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GC-MS IDENTIFICATION OF PHYTOCOMPOUNDS IN THE METHANOLIC EXTRACT OF *ERYTHRINA INDICA*

Amargeetha A[1] and Velavan S[2]

1 Research Scholar, P.G and Research Department, Marudupandiyar College, Thanjavur,

2Associate Professor, P.G and Research Department, Marudupandiyar College, Thanjavur

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For Correspondence:

Dr. Velavan S

P.G and Research Department, Marudupandiyar College, Thanjavur

E-mail:

mayavelvan@gmail.com

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 whole plant methanolic extract of *Erythrina indica* 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 3, 7, 11, 15-Tetramethyl, cis-10-Nonadecenoic acid, n-Hexadecanoic acid Methyl 9-cis,11-trans, 9,12,15-Octadecatrienoic acid, Octadecanoic acid and Squalene in the methanolic extract of *Erythrina indica* . These findings support the traditional use of *Erythrina indica* in various disorders.

1. 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 [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]. 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 [3].

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]. 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 [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 Erythrina indica flower belongs to the Fabaceae family. The name "coral tree" is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. [7]. *E. Indica* shows several other characteristic pharmacological effects like neuromuscular blocking, smooth muscle relaxant, CNS depressant, analgesic and hydrocholerectic action, which are consistent

with the reported uses of the plant extracts in the indigenous system of medicine. It has shown potential effects in convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds [8 -11]. The aim of this study is to determine the organic compounds present in the *Erythrina indica* extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

2. MATERIAL AND METHODS

2.1 Plant materials:

The fully mature *Erythrina indica* flower were collected in April 2013 from Tamil University, Thanjavur District, 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.

2.2 Preparation of extracts:

The collected *Erythrina indica* flower 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% ethanol 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.

2.3 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

3. RESULTS AND DISCUSSION

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 biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions [14].

3.1 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 (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3.2 GC-MS ANALYSIS

Twenty compounds were identified in *Erythrina indica* 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 3,7,11,15-Tetramethyl, cis-10-Nonadecenoic acid, n-Hexadecanoic acid Methyl 9-cis,11-trans, 9,12,15-Octadecatrienoic acid, Octadecanoic acid and Squalene. Biological activity of the important compounds represent in table 2.

