,4Dimethyl	

Table 4. Structure of phytocomponents from *M. concanensis* bark by GC-MS analysis

 Isooctanol	 4-Nonene, 3-methyl-	1,14-Tetradecanediol
 Dibutyl phthalate	 Heptanoic acid,2 – ethyl-	 1-Hepten-4-ol
 4- Dodecanol	 Hexanedioic acid, bis(2-ethylhexyl)	
 Heptane, 2,2,3,3,5,6,,6-Heptamethyl-	 1,2 Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	 1-Nonene, 4,6,8- trimethyl-
 1-Hexanol, 2-ethyl-2-propyl-	 Squalene	 Trimethyl(4-tert-butyphenoxy) silane)

4. Discussion

Now a day the study of the organic compounds from plants and their activity has increased. Gas Chromatography - Mass Spectrometry (GC - MS) is a valuable tool for reliable identification of bioactive compounds (Johnson *et al*., 2011) [6]. In the present study, 15 compounds have been identified from the ethanol extract of leaf and 16 from bark of *M. concanensis* by GC - MS analysis. The most abundant components found in the leaf were Butanic acid, 1,5-Hepatadiene, 3,3, Dimethyl-(E) and 2- propanoic acid, 2 propanyl ester. Whereas bark extract contain important phytochemicals like, Squalene, 1-Hexanol, 2-ethyl-2-propyl, 1, 2-Benzenedicarboxylic acid, Hexanedioic acid, Heptane, Heptanoic acid, and Isooctanol.

The identified phytochemicals and squalene has an antioxidant activity. It has been found that squalene possesses chemo-preventive activity against the colon carcinogenesis (Rao *et al*., 1998) [13]. Phytol, a bioactive principle, detected from *Sarcostemma secamone* (L.) and *M.concanensis* is also found to be effective at different stages of arthritis. It is found to give good as well as preventive and therapeutic results against arthritis. Reactive oxygen species-promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al*., 2009) [11]. Different types of compounds were found in this fraction. Dehydroabietic acid was present in considerable amounts. Dehydroabietic acid is an antibacterial (Soderberg *et al*., 1990) [15], anti-inflammatory (Li and McChesney, 1992) [8] and potential antitumor-promoting agents (Kinouchi *et al*., 2000) [7]. Flavonoids possess anticarcinogenic and anti-inflammatory properties. Chrysin is an anti-inflammatory and antibacterial agent and have been found to exhibit anti-HIV-activity (Wang *et al*., 1998) [16].

5. Conclusion

The result shows that the work is significant with respect to its content of various phytochemical compounds as well. In the present work nearly thirty one biochemical compounds were identified in *M. concanensis* leaf and bark extracts by GC-MS analysis. Further studies to be carry out in isolation and quantification of the compounds to analyze the antioxidant potential and need to evaluate *in vivo* studies are most significant to evaluate their natural biological activity.

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7. Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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