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Determination of bioactive compounds from *Erythrina variegata* leaf extract using gas chromatographic and mass spectroscopic techniques

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Abstract
The phytocomponents of *Erythrina variegata* leaves were evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. The mass spectrum 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 via Hexadecanoic acid, 7,10-Hexadecadienoic acid, methyl ester, 3-eicosyne, Butanoic acid, 3-methyl-, 3,7-dimethyl -6-octenyl ester, 1,3-Propanediol, 2-ethyl-2-(hydroxyl met hyl), 1,2-Benzenedicarboxylic acid, dibutyl ester, Phytol, 17-Octadecenoic acid, methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester and 2-Hexadecen-1-ol, 3,7,11,15-tetramethylcompounds in the ethanolic extract of *Erythrina variegata*. These findings support the traditional use of *Erythrina variegata* for various disorders.

Keywords: gas chromatography mass spectroscopy, *Erythrina variegata*, phytocompounds

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 (De-Fátima *et al*., 2006) [1]. Different medicinal plants and their medicinal values are widely used for various ailments throughout the world. Various chemical compounds isolated and characterized from Boraginaceous plant species are described. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi, 2000: Shahidi *et al*., 2008) [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 (Meurer-Grimes *et al*., 1996: Koduru *et al*., 2006) [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 (Mathekaga *et al*., 1998) [6].
Within a decade, there were a number of dramatic advances in analytical techniques including FTIR, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals. Gas Chromatography Mass Spectroscopy is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites *et al*., 1997) [7]. The chosen medicinal plant namely as *Erythrina variegata* leaves belongs to Fabaceae Family. *Erythrina variegata* leaves is widely distributed in southern India and Sri Lanka. The aim of this study is to determine the organic compounds present in the *Erythrina variegata* leaves extract with the aid of GC-MS Technique.

Materials and Methods
Plant Materials
Erythrina variegata leaves were collected in the month of January -2018 from Thanjavur. The leaf was identified and authenticated by Dr. S. John Britto. The Director, the Rabiant Herbarium and Centre for Molecular Systematic. St. Joshphe's College, Trichy - Tamil Nadu, India. A voucher specimen has been deposited at the Rabinat Herbarium St. Joshph's college, Trichy, Tamil Nadu, India.

Preparation of alcoholic extract
The leaves of *Erythrina variegata* was first washed several times with distilled water and traces of impurities were removed from the plant. The leaves were dried at room temperature and coarsely powered. The powder extracted with ethanol extract for 24 hours a semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was then concentrated in vacuum until the solvent was completely removed.

GC MS Analysis
GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32 mm, column length is 30 m, column thickness 0.50 µm), operating in electron impact mode at 70eV; Helium

gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min 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 51.25 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 Srinivasan *et al*., (2013) [8].

Identification of components

Interpretation on GCMS 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 Dr. Dukes, (2013) [9].

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) [10]. 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*., 2001) [11]. 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 defence mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions[1].

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. Gas chromatography mass spectrometry (GCMS) is a technique that combines the features of gas liquid chromatography and mass spectrometry to recognize different substances within a test sample. The results pertaining to GC-MS analysis of the ethanolic extract of *E. variegata* lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC.

The various compounds present in the entire herb of *E. variegata* detected by the GC-MS are shown in Table 1. In the GC-MS analysis, Hexadecanoic acid, 7,10-Hexadecadienoic acid, methyl ester, 3-eicosyne, Butanoic acid, 3-methyl-, 3,7-dimethyl -6-octenyl ester, 1,3-Propanediol, 2-ethyl-2-(hydroxyl met hyl), 1,2-Benzenedicarboxylic acid, dibutyl ester, Phytol, 17-Octadecenoic acid, methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester and 2-Hexadecen-1-ol, 3,7,11,15-tetramethylcompounds were identified and highly in the ethanolic extract of *Erythrina variegata* leaves and were mentioned in table above. The identification of compounds is based on the peak area, molecular weight and molecular formula. These compounds are responsible for pharmacological activities. Out of 20 compounds, 05 compounds have highest peak area.

