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GC-MS analysis of phytocomponents in methanolic extract of *Coleus aromaticus*

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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 leaves methanolic extract of *Coleus aromaticus* 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 1,2-benzenedicarboxylic acid, diethyl ester, Phytol, Octadecenal, Dibutyl phthalate, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl, hexadecanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, 9,12,15-Octadecatrienoic acid, ethyl ester and solanesol in the methanolic extract of *Coleus aromaticus*. These findings support the traditional use of *Coleus aromaticus* in various disorders.

Keywords: Gas chromatography and mass spectroscopy, *Coleus aromaticus*, 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) [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 (Mathekaga, and Meye, 1998) [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 (Harborne, 1986) [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, amino acids, 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) [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 (Hamburger and Hostettmann, 1991) [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 (Roberts and Xia, 1995) [6].
The chosen medicinal plant namely as *Coleus aromaticus* leaves L belongs to the Solanaceae family. *Coleus aromaticus* is cultivated in many of the drier regions of India. It is also found in Nepal, China and Yemen (Khare, 2007) [7]. The biological activity was screened against the micro organisms causing skin allergies, diarrhea and dysentery. A recent study with methanol extract of mature leaves reported anti-inflammatory and antinociceptive activity (Merish *et al*., 2014 Sadique *et al*., 1987, Chatterjee, 1990, Nadkarni, 1982, Nadkarni, 2003, Asolkar, 1992)

[8-13]. The aim of this study is to determine the organic compounds present in the *Coleus aromaticus* 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 plant of *Coleus aromaticus* leaves (Tamil -Karpooravalli) were collected in June 2019 from Thanjavur, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabiant 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 *Coleus aromaticus* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted with 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 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. 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

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) [14].

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.

GC-MS analysis

Twenty six were identified in *Coleus aromaticus* 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 The GC-MS analysis revealed the presence of various compounds like 1,2-benzenedicarboxylic acid, diethyl ester, phytol, octadecenal, dibutyl phthalate, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl, hexadecanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, (z,z,z), 9,12,15-octadecatrienoic acid, ethyl ester, (z,z,z) and solanesol. Table 2 represents the activity of phyto-components identified in the methanolic extracts of the *Coleus aromaticus* leaves by GC-MS.

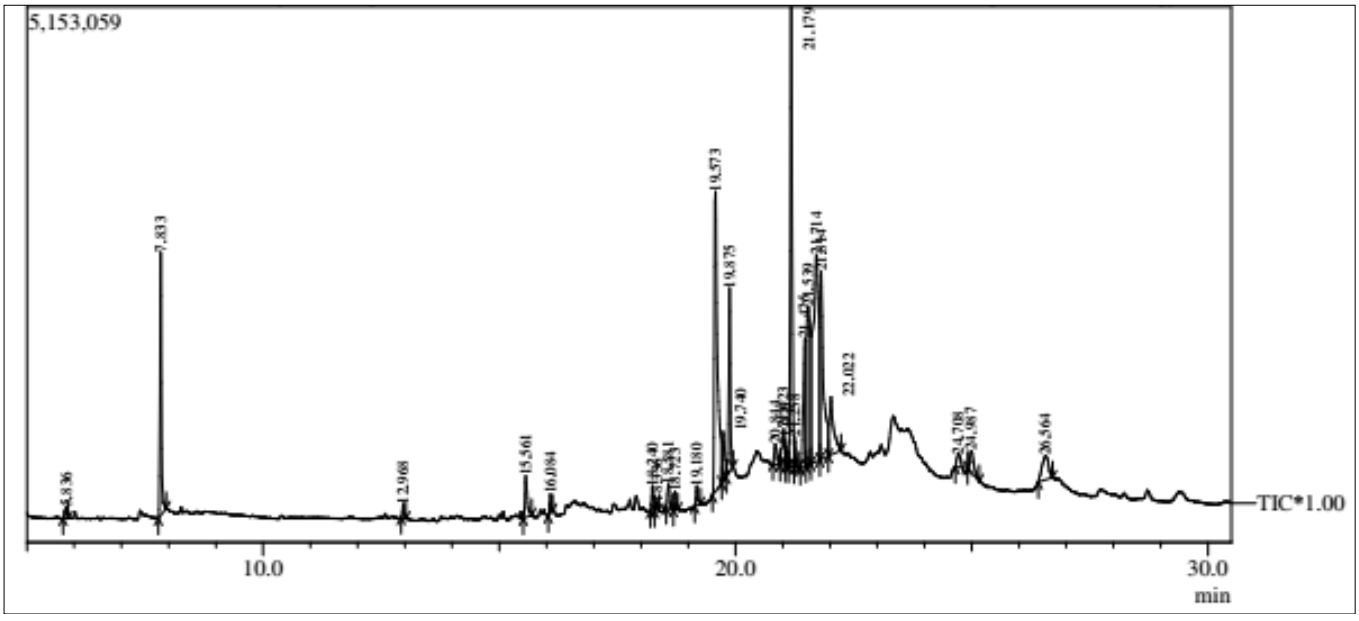

Fig 1: Chromatogram obtained from the GC/MS with the extract of *Coleus aromaticus* leaves.

