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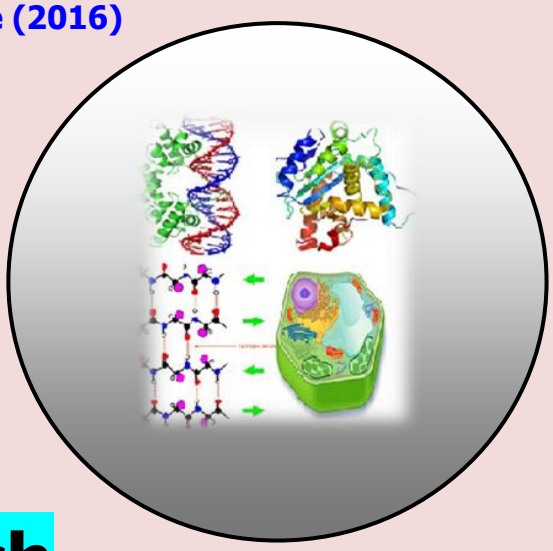

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T. Antony
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RESEARCH PAPER

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Bioactive Compounds in Ethanolic Extract of *Phoenix pusilla* Fruit Using GC-MS Technique

T. Antony Thangadurai and *S. Velavan

P.G and Research Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India

***P.G and Research Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India**

ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from the plant fruit ethanolic extract of Phoenix pusillaby Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of ethanolic 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 Tetradecane, 9-Eicosene, Hexadecanoic acid, Hexadecanoic acid methyl ester and 1,2-Benzenedicarboxylic acid, diethyl ester of Phoenix pusilla fruit. These findings support the traditional use of Phoenix pusilla fruitin various disorders.
Keyword: Gas chromatography and Mass spectroscopy, Phoenix pusilla and Phytochemistry.

INTRODUCTION

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 (Sathyaprabha *et al.,* 2010). 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 (Mathekaga and Meyer, 1998).

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 (Harborne, 1986).

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)(Liu, 2004). Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits (Hamburger and Hostettmann, 1991). 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 (Roberts and Xia, 1995). Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants (Ronald Hites, 1997). The chosen medicinal plant namely as *Phoenix pusilla* fruit belongs to Arecaceae Family. *Phoenix pusilla* fruitis widely distributed in southern India and Sri Lanka. The aim of this study is to determine the organic compounds present in the *Phoenix pusilla* fruit extract with the aid of GC-MS Technique.

MATERIAL AND METHODS

Plant materials

The *Phoenix pusilla*fruit were collected from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center 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 *Phoenix pusilla* fruit were washed several times with distilled water to remove the traces of impurities from the fruit. Then examined carefully old, infected and fungus damaged portion of the fruit were removed. Healthy fruit were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Phoenix pusilla* fruit extract was stored in refrigerator until 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 Turbo Mass Ver 5.2.0

RESULTS AND DISCUSSION

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample (Kell *et al*., 2005). In the last few years, GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Fernie *et al*., 2004). 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.

Table 1. Identification of bioactive compounds in ethanolic extract of *Phoenix pusilla* fruit extract using GC MS.

Peak#	R. Time	Area%	Molecular formula	Molecular weight	Molecular name
1	5.356	1.60	$C_{12}H_{27}N$	185	1-Hexanamine, N-hexyl- (CAS) Di-n-hexylamine
2	5.455	2.17	$C_5H_{10}O_4$	134	1,2,3-Propanetriol, monoacetate
3	7.207	1.10	$C_{16}H_{32}$	224	3-Hexadecene, (Z)-
4	7.305	1.98	$C_{15}H_{15}NO_4$	273	Tricyclo[4.2.2.0(2,5)]deca-7,9-diene-7,8-dicarboxylic acid, 3-cyano-, dimethyl ester
5	7.492	5.17	$C_{10}H_{10}O_4$	194	1,2-Benzenedicarboxylic acid, dimethyl ester
6	7.883	3.33	$C_{10}H_{13}N_5O_5$	283	Guanosine
7	7.938	3.38	$C_{19}H_{54}O_7SI_7$	590	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane
8	9.183	2.07	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure, Di-(Hex-1-En-5-Yl-Ester
9	9.250	9.19	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate
10	9.571	11.82	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure, Di-(Hex-1-En-5-Yl-Ester)
11	9.662	28.29	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic Acid, Diethyl Ester
12	9.725	2.90	$C_{20}H_{40}$	280	9-Eicosene
13	9.808	3.96	$C_{17}H_{36}$	240	Tetradecane,
14	9.947	9.28	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure, Di-(Hex-1-En-5-Yl-Ester)
15	10.847	1.03	$C_{21}H_{44}$	296	Pentadecane, 8-Hexyl- (CAS) 8-N-Hexylpentadecane
16	11.413	1.90	$C_{15}H_{17}Cl$	232	1-[2-Butyl-3-(1-Chlorovinyl)-2-Cyclopropenyl]Benzene
17	11.713	1.24	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
18	13.896	4.45	$C_{17}H_{34}O_2$	258	Hexadecanoic acid methyl ester
19	14.088	3.07	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, monobutyl ester.
20	14.225	2.05	$C_{28}H_{56}O_2$	424	Hexacosanoic acid, 2-methyl-, methyl ester

Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions (De-Fatima *et al*., 2006). 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.

