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Analysis of bioactive compounds in ethanolic extract of *Commelina maculata* leaves using GC-MS technique

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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 leaves ethanolic extract of *Commelina maculata* by 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 Hexadecanoic Acid, 2-Hexadecen-1-ol, Heptadecane, 3,7,11,15-Tetramethyl in the ethanolic extract of *Commelina maculata* leaves These findings support the traditional use of *Commelina maculata* leaves in various disorders.

Keywords: Gas chromatography and mass spectroscopy, *Commelina maculata*, phyto chemistry

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) [18]. 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) [14].

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) [9].

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) [13]. 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) [8]. 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) [15].

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) [16]. The chosen medicinal plant namely as *Commelina maculata* leaves belongs to Commelinaceae Family. *Commelina maculata* is widely family found in India, Burma, Bhutan, and southern

China Hong *et al*. (2000) [11]. The aim of this study is to determine the organic compounds present in the *Commelina maculata* leaves extract with the aid of GC-MS Technique.

Material and methods

Plant materials

The whole plant of *Commelina maculata* leaves 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, Tiruchirappalli, Tamil Nadu, India.

Preparation of extracts

The collected *Commelina maculata* leaves were washed several times with distilled water to remove the traces of impurities from the plant. Then examined carefully old, infected and fungus damaged portion of the leaves were removed. Healthy leaves 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 *Commelina maculata* leaves 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) [12]. 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) [7]. 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 De-Fatima *et al*. (2006) [5]. 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.

Twenty compounds were identified in *Commelina maculata* leaves 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 Hexadecanoic acid, 2-Hexadecen-1-OL, Heptadecane, 3,7,11,15-Tetramethyl. 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. The investigation concluded that the stronger extraction capacity of ethanol could have produced a number of active constituents healthy environment Daffodil *et al.* (2012) [4]; Thanga Krishna Kumari *et al.* (2012) [20]; Sheela and Uthayakumari (2013) [19].

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) [3]; Falodun *et al*. (2009) [6]. 1, 2- benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent Heinonen *et al*. (1998) [10]. 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) [17]. 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) [1]; Balaji and Kilimozhi (2014) [2].

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.

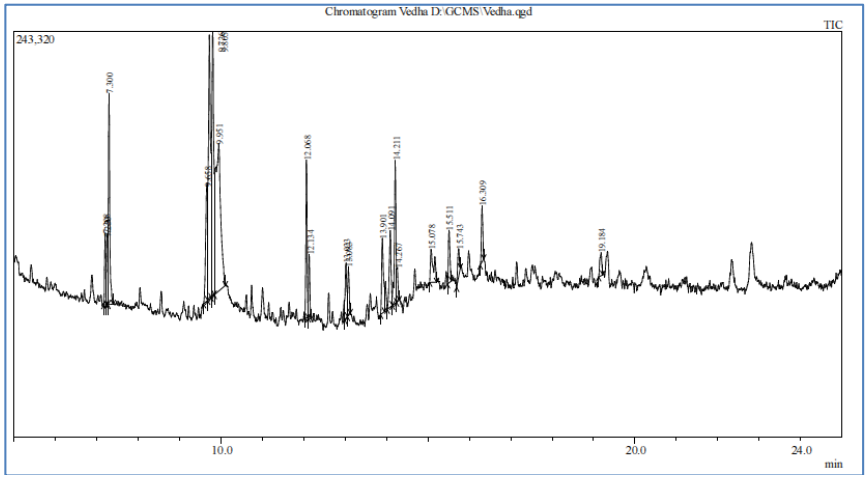

Fig 1: GC-MS Chromatogram *Commelina maculata* leaves extract

Table 1: Identification of bioactive compounds in ethanolic extract of *Commelina maculata* leaves extract using GC-MS

Peak#	R. Time	Area%	Molecular formula	Molecular weight	Molecular name	Nature of the Compound
1	7.208	2.40	$C_8H_{18}O$	130	Isooctanol	Chiral alcohol/Fatty acid
2	7.250	2.41	$C_{11}H_{22}O_2$	186	Nonanoic Acid, Ethyl Ester	Fatty acid Ester
3	7.300	7.17	$C_{17}H_{36}$	240	Heptadecane	Alkane
4	9.658	6.14	$C_9H_{11}N_3O_2$	193	Benzoic Acid	Aromatic acid
5	9.726	14.16	$C_{16}H_{32}$	224	3-Hexadecene	Alkane
6	9.805	13.21	$C_{17}H_{36}$	240	Heptadecane	Alkane
7	9.951	20.34	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure, Di-(Hex-1-En-5-Yl-Ester)	
8	12.068	5.10	$C_{16}H_{34}O$	242	1-Hexadecanol	Terpen alcohol
9	12.134	1.94	$C_{15}H_{32}$	212	Pentadecane	Alkane
10	13.023	2.28	$C_8H_{16}O$	128	4-Octanone	Ketone
11	13.083	1.60	$C_8H_{16}O$	128	2-Octen-1-Ol	Alkenyl alcohol
12	13.901	3.52	$C_{16}H_{32}O_2$	256	Hexadecanoic Acid	Palmitic Acid
13	14.091	3.65	$C_{18}H_{26}O_5$	322	1,2-Benzenedicarboxylic Acid, 2-Butoxyethyl Butyl Ester	Acid/Polymer ester
14	14.211	5.65	$C_{36}H_{75}O_3P$	587	Phosphonic Acid, Dioctadecyl Ester	Ester
15	14.267	1.16	$C_{11}H_{24}$	156	Nonane, 3,7-Dimethyl	Alkane hydrocarbon
16	15.078	2.71	$C_7H_{14}O$	144	Cyclohexanol, 4-MethylMethylcyclohexanol	Cyclohexane
17	15.511	2.10	$C_{20}H_{40}O$	296	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	Fatty alcohol
18	15.743	1.32	$C_9H_{20}O_2$	160	1,9-Nonanediol (Cas) N-Nonane-1,9-Diol	Alcohol
19	16.309	1.95	$C_{18}H_{38}O$	270	1-Octadecanol	Alcohol
20	19.184	1.20	$C_9H_{19}NO_3$	189	Nitric Acid, Nonyl Ester	Acid

Table 2: Biological activity of phytocomponents identified in the ethanol leaf extract of *Commelina maculata* leaves

S. No	Compound Name	Biological activity
1	Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
2	1-Hexadecanol	Antimicrobial, Anti-inflammatory.
3	Heptadecane	Antioxidant
4	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	Antimicrobial, Anti-inflammatory, Cancer-Preventive, anti-diuretic Antioxidant

**Duke's. Phyto chemical and Ethno botanical 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. The *Commelina maculata* leaves has long been investigated for its phytochemicals and pharmacological activities supporting its vast ethno botanical and alternative medicinal use. The phytochemicals of this plant has been reported extensively as an anti-cancerous, antimicrobial, anti-inflammatory and antioxidant agent.

Therefore, it is recommended as a plant of phyto pharmaceutical importance.

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