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R Saahithya
Postgraduate Research Scholar,
Department of Veterinary
Pathology, Madras Veterinary
College, TANUVAS, Chennai,
Tamil Nadu, India

R Sridhar
Professor and Head,
Department of Veterinary
Pathology, Veterinary College
and Research Institute,
Tirunelveli, Tamil Nadu, India

P Sriram
Professor and Head, Department
of Veterinary Pharmacology and
Toxicology, Madras Veterinary
College, TANUVAS, Chennai,
Tamil Nadu, India

Gas chromatography and mass spectroscopy analysis (GC-MS) of the phytochemical constituents of *Momordica charantia* (Indian variety Co.1)

R Saahithya, R Sridhar and P Sriram

Abstract

The aim of this study was to delineate various bioactive compounds present in the *Momordica charantia* (Indian variety Co.1) available at the Chennai locality based on characterisation using gas chromatography and spectroscopy analysis.

The aqueous extract of *Momordica charantia* (MC) was prepared from the unripened fruits. They were split open, deseeded, grounded in a blender and extract collected was centrifuged and stored. Part of the extract was freeze dried and GC-MS analysis was performed and analysed for the active phytochemical compounds. Eleven compounds were identified through GC-MS analysis of MC extract.

The major compound present in the aqueous extract was 10-Octadecanoic acid, methyl ester and Pentadecanoic acid, methyl ester. The RT of the compounds were 18.7 and 17.05 minutes respectively and the relative peak area was 10.7 and 9.8 per cent respectively. The other compounds identified were based on NIST library search.

Keywords: *Momordica charantia*, aqueous extract, Gas chromatography and mass spectroscopy, Indian variety Co.1, unripened fruits, phytochemical bioactive compounds

1. Introduction

Momordica charantia L. (Bitter gourd) is a common vegetable grown widely in Asia that is used as a traditional medicine. It is commonly known as bitter gourd and belongs to the family Cucurbitaceae. Two varieties of this plant are cultivated in India viz., *M. charantia* var. *charantia* which were fusiform in shape and *M. charantia* var. *muricata* which are identified by small round fruit. The bitter flavour of both varieties is due to the alkaloid momordicine produced in fruits and leaves.

The unripened fruits of vegetable MC had been used as folklore medicine for the management of ailments such as leprosy, eczema, piles, rheumatism, malaria, amenorrhoea, hypertension, stomach pain, infections, cold, and cough and most efficiently for diabetes due to its antioxidant (Kubola and Siriamornpun, 2008) [8], antimicrobial (Mwambete, 2009) [9], hypoglycemic and hypolipidaemic action (El-Bakyn *et al.* 2009) [4]; anticancer activity, antiplasmodial, antileishmania (Gupta *et al.* 2010) [5] and antiulcer activity (Paul *et al.* 2010) [11].

2. Materials and Methods

2.1 Collection of unripened fruits of *Momordica charantia* (Indian variety Co.1)

The unripened fruits of *Momordica charantia* (MC) were procured from the local market of Chennai and were identified by a botanist. They were identified as Indian variety Co.1.

2.2 Preparation of aqueous extract of *Momordica charantia*

The aqueous extract from the unripened fruits of *Momordica charantia* were prepared by manually. The unripened fruits were cleaned thoroughly in fresh water, air dried and then weighed.

The unripened fruits were slit horizontally to remove the pulp and seeds. The remaining portions of the fruits were chopped and was thoroughly grounded using a blender. It was allowed to strain through a muslin cloth into a beaker. The collected extract was filled in centrifuge tubes and centrifuged at 3000g for 30 minutes at 4 °C. The sediment at the bottom was discarded and the clear supernatant at the top was collected in centrifuge tubes at -20 °C.

A fraction of the collected aqueous extract was subjected for freeze drying at -56 °C and GC-MS analysis carried out. The freeze dried powder was stored at -20 °C until further analysis.

Correspondence

R Saahithya
Postgraduate Research Scholar,
Department of Veterinary
Pathology, Madras Veterinary
College, TANUVAS, Chennai,
Tamil Nadu, India

2.3 Gas Chromatography and Mass Spectroscopy (GC-MS)

The freeze dried fraction of the *Momordica charantia* aqueous extract was analyzed by GC-MS. A bench top Agilent gas chromatograph (Model 6890N) equipped with a mass selective detector (Model 5973N) was used. A volume of 1 μ L sample solution was injected manually into the GC-MS system. Injection port and detector temperatures were 230 $^{\circ}$ C and 220 $^{\circ}$ C respectively. The column temperature was initially held at 100 $^{\circ}$ C for 5 min and then increased at 10 $^{\circ}$ C/min to 250 $^{\circ}$ C with isothermal hold at this temperature for 10 min. Mass spectrum (MS) was set with electron impact (EI) ionization mode setting at 1500 V with ion source temperature of 240 $^{\circ}$ C and MS quad temperature 150 $^{\circ}$ C.