Table 1: Shows the components identified in methanolic extract of *Coleus aromaticus* leaves (GC-MS study)

Peak#	R. Time	Area%	Molecular formula	Molecular weight	Molecular Name
1	5.836	0.38	$C_9H_{20}O_2$	160	Butane, 1,1-Diethoxy-3-Methyl
2	7.833	6.12	$C_9H_{20}O_3$	176	Propane, 1,1,3-Triethoxy
3	12.968	0.34	$C_{13}H_{18}O_2$	206	1-(3,6,6-Trimethyl-1,6,7,7a-Tetrahydrocyclopenta[C]Pyran-1-Yl)Ethanone
4	15.561	1.53	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic Acid, Diethyl Ester
5	16.084	0.51	$C_{13}H_{18}O$	190	Megastigmatrienone
6	18.240	0.57	$C_{22}H_{42}O_2$	338	Phytol
7	18.336	0.28	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-Trimethyl
8	18.581	0.82	$C_{14}H_{20}O$	204	2-(3-Isopropyl-4-Methyl-Pent-3-En-1-Ynyl)-2-Methyl-Cyclobutanone
9	18.723	0.47	$C_{18}H_{34}O$	266	13-Octadecenal,
10	19.180	0.56	$C_{16}H_{32}O_2$	256	Pentadecanoic Acid, Methyl Ester
11	19.573	14.23	$C_{18}H_{34}O_2$	282	9-Octadecenoic Acid
12	19.740	1.93	$C_{16}H_{22}O_4$	278	Dibutyl Phthalate
13	19.875	4.93	$C_{20}H_{40}O_2$	312	Octadecanoic Acid, Ethyl Ester
14	20.844	0.97	$C_{21}H_{44}O$	312	1-Heneicosanol
15	21.023	1.55	$C_{19}H_{36}O_2$	296	11-Octadecenoic Acid, Methyl Ester
16	21.075	0.60	$C_{19}H_{36}O_2$	296	9-Octadecenoic Acid ter
17	21.179	13.27	$C_{20}H_{40}O$	296	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-,
18	21.258	0.64	$C_{17}H_{34}O_2$	270	Hexadecanoic Acid, Methyl Ester
19	21.475	5.03	$C_{18}H_{34}O_2$	282	Oleic Acid
20	21.539	9.60	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic Acid,
21	21.714	18.15	$C_{33}H_{66}O_3$	510	Octadecanoic Acid, 3-Hydroxy-2-Tetradecyl-, Methyl Ester, (2R,3R)
22	21.814	9.49	$C_{20}H_{34}O_2$	306	9,12,15-Octadecatrienoic Acid, Ethyl Ester
23	22.022	3.75	$C_{19}H_{38}O_2$	298	Heptadecanoic Acid, Ethyl Ester
24	24.708	0.47	$C_{20}H_{34}O$	290	Trans-Geranylgeraniol
25	24.987	1.37	$C_{20}H_{34}O$	290	Neryl Linalool Isomer
26	26.564	2.43	$C_{45}H_{74}O$	630	Solanesol

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Coleus aromaticus* leaves by GC-MS.

Compound name	Biological activity
1,2-Benzenedicarboxylic Acid, Diethyl Ester	Plasticizers
Phytol	Antimicrobial, Anticancer, Cancer preventive, Diuretic, Antiinflammatory.
Octadecenal	Antimicrobial, Anti-inflammatory
Dibutyl Phthalate	Antimicrobial, Antifouling.
2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents.
Hexadecanoic Acid, Methyl Ester	Antioxidant, Flavor, Hypocholesterolemic Pesticide, 5-Alpha reductase inhibitor
Oleic Acid	Antiinflammatory**,** Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge.
9,12,15-Octadecatrienoic Acid	Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary,
9,12,15-Octadecatrienoic Acid, Ethyl Ester	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Anti androgenic, Nematicide 5-Alpha reductase inhibitor, Antihistaminic Anticoronary, Insectifuge, Antieczemic Anticancer.
Solanesol	Used in synthesis of high-value bio chemicals such as vitamin-K.

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

Conclusion

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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