Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 748-751
Received: 24-03-2017
Accepted: 25-04-2017

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Identification of bioactive components in *Moringa oleifera* fruit by GC-MS

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Abstract
In this study, the flavoring components of *Moringa oleifera* fruit have been evaluated using Gas Chromatography–Mass Spectrometry. The chemical compositions of the hexane extract of drumstick were investigated using Shimadzu system Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology library. The extract of *Moringa oleifera* fruit contained 28 compounds of which the maximum quantum was 2,6-dihydroxybenzoic acid, 3TMS derivative (38.8%) followed by tetrapentacontane (20.6%), Other notable compounds were hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl (8%), 2-propenoic acid, pentadecyl ester (5.97%), 3,4-dihydroxymandelic acid, 4TMS derivative (5.29%), bis(2-ethylhexyl) phthalate (2.56%), 1-dodecanol (2.53%) and glycidyl oleate (2.51%). The results of this study offer usage of *Moringa oleifera* fruit will promote the functional properties in human body.

Keywords: *Moringa oleifera* fruit, bioactive compounds, GC-MS, health benefits

Introduction
Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [1]. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides [2, 3]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care. The screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes [4, 5]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [6].

Moringa oleifera or sahijan, Lam is one of the most widely cultivated species of the monogenic family Moringaceae in recent times. This is also called for "Miracle Tree" or "Drumstick Tree". The moringa tree had spread to most part of Asia, nearly the whole of Africa, South America, Southern parts of North America and some parts in Europe. The plant is cultivated in the semi-arid, tropical and sub-tropical areas of the world [7]. The moringa plant originated initially in the Northern parts of India and soon moved into Southern parts, the name itself in Tamil is pronounced as "*murungai keerai*" (moringa leaves) and "*murungai kaai*" (moringa vegetable) which is the literal translation of *Moringa oleifera*. The *Moringa oleifera* fruit (Drumstick) is a three-sided pod with nine longitudinal ridges that are about 20 to 50 cm long and 2.0 to 2.5 cm wide [8] and its immature pods are green or reddish, and mature pods are brown with 15-20 large brown to black seeds with three papery wings [9]. *M. oleifera* is well known for its medicinal properties and rich bioactive molecules. Its pharmacological significance (particularly hypotensive property) has been known since ancient times [10].

The fresh drumstick contained 85.39g of water, 2.62g of protein, 1.27g of ash, 0.12g of total fat, 6.83g of total fibre, 5.60g of insoluble fibre, 1.23 g of soluble fibre, 3.76g of carbohydrate, 123 KJ energy, 1.67µg of ergocalciferol, 358µg of vitamin K_1, 17.28µg of β-carotene, 350 µg of total carotenoids, 33.30mg of calcium, 0.73mg of iron, 52.87mg of phosphorus and 139mg of total polyphenol per 100g [11]. The most volatile components present for isobutyl isothiocyanate, isopropyl isothiocyanate, sec-butyl isothiocyanate, n-butylisothiocyanate and benzyl isothiocyanate in seed kernel, isobutyl isothiocyanate, isopropyl isothiocyanate, n-butyl isothiocyanate and sec-butyl isothiocyanate in isolated oil of the leaf from *Moringa peregrine* [12]. The various parts of moringa plant such as leaves, roots, seed, fruits, flowers and immature pods act as circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory [13].

In southern Asia it also used as an indigenous system of medicine for treatment of antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering antioxidant, antidiabetic, hepatoprotective [14, 15, 16].

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 [17, 18]. Gas chromatography and mass spectrum is a reliable technique to identify the phyto constituents of volatile matter, long chain branched hydrocarbons, alcohols, acids and esters [19]. The main aim of the present work was to GC-MS analysis of the hexane extract of drumstick for confirmation of volatile components.

Materials and Methods

Plant material and extraction procedure

Fully mature, fresh PKM_2 drumstick variety was purchased from Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Tamil Nadu. It was ground into electrical mixer (Preethi, 2008 Model) and an aliquot of 250 ml was extracted with 500 ml of hexane in Yoga ERS-45 electrical shaker on 250 rpm for 24 hours. The mixed sample was filtered by using Whatman® No. 41 filter paper (pore size 20 - 25 m), rinsed with two part of hexane and evaporated under reduced pressure to a volume of 30 ml using evaporator [20].