Mass spectral acquisition was performed using GC-MSD Agilent Chem Station Software. The spectra were acquired by scanning all ions from 35 to 600 m/z. Identification of the separated compounds was made by matching MS values of the compounds in the National Institute of Standards and Technology (NIST) library.

3. Results and Discussion

The results of the GC-MS analysis revealed eleven bioactive compounds which are presented in Table 1, Fig. 1. The identification of the compounds was performed by similarity searches and mass spectra data in the NIST (National Institute for Standard and Technology) MS Search 2.0 Library. It was confirmed based on the bioactive compound, retention time (RT), molecular formula and peak area.

Eleven compounds were identified by spectral analysis and the overall spectrum is presented in Fig. 1. The major compound present in the aqueous extract was 10-Octadecenoic acid, methyl ester and Pentadecanoic acid,

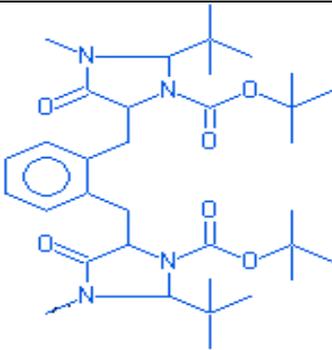
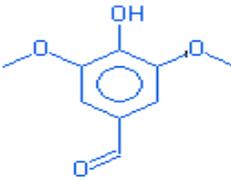
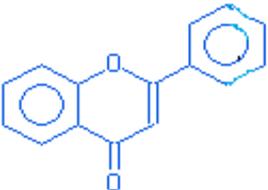
methyl ester. The RT of the compounds were 18.7 and 17.05 minutes respectively and the relative peak area was 10.7 and 9.8 per cent respectively.

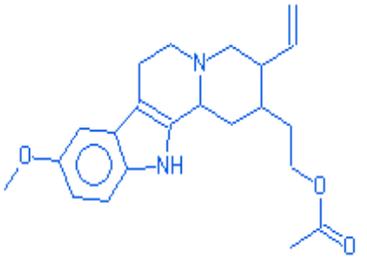
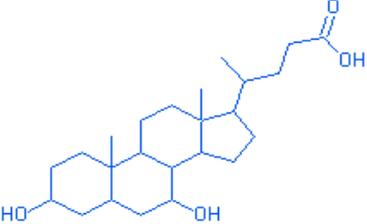
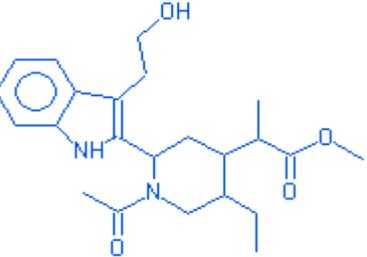
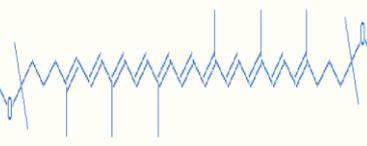
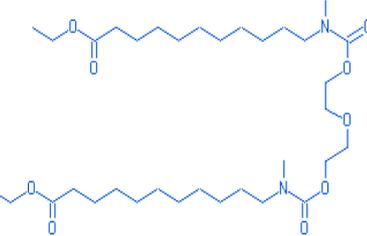
The other compounds identified were 1- Imidazolidine carboxylic acid, 5,5'-[1,2-phenylenebis (methylene)] bis[2-(1,1-dimethylethyl)-3-methyl-4-oxo-bis (1,1-dimethyl)ester, (2S-[2 α , 5 α]; Benzaldehyde, 4-hydroxy-3,5-dimethoxy; Flavone; E,E,Z-1,3,12-Nonadecatriene-5,14-diol; Corynan-17-ol, 18,19-didehydro-10-methoxy acetate (ester); Ursodeoxycholic acid; 4 - Piperidine acetic acid, 1-acetyl-5-ethyl-2[3-(2-hydroxyethyl)-1H-indol-2-yl]- α - methyl-methyl ester; Lycopene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-all-trans and Diethylene glycol, bis [N-methyl-N-(10-ethoxycarbonyldecyl) carbamate].

Basic pubmed search tool was used to find the properties of the individual compounds obtained in our results. Among the various compounds, five compounds were shown to possess anticancer effects. They were Flavones, Pentadecanoic acid methyl ester, 10-Octadecenoic acid methyl ester, Ursodeoxycholic acid and Lycopenes (Ujiki *et al.* 2006; Bobe *et al.* 2008; Arem *et al.* 2013) [12, 3, 1]. They stated that there was a statistically significant reduced cancer risk with high total flavones intake.

In addition, these compounds inhibited carcinogenesis by inhibition of proliferation, cell cycle arrest, induction of apoptosis, promotion of differentiation, antioxidant activity and inhibition of angiogenesis and decrease the metabolic activation of procarcinogens. Pentadecanoic acid methyl ester and 10-Octadecenoic acid methyl ester had properties related to antioxidant effects and also capable of alleviating cancer (Belakhdar *et al.* 2015; Nkondjock *et al.* 2005; Han *et al.* 2013; Koh *et al.* 2017) [2, 10, 6, 7].