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Erythrina indica.*

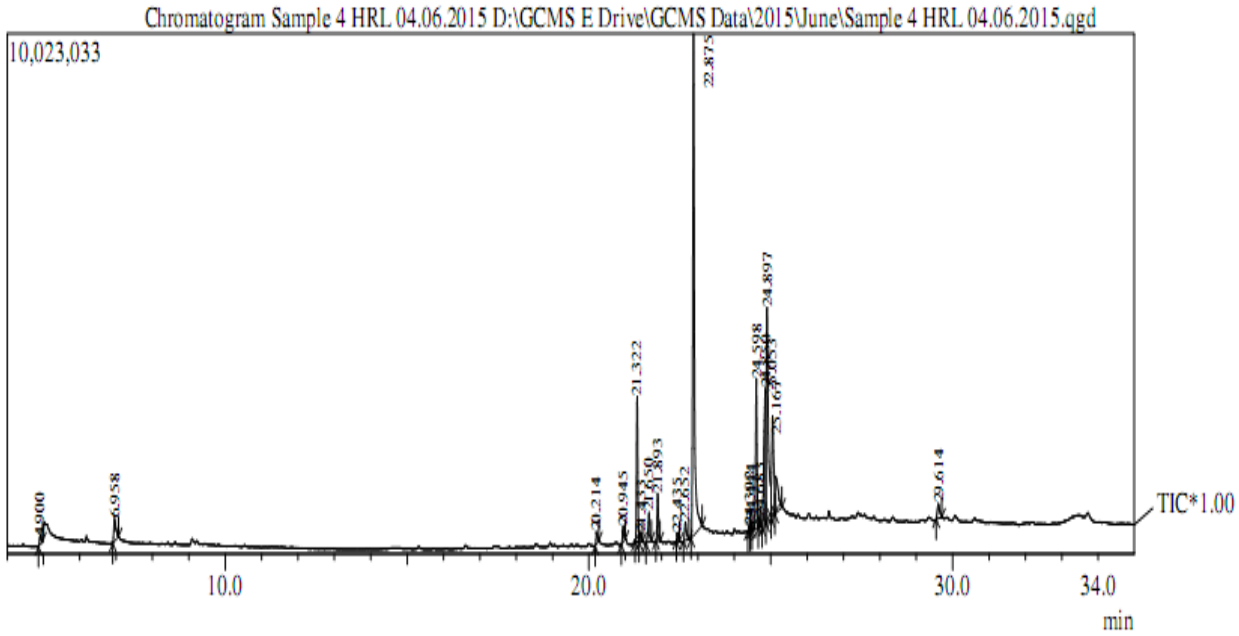

Table 1 Shows the components identified in methanolic extract of *Erythrina indica* (GC MS study)

Peak	R. Time	Area%	Name	Molecular formula	Molecular weight
1	4.900	0.47	1-Butyl(dimethyl)silyloxypropane	$C_9H_{22}OSi$	174
2	6.958	1.96	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130
3	20.214	0.63	Tridecanoic acid	$C_{13}H_{26}O_2$	214
4	20.945	0.95	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl	$C_{13}H_{18}O_3$	222
5	21.322	7.08	2,6,10-trimethyl,14-ethylene-14-PE	$C_{20}H_{38}$	278
6	21.433	0.68	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268
7	21.650	1.82	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268
8	21.893	2.28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
9	22.435	0.40	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
10	22.652	1.38	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296

11	22.875	32.64	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
12	24.392	0.27	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294
13	24.444	0.40	9-Octadecenoic acid, methyl este	$C_{19}H_{36}O_2$	296
14	24.598	7.76	Phytol	$C_{20}H_{40}O$	296
15	24.683	0.41	Methyl stearate $$ Octadecanoic acid,	$C_{19}H_{38}O_2$	298
16	24.850	10.31	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240
17	24.897	17.07	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278
18	25.053	7.93	Octadecanoic acid	$C_{18}H_{36}O_2$	284
19	25.167	4.28	Squalene	$C_{30}H_{50}$	410
20	29.614	1.30	3-Pentadecylphenol	$C_{21}H_{36}O$	304

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

S.no	Compound name	Biological activity**
1.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial
2.	cis-10-Nonadecenoic acid	Antitumor
3.	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
4.	Methyl 9-cis,11-	Anti cancer

	trans- octadecadienoate	
5.	9,12,15- Octadecatrienoic acid	Flavour, Fungicide, pesticide, perfumery Anti -inflammatory
6.	Octadecanoic acid	Lower LDL Cholesterol level
7.	Squalene	Antibacterial, Antioxidant,Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor
8.	Phytol	Anti cancer, Anti-inflammatory Hypocholesterolemic, Nematicide, Anticoronary, Antiarthritic, Hepatoprotective, Anti -androgenic,
9.	cis-10-Nonadecenoic acid	Antitumor activity

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

Karpagasundari and Kulothungan[12] screened the bioactive components of *Physalis minima* leaves have been evaluated using GCMS. GC/MS analysis of extract of *Physalis minima* 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). This study supports our finding compounds. Prabhadevi *et al* [13] explored the phytochemical constituents present in *Allamanda cathartica (A. cathartica)* L. using GC-MS. The GC-MS analyses determined the presence of 28 different phytochemical compounds in the ethanolic leaf extract of *A. cathartica*. The major phytoconstituents were 9,12,15-octadecatrienoic acid (Z,Z,Z)- (16.39%), n-hexadecanoic acid (14.08%), 3-O-methyl-d-glucose (11.03%) and 9,12,15-octadecatrienoic acid ethyl ester (Z,Z,Z)- (10.58%). The ethanolic stem extract of *A. cathartica* showed the presence of 26 different bioactive compounds. The major ones are 3-O-methyl-d-glucose (29.86%), 2-furancarboxaldehyde 5-(hydroxymethyl)- (14.87%), n-hexadecanoic acid (9.13%) and 9,12,15-octadecatrienoic acid (Z,Z,Z)- (7.34%). Similar types of compounds were agreement with our 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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