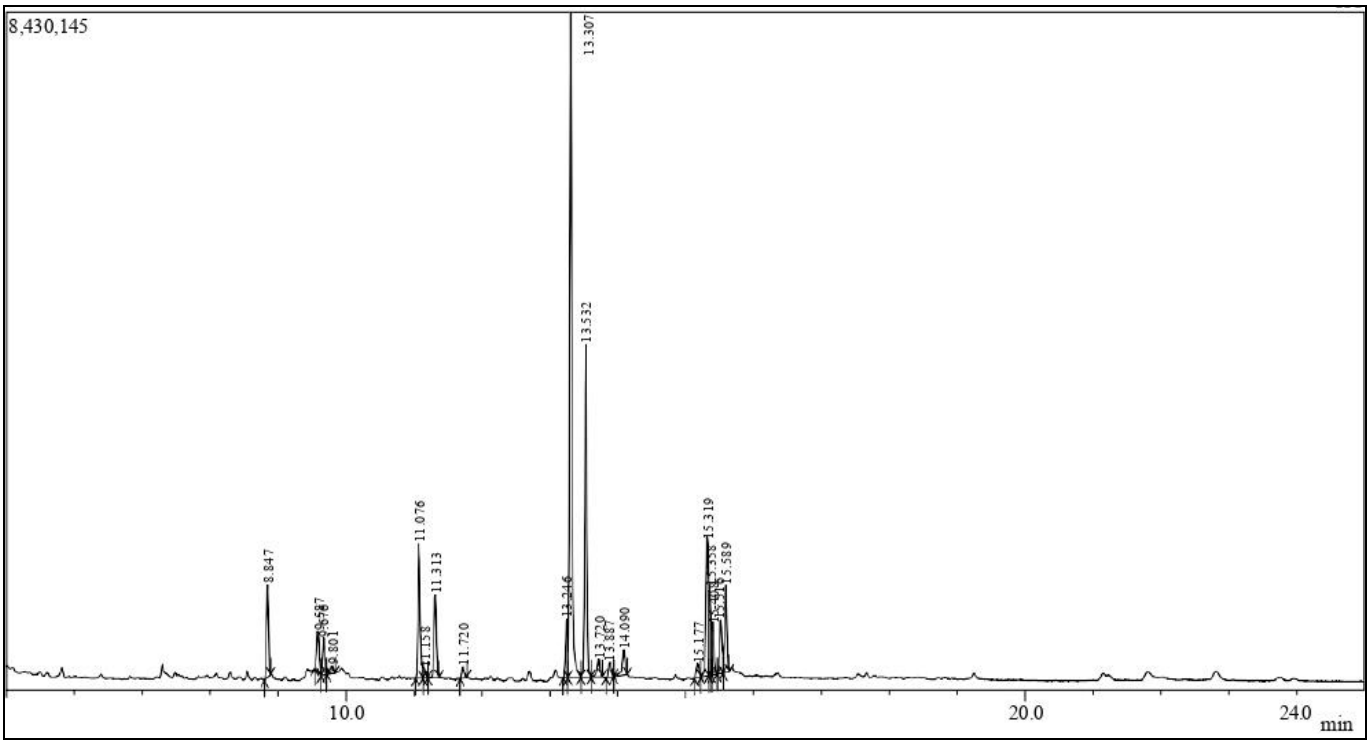

Fig 1: GC MS Chromatogram of *Erythrina variegata* leaves extract

Table 1: Identification of bioactive compounds in ethanolic extract of *Erythrina variegata* leaves extract using GC MS

