Gc-Ms analysis of bioactive compounds in methanolic extract of *Acorus calamus* Linn.

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Abstract

***Acorus calamus* leaf extracts were prepared in methanolic solvent to study the phytochemical profile using Gas Chromatography - Mass Spectrometry method. GC-MS analysis of *Acorus calamus* showed 38 peaks in the chromatogram of which 16 of the compounds where identified. From this study it is obvious that *Acorus calamus* leaf extracts contains many biologically active compounds and also it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drugs and insecticides.**

Keywords: *Acorus calamus*, active compounds, GC-MS, methanol solvent, phytochemical.

Introduction

Medicinal and aromatic plants contain substances that can be used for therapeutic purposes. These compounds are called "active ingredients" and the plant is considered as the source for this compound [1]. The plant *Acorus calamus* Linn commonly called Vasambu belongs to the family Acoraceae. It is a herbaceous perennial has been used medicinally for a wide variety of ailments, such as gastrointestinal diseases [2] and treating pain [3], and its aroma makes calamus essential oil valued in the perfume industry [4]. The leaves, stems, and roots are used in various Siddha and Ayurvedic medicines [5].

Acoraceae, in the order of Acorales, is a family of monocotyledonous flowering plants, with solitary genus, namely, Acorus Linn., comprising two species, distributed in temperate and subtropical Asia, North America, and tropical Asia. They are introduced and naturalized in Europe, New Guinea, and North America. Plants are perennial herbs, glabrous, and aromatic and often occur in marshes, shallow waters, or wetlands. Laticifers and raphides are absent, while aerenchyma are present [6]. Rhizomes are creeping, much branched, and lacunose.

Gas Chromatography - Mass Spectrometry

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

Mass spectrometry - Is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental

composition of a sample or molecule. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios by using the one of a variety of techniques.

Materials and Methods

The entire parts of *Acorus calamus* Linn were collected from Nagercoil, Tamilnadu, India and were authenticated by using local floras Gamble. The herbariom specimens kept in the herbarium of PG and Research department of Botany, ST Hindu collage, Nagercoil, Tamilnadu, India.

The plant parts was collected, washed, air dried in shadow and grinded by mixer grinder. After grinding, 5 gm of plant material was extracted in 50 ml of metonolic solvent by hot extraction using Soxhlet apparatus [7]. The organic solvent was filtered by whatman filter paper till clear solution was obtained.

GC-MS Analysis

Separation is due to differential distribution coefficients. In this chromatography, moving phase or mobile phase) is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column [8]. The instrument used to perform gas chromatography is called a gas chromatograph (or “aerograph”, “gas separator”). The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases [9]. This causes each compound to elute at a different time, known as the retention time of the compound Secondly, the column through which the gas phase passes in located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the gas phase is solely a function of the vapour pressure of the gas.

Protocol:

The plant leaves powder was extracted with methanol and analyzed using GC-MS (GC Clarius 500 Perkin Elmer) analyzer. The data were obtained on an Elite-1 (100% Dimethyl poly siloxane) column (30 0.25mm 1µmdf). Helium (99.999%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2µl of Methanol solution of the sample was injected into the column with the injector temperature at 250°C. GC oven temperature started at 110^{o}C and holding for 2min and it was raised to 2000C at the rate of 100C/min, without holding. Holding was allowed at 280°C for 9 min with program rate of 5°C/min. The injector and detector temperatures were set at 250°C and 280°C respectively. An ion source temperature was maintained at 200°C. The mass spectrum of compounds in samples was obtained by electron ionization at 70eV and the detector was operated in scan mode from 45-450 amu (atomic mass units). A scan interval of 0.5 seconds and fragments from 45 to 450 Da was maintained. The total running time was 36 minutes.

Results and Discussion:

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns [10]. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2. This is done in order to determine whether this plant species contains any individual compound, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance.

The methanol extract of *Acorus calamus* showed 38 peaks in the chromatogram. Of which, 16 of the compounds were identified as Cis-dimethylstilbene (0.446%), 1,5,10- trioxa-9,11-diazatricyclo[7.5.0(6,14) .0(8,1 (19.63%), 2,3 – dimethoxy-5(2-propenyl)-2,5-cyclohexadiene-1 (1.202%), Butyl 3,4,4,4-tetrafluro-3-triflurомethylbutanate (0.506%), Neophytadiene (0.354%), Phthalic acid, butylhexyl ester (0.775%), Phthalic acid, 6-ethyl-3-octylbutyl ester (0.155%), Hexadecanoic acid (CAS) (0.743%), Phthalic acid,2-isopropylphenylmethyl ester (0.507%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester(CAS),(0.594%), 2,3-Dihydroxypropyl elaidate (2.376%), 2-hexadecen-1-ol,3,11,15-tetramethyl-,[R-[R*,R*-(E)]] (0.683%), 2.beta.-(3'-oxobutyl)-1.alpha.,3,3-trimethyl-7-oxa (1.398%), Phthalic acid, decylisohexyl ester (0.553%), 1,2-benzenedicarboxylic acid bis(1-methylheptyl) ester (0.596%) and Phthalicacid,didecyl ester(0.675%).