Table 1: Bioactive compounds detected in the aqueous extract of *Momordica charantia* (Indian variety Co.1) by GC-MS analysis

Sl. No	Name of the compound	Molecular structure	Retention time (minutes)	Relative peak area (%)
1.	1- Imidazolidine carboxylic acid, 5,5'-[1,2-phenylenebis (methylene)] bis[2-(1,1-dimethylethyl)-3-methyl-4-oxo-bis (1,1-dimethyl)ester (2S-[2 α , 5 α]		29.03	5.5
2.	Benzaldehyde, 4-hydroxy-3,5-dimethoxy		12.83	1.2
3.	Flavone		15.08	1.8

4.	Pentadecanoic acid, methyl ester		17.05	9.8
5.	10-Octadecenoic acid, methyl ester		18.7	10.7
6.	E,E,Z-1,3,12-Nonadecatriene-5,14-diol		19.58	4.3
7.	Corynan-17-ol, 18,19-didehydro-10-methoxyacetate (ester)		20.73	3.6
8.	Ursodeoxycholic acid		21.97	3.6
9.	4 - Piperidine acetic acid, 1-acetyl-5-ethyl-2[3-(2-hydroxyethyl)-1H-indol-2-yl]-à - methyl-methyl ester		24.15	2.7
10.	Lycopene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-all-trans		27.4	2.2
11.	Diethylene glycol, bis[N-methyl-N-(10-ethoxycarbonyldecyl)carbamate]		32.23	6.9

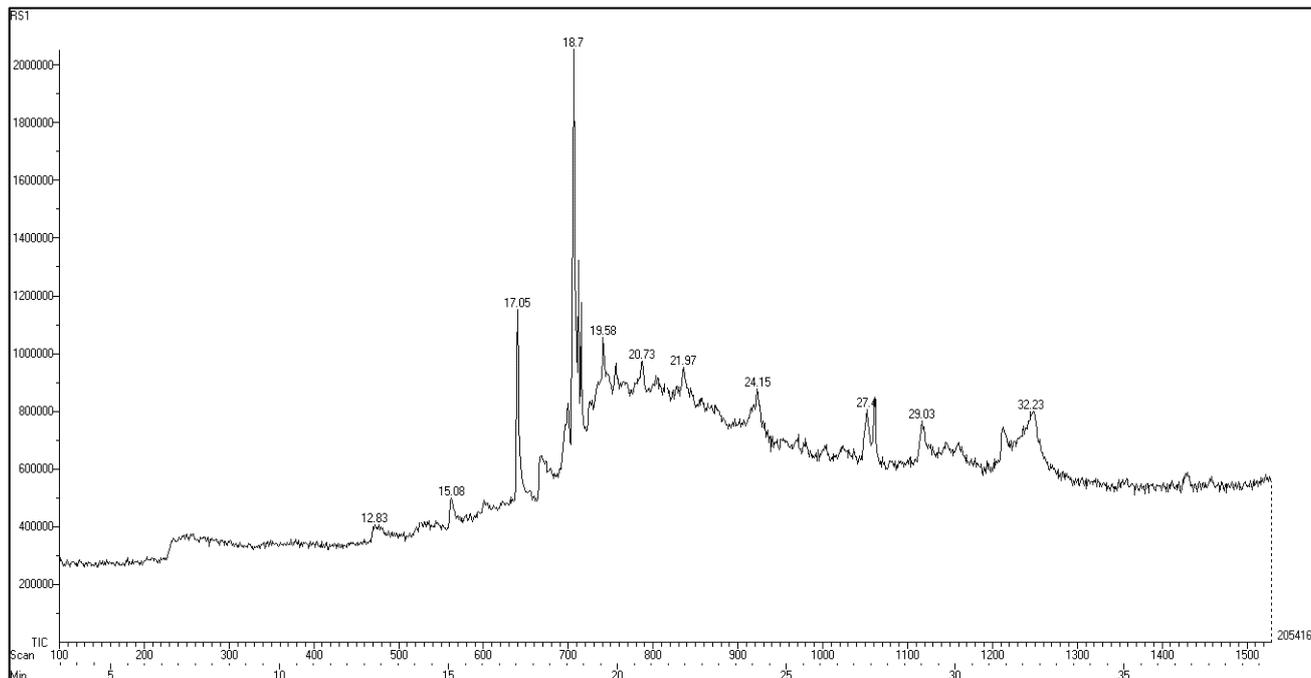


Fig 1: GC-MS chromatogram of aqueous extract of unripened fruit of *Momordica charantia* (Indian variety Co.1)

4. Conclusions

This study revealed the phytochemical constituents of aqueous extract of *Momordica charantia* (Indian variety Co.1) available in the Chennai locality and its various properties which could be significantly made use of in the future studies in conventional and alternative medicine apart from its use as part of day to day vegetable in Asian cuisine.

5. Acknowledgments

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