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Research Article

Determination of Phytocomponents in Methanolic Extract of *Annona muricata* Leaf Using GC-MS Technique

K. Shibula [1], S. Velavan[2*]

[1]*Research Scholar, Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, S. India*
[2]*Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, S. India*

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ABSTRACT
The aim of this study was to carry out for identification of bioactive compounds from the methanolic extract of *Annona muricata* leaves by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer 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 (NIST) library. The GC-MS analysis revealed the presence of various compounds like 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, Tetradecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Phytol and Octadecanoic acid in the methanolic extract of *Annona muricata*. These findings support the traditional use of *Annona muricata* in various disorders.

Keyword: Gas chromatography and Mass spectroscopy, *Annona muricata*, Phytochemistry

INTRODUCTION

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function[1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations[2]. Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines[3].

Plant produces many chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates)[4]. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits[5]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals[6]. The chosen medicinal plant namely as *Annona muricata*.

Annona muricata L. belongs to the family of Annonaceae has a widespread pantropical distribution and has been pridely known as corossol. It is a widespread small tree and has its native in Central America[8]. The fruit of *Annona muricata* Linn. is found to be edible in Yunnan province of China9 and their fruits is used commercially for the production of juice, candy and sherbets. Intensive chemical investigations of the leaves and seeds of this species have resulted in the isolation of a great number of acetogenins. The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties. Roots of these species are used in traditional medicine due to their antiparasitical and pesticidal properties[7 -12]. The aim of this study is to determine the organic compounds present in the *Annona muricata* leaf extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

MATERIAL AND METHODS

Plant materials

The fully mature *Annona muricata* leaves were collected in April 2013 from Tamil University, Thanjavur District,

*Author for Correspondence

Table 1: Shows the components identified in methanolic extract of *Annona muricata* (GC MS study)

Peak	R.Time	Area %	Height %	Molecular Formula	Name of the compounds
1	4.620	0.65	0.78	$C_6H_{14}N_2$	1-Pyrrolidineethanamine
2	5.120	1.27	1.55	$C_6H_8O_4$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
3	7.744	4.32	3.78	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
4	8.595	1.73	1.65	$C_9H_{17}N$	1-(3-Methyl-3-butenyl) Pyrrolidin
5	8.833	2.27	1.65	C_8H_8O	1-(3-Methyl-3-butenyl)pyrrolidine
6	12.344	1.51	1.16	$C_{15}H_{24}$	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-
7	13.285	2.15	1.67	$C_{15}H_{24}$	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-
8	6.034	0.73	0.85	$C_{15}H_{24}$	Naphthalene, 1,2,3,5,6,8A-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- $$
9	20.208	1.03	1.00	$C_{15}H_{24}$	Tetradecanoic acid
10	20.400	4.08	2.67	$C_5H_6N_{2O}$	2-Amino-3-hydroxypyridine
11	21.320	22.65	24.96	$C_{20}H38$	2,6,10-Trimethyl,14-ethylene-14-pentadecne
12	21.406	2.49	2.28	$C_{20}H_{40}O$	(2E)-3,7,11,15-tetramethyl-2-hexadecene
13	21.649	3.50	3.94	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	21.891	6.16	7.14	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	22.438	0.68	0.80	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
16	22.864	20.86	19.87	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
17	24.597	9.11	10.12	$C_{22}H_{42}O_2$	Phytol
18	24.845	5.42	5.73	$C_{17}H_{32}O_2$	cis-10-Heptadecenoic acid
19	24.892	6.06	5.03	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
20	25.050	3.29	3.37	$C_{18}H_{36}O_2$	Octadecanoic acid
		100.00	100.00		

Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr.S.John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts

The collected *Annona muricata* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. 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 TurboMass Ver 5.2.0

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. Additionally, it can identify trace in materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample [13,14.] Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Annona muricata* by GC-MS.

S.NO.	R.Time	Name of the compound	Biological activity **
	7.744	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	Antimicrobial,Anti inflammatory
	20.208	Tetradecanoic acid	Antioxidant, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic
	21.891	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic Antioxidant
	22.438	Hexadecanoic acid, methyl ester	Antioxidant, hypocholesterolemic , Anti androgenic , hemolytic, Alpha reductase inhibitor.
	22.864	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
	24.597	Phytol	Cancer-preventive
	25.050	Octadecanoic acid	Antibacterial Antifungal

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions[15].