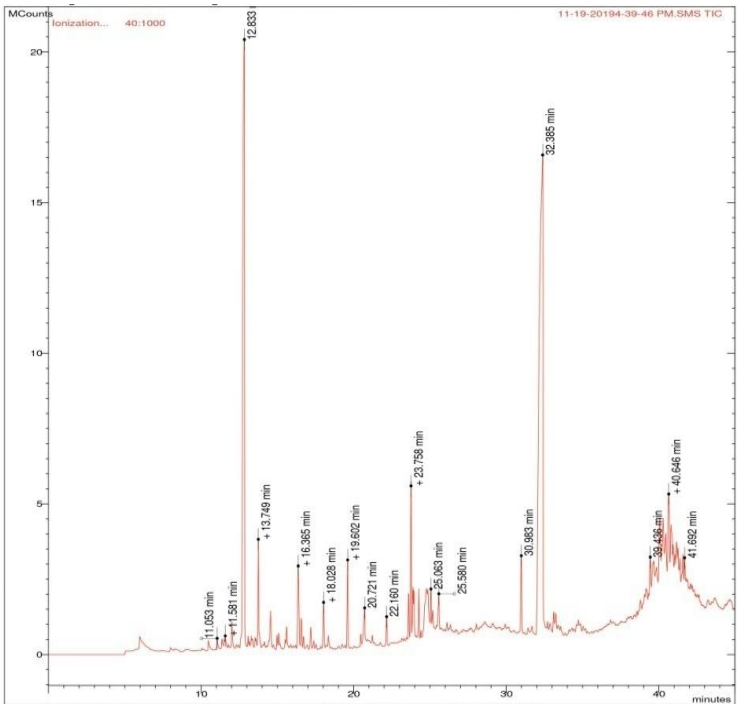
Conclusion:

Many of the drugs that are derived from the secondary metabolites are simple synthetic modifications or copies of these naturally obtained substances. In the present study it is revealed that *A. calamus* has many biologically active compounds and therefore it is an attractive subject for further experimental and clinical investigations.

Biologically active phyto-compounds identified from methanol extract Of *Acorus calamus* leaves by GC-MS analysis

RT	Name of the compound	MF	MW	Peak area %
12.015	Cis-dimethylstilbene	$C_{14}H_{12}$	180.24 g/mol	0.446
12.833	1,5,10- trioxa-9,11-diazatricyclo[7.5.0(6,14) .0(8,1	$C_{20}H_{40}O$	296.5 g/mol	19.63
13.749	2,3 – dimethoxy-5(2-propenyl)-2,5-cyclohexadiene-1	$C_{17}H_{16}O_4$	284.31 g/mol	1.202
16.570	Butyl 3,4,4,4-tetrafluro-3-trifluromethylbutanate	$C_{21}H_{12}F_{18}O_6$	702.3 g/mol	0.506
17.187	Neophytadiene	$C_{20}H_{38}$	278.5 g/mol	0.354
18.028	Phthalic acid, butylhexyl ester	$C_{18}H_{26}O_4$	306.3966	0.775
20.464	Phthalic acid, 6-ethyl-3-octylbutyl ester	$C_{22}H_{34}O_4$	362.5 g/mol	0.155
20.721	Hexadecanoic acid (CAS)	$C_{16}H_{32}O_2$	256.42 g/mol	0.743
22.160	Phthalic acid,2-isopropylphenylmethyl ester	$C_{18}H_{18}O_4$	298.333 g/mol	0.507
23.601	9,12-octadecadienoic acid (Z,Z)-, methyl ester(CAS)	$C_{19}H_{34}O_2$	294.5 g/mol	0.594
23.758	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.5 g/mol	2.376
23.946	2-hexadecen-1-ol,3,11,15-tetramethyl-,[R-[R*,R*-(E)]]	$C_{20}H_{40}O$	296.5 g/mol	0.683
30.983	2.beta.-(3'-oxobutyl)-1.alpha.,3,3-trimethyl-7-oxa	$C_{14}H_{24}O_2$	224.34 g/mol	1.398
39.436	Phthalic acid, decylisohexyl ester	$C_{24}H_{38}O_4$	390.6 g/mol	0.553
40.935	1,2-benzenedicarboxylic acid, bis(1-methylheptyl) ester	$C_{18}H_{26}O_4$	306.4 g/mol	0.596
41.258	Phthalic acid, didecyl ester	$C_{28}H_{46}O_4$	446.6624 g/mol	0.675

GC-MS Total Ion Chromatogram (TIC) of hot methanol extract for leaves of *Acorus calamus*



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