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

GC-MS analysis was carried out on GC-MS-QP2020 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions : column VF-5MS fussed silica capillary column (30.0m x 0.25mm x 0.25µm, composed of 5 per cent phenyl/95 percent dimethyl polysiloxane), operating in electron impact mode at 70ev; helium (99.999%) was used as carrier gas at a constant flow of 1.0 ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 240 ^{0}C ion-source temperature 200 ^{0}C. The oven temperature was programmed from 70 ^{0}C (isothermal for 3 min), with an increase of 10 ^{0}C/minutes to 240 ^{0}C, ending with a 9 minutes isothermal at 280 ^{0}C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 440 Da. Total GC running time was 40min. The relative percentage 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 Shimadzu GC-MS Real time analysis.

Results and Discussion

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with 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.

Twenty eight major compounds were identified in *Moringa oleifera* fruit extract by GC-MS analysis. The active principles with their retention time, molecular formula, molecular weight and concentration (%) are presented in (Table 1 and Fig 1). The extract of *Moringa oleifera* fruit contained 28 major compounds of which the maximum quantum was 2,6-dihydroxybenzoic acid, 3TMS derivative (38.8%) followed by tetrapentacontane (20.6%), Other notable compounds were hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl (8%), 2-propenoic acid, pentadecyl ester (5.97%), 3,4-dihydroxymandelic acid, 4TMS derivative (5.29%), bis(2-ethylhexyl) phthalate (2.56%), 1-dodecanol (2.53%), and glycidyl oleate (2.51%).

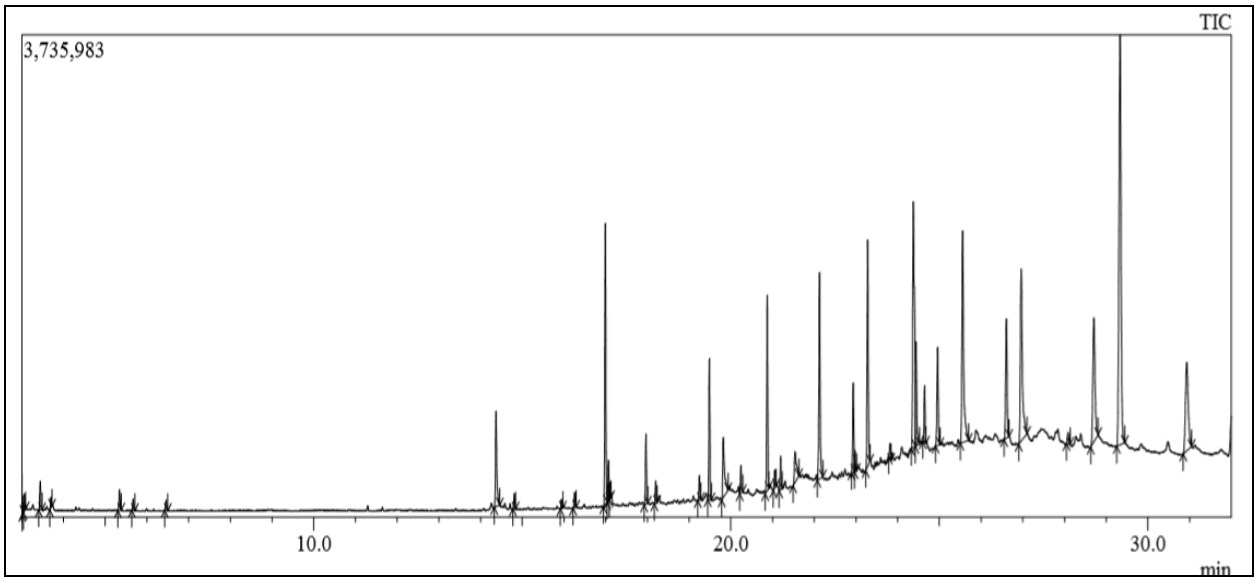
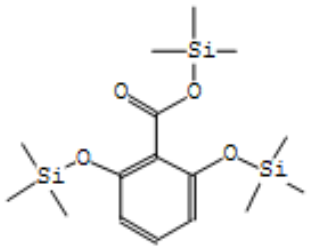


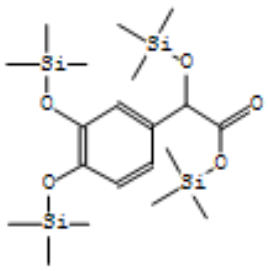
Fig 1: Chromatogram obtained from the GC-MS with the extract of *Moringa oleifera* fruit

Table 1: Total ionic chromatogram (GC–MS) of hexane extract of *Moringa oleifera* fruit obtained with 70 eV using a VF-5MS fused silica capillary column with He gas as the carrier.