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. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

GC-MS Analysis

Twenty compounds were identified in *Annona muricata* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, Tetradecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Phytol and Octadecanoic acid. Biological activity of phyto-components identified in the methanolic extracts of the *Annona muricata* represented in table 2.

Karpagasundari and Kulothungan[16] screened the bioactive components of *Physalisminima* leaves have been evaluated using GCMS. GC/MS analysis of extract of *Physalisminima* leaves revealed the existence of Heneicosanoic acid (25.22), Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9, 12-dienoic acid (32.02).

The GC-MS analysis of *Caesalpinia italica* leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-Linolenic acid possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol Diterpene is an antimicrobial, anticancer, antiinflammatory and diuretic agent[17.] 9, 12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and di-isooctyl ester were present in *Caesalpinia sappan* ethanol extract [18]. Similar types of compounds were identified among the twenty compounds of this present study.

Similarely the work was done in GC-MS analysis of bioactive components of *Hugonia mystax L*. (Linaceae). Thirteen compounds were identified. 1,2-Benzenedicarboxylic acid, diisooctyl ester (48.75%) was found to be major component followed by n-Hexadecanoic acid (13.52%), Phytol (9.25%), Squalene (6.41%), Vitamin E (4.09%), Dianhydromannitol (3.56%), 9,12 – Octadecadienoic acid (Z,Z) – (3.20%) and 3,7,11,15 – tetramethyl -2- hexadecen -1-ol (2.85%) .The presence of varios bioactive compounds justifies the use of the leaf for various ailments by traditional practitioners. So it is recommended as a plant of phytopharmaceutical importance[19].

Phytol is one among the twenty compounds of the present study. Similarly Maria Jancy Rani *et al.*,[20] investigated the presence of phytol in the leaves of *Lantana camara.* Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells [21]. Phytol, Phenol, 2, 4-bis (1-phenylethyl) which are all have medicinal properties and is a key acyclic diterpene alcohol that is a precursor for vitamins E and K. It is used along with simple sugar or

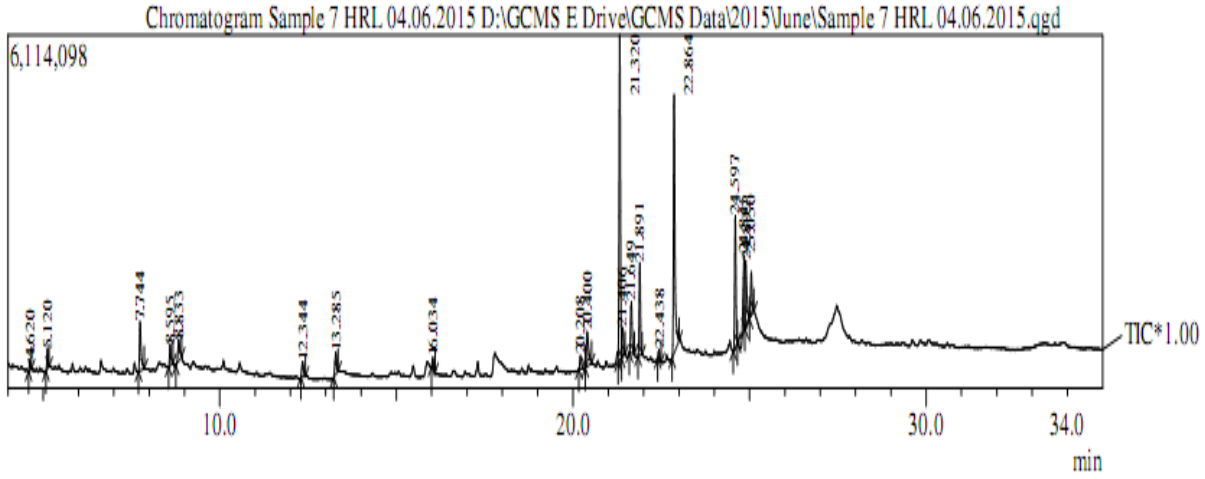

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Annona muricata.*

corn syrup as a hardener in candies. Similar types of compounds were identified among the twenty compounds of this present study.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

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