Peak#	R. Time	Area	Area%	Height	Height%	A/H	Name
1	8.847	2348786	4.18	1152027	4.71	2.04	Phenol, 3,5-bis(1,1-dimethylethyl)
2	9.587	1178370	2.10	511946	2.09	2.30	1,2-benzoldicarbonsaeure, di-(he
3	9.676	1075627	1.91	463599	1.90	2.32	1,2-benzoldicarbonsaeure, di-(he
4	9.801	288595	0.51	94926	0.39	3.04	Hexane, 2,4,4-trimethyl
5	11.076	3922163	6.98	1673580	6.85	2.34	1-Tetradecanamine, N,N-dimethyl
6	11.158	317083	0.56	118967	0.49	2.67	Dodecane, 4,6-dimethyl
7	11.313	2466285	4.39	1025053	4.19	2.41	Pentanoic acid, 4-methyl-, methyl ester
8	11.720	398745	0.71	148478	0.61	2.69	Hexadecanoic acid
9	13.246	1544544	2.75	730079	2.99	2.12	7,10-Hexadecadienoic acid, methyl ester
10	13.307	18918617	33.65	8260228	33.79	2.29	3-eicosyne
11	13.532	8818519	15.69	4143535	16.95	2.13	Butanoic acid, 3-methyl-, 3,7-dimethyl -6-octenyl ester
12	13.720	627388	1.12	219124	0.90	2.86	(3-Tert-butyl-5-hydroxymethyl-
13	13.887	635645	1.13	189348	0.77	3.36	1,3-Propanediol, 2-ethyl-2-(hydroxyl met hyl)
14	14.090	897833	1.60	317280	1.30	2.83	1,2-Benzenedicarboxylic acid, dibutyl ester
15	15.177	460610	0.82	174285	0.71	2.64	9-Octadecene
16	15.319	4306459	7.66	1732535	7.09	2.49	Phytol
17	15.358	2202194	3.92	1099958	4.50	2.00	17-Octadecenoic acid, methyl ester
18	15.408	1462730	2.60	633946	2.59	2.31	9,12,15-Octadecatrienoic acid, methyl ester
19	15.516	1735473	3.09	667660	2.73	2.60	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-
20	15.589	2609401	4.64	1090064	4.46	2.39	Methyl stearate
		56215067	100.00	24446618	100.00		

Among the identified phytochemicals hexadecanoic acid is suggested to be a fatty acid ester and it may employ as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities (Bodoprost *et al*., 2007: Falodun *et al*., 2009) [12, 13]. 1, 2- bnzenedicarboxylic acid, diisooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent (Heinonen *et al*., 1998) [14]. 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 *Vitex altissima*, a Verbenaceae member (Sathish *et al*., 2012) [15]. 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 *Clerodendrum inerme* and *C. phlomidis* leaves (Anandhi and Ushadevi, 2013; Balaji *et al*., 2013) [16, 17].

Table 2: Biological activity of phytocomponents identified in the ethanol leaf extract of *Erythrina variegata*

S. No	Compound Name	Biological activity
1.	Hexadecanoic acid	Hypercholesterolemic, Lubricant, Antimicrobial, Flavor, Cosmetic and Perfumery
2.	7,10-Hexadecadienoic acid, methyl ester	Antioxidant, anti-inflammatory, hypocholesterolemic and anti-cancer
3.	3-eicosyne	Antimicrobial
4.	Butanoic acid, 3-methyl-, 3,7-dimethyl -6-octenyl ester	Antimicrobial
5.	1,3-Propanediol, 2-ethyl-2-(hydroxyl met hyl)	Antioxidant and Antimicrobial. Activities
6.	1,2-Benzenedicarboxylic acid, dibutyl ester	Antimicrobial and Antifouling
7.	Phytol	Anti-nociceptive, Antioxidant, anticancer, anti-inflammatory, antimicrobial, diuretic, chemo preventive properties
8.	17-Octadecenoic acid, methyl ester	Antimicrobial and Anti-inflammatory
9.	9,12,15-Octadecatrienoic acid, methyl ester	Anti-inflammatory, Hypocholesterolemic, anti-cancer, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Anti-eczemic, Anti-acne, 5-Alpha Reductase inhibitor, Anti-androgenic, Anti-arthritic, Anti-coronary and Insectifuge.
10.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	Anti-nociceptive and antioxidant Anti-inflammatory

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

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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