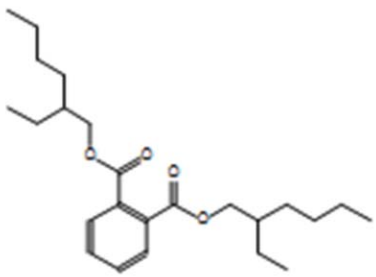
S. No.	Retention time	Name of the component	Molecular formula	Molecular weight	Peak Area %
1.	3.020	Cyclohexane, 1,2-dibromo-4-(1,2-dibromoethyl	$C_8H_{12}Br_4$	424	0.10
2.	3.061	2-Butanol, 2,3-dimethyl-	$C_6H_{14}O$	102	0.35
3.	3.443	Toluene	C_7H_8	92	0.59
4.	3.700	Cyclopentanol, 1-methyl-	$C_6H_{12}O$	100	0.20
5.	5.342	Pentane, 3-ethyl-2,4-dimethyl-	C_9H_{20}	128	0.44
6.	5.671	1-Pentanol, 2,2-dimethyl-	$C_7H_{16}O$	116	0.24
7.	6.459	3-Hexen-2-one	$C_6H_{10}O$	98	0.23
8.	14.371	1-Dodecanol	$C_{12}H_{26}O$	186	2.53
9.	14.810	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	0.31
10.	15.490	Heneicosane	$C_{21}H_{44}$	296	0.17
11.	16.254	3,4-Dihydroxyphenylglycol, 4TMS derivative	$C_{20}H_{42}O_4$	458	0.32
12.	16.993	2-Propenoic acid, pentadecyl ester	$C_{18}H_{34}O_2$	282	5.97
13.	17.067	Propanoic acid, decyl ester	$C_{13}H_{26}O_2$	214	0.89
14.	17.100	Heneicosane	$C_{21}H_{44}$	296	0.22
15.	19.820	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	2.12
16.	21.055	Behenic alcohol	$C_{22}H_{46}O$	326	0.37
17.	21.195	Eicosane	$C_{20}H_{42}$	282	2.09
18.	21.539	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	1.38
19.	22.935	Glycidyl palmitate	$C_{19}H_{36}O_3$	312	1.93
20.	23.281	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	$C_{12}H_{38}O_5Si_6$	430	8.00
21.	23.815	Tetracosane	$C_{24}H_{50}$	338	0.27
22.	24.442	Glycidyl oleate	$C_{21}H_{38}O_3$	338	2.51
23.	24.646	Pentatriacontane	$C_{35}H_{72}$	492	1.17
24.	24.958	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	2.56
25.	28.086	Squalene	$C_{30}H_{50}$	420	0.34
26.	28.075	2,6-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	38.8
27.	29.333	Tetrapentacontane	$C_{54}H_{110}$	758	20.6
28.	30.932	3,4-Dihydroxymandelic acid, 4TMS derivative	$C_{20}H_{40}O_5Si_4$	472	5.29



2,6-Dihydroxybenzoic acid, 3TMS derivative



3,4-Dihydroxymandelic acid, 4TMS derivative



Bis(2-ethylhexyl) phthalate

The presence of component 2,6-dihydroxybenzoic acid, tetrapentacontane, 2-propenoic acid, pentadecyl ester, 3,4-dihydroxymandelic acid has acted as a various therapeutic and pharmaceutical benefits such as hydroxylation of liver enzymes during phase I metabolism, hair growth promoter, inhibit production of uric acid and arachidonic acid inhibitor in human body respectively (PubChem Database). Dihydroxybenzoic acids are used as intermediates for pharmaceuticals, especially for antipyretic analgesic, ant rheumatism and antimicrobial activity [21].

Conclusion

Among this study, there are twenty eight major compounds were identified from *Moringa oleifera* fruit extract by GC-MS. The compounds possess various potential health benefits. It is recommended as the production of nutraceutical based food manufacturers and drug developers and further studies also needed to explore these compounds in a manner of *in vivo*.

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