Table 2. Biological activity of phyto components identified in the ethanol leaf extract of *Phoenix pusilla* fruit.

S. No	Compound Name	Biological activity
1.	Tetradecane	Anti-tuberculosis activity1
2.	9-Eicosene	Anti-microbial and cytotoxic properties
3.	Hexadecanoic acid	**Antioxidant**, Nematicide, 5-Alpha-Reductase-Inhibitor, Flavor, Hemolytic Hypercholesterolemic, Pesticide Antialopecic, Antiandrogenic Antifibrinolytic
4.	Hexadecanoic acid methyl ester	**Antioxidant** Hypocholesterolemic, Nematicide, Insecticide, Lubricant, Antiandrogenic Flavor, Hemolytic
5.	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate	Plasticizers, Used as a plasticizer for vinyl foams, which are often used as floor tiles. Other uses are in traffic cones, food conveyor belts, and artificial leather

****Duke's. Phytochemical and Ethnobotanical Databases, www.ars-gov/cgi-bin/duke/, 2013.**

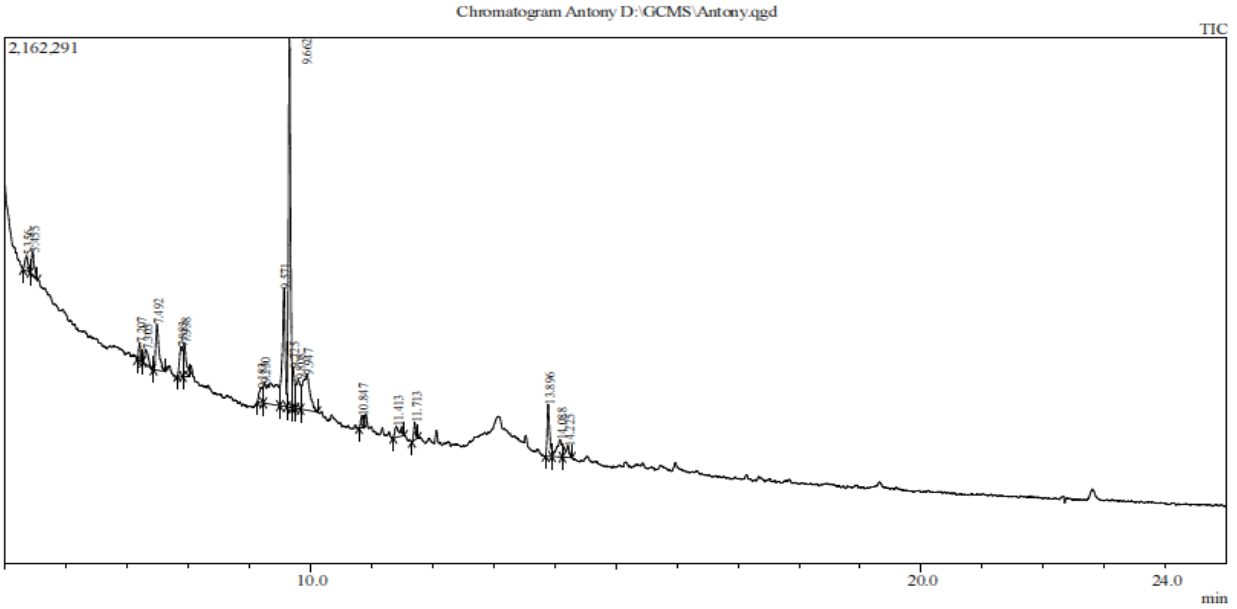

Figure 1. GC MS Chromatogram of *Phoenix pusilla* fruit extract.

Twenty compounds were identified in *Phoenix pusilla* fruit 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 Figure 1). The prevailing compounds were Tetradecane, 9-Eicosene, Hexadecanoic acid, Hexadecanoic acid methyl ester and1,2-Benzenedicarboxylic acid, diethyl ester. The biological activities of identified compounds were listed (Table 2) are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

Among the identified phytochemicals hexadecanoic acid is suggested to be a fatty acid ester and it may employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities (Bodoprost and Rosemeyer, 2007; Falodun *et al*., 2009). 1, 2- benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent (Heinonen *et al*., 1998).

Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic acid, 1,2-Benzenedicarboxylic acid, dibutyl ester, hexadecanoic acid, ethyl ester and 9, 12-octadecadienoic acid (Z,Z) were identified in the ethanolic leaf extract of *Vitexaltissima*, a Verbenaceae member (Sathish *et al*., 2012). Likewise, hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, 9,12- octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2-benzenedicarboxylic acid and 9-octadecenoic acid (Z)-ethyl ester were reported in *Clerodendruminerme* and *C. phlomidis* leaves (Anandhi and Ushadevi, 2013; Balaji and Kilimozhi, 2014).

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.

CONCLUSION

The investigation concluded that the stronger extraction capacity of ethanol 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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Corresponding author: T. Antony Thangadurai, Research Scholar, P.G and Research Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India
Email:mayavelvan@gmail.com

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