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Research Article

GC-MS ANALYSIS OF *CASSIA ITALICA* LEAF METHANOL EXTRACT

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. *Cassia italica* has medicinal values; methanol leaf extract of this plant was analyzed using 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. Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of 17 compounds. In GC-MS analysis, some of the phytocomponents screened were Phytol, Squalene and n-Hexadecanoic acid. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Many of them are used in industry for various applications like flavor, antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.

Keywords: *Cassia italica*, GC-MS, Phytol and various applications

INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli *et al*., 2009).

Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Vyas, 1999; Kaushik *et al*., 2002; Chaman Lal and Verma, 2006)

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-Fatima *et al*., 2006).

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Milne *et al.,* 1993). Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids (Jie *et al.,* 1988) and alkaloids (Betz *et al.,* 1997).

Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase. It also plays a fundamental role as an analytical technique for quality control and standardization of phyto therapeuticals (Andrew, 2007). There are a number of different kinds of chromatography, which differ in the mobile and the stationary phases used. Gas chromatography - specifically gas-liquid chromatography - involves a sample being vapourised 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. The principle of gas chromatography is adsorption and partition. Within the family of chromatography- based methods gas chromatography (GC) is one of the most widely used techniques. It was first described by James and Martin in 1952 and has become one of the most important tools for the separation of volatile compounds. Gas chromatography has gained widespread acceptance in numerous application areas, such as process control in chemical plants, quality control in the food industry, monitoring sample composition in the oil-industry, environmental and bio medical sciences. These are just a few examples in which gas chromatography has been applied. The combination of speed, sensitivity and a high resolving power in gas chromatography provides a very adequate technique for the separation of complex samples. Moreover, the coupling to spectrometric methods such as mass spectrometry (MS) for direct identification of unknown compounds is easy to establish.

Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents. Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients.

Gas Chromatography Components

A gas is the mobile phase and the stationary phase can be either a solid or a non- volatile liquid. There are five basic GC components:

1) Pneumatic system – gas supply (flow control and measurement).

2) Injection system – a heated injector port, where the sample is vaporized if necessary.

3) Column – where the separation occurs.

4) Oven –The coiled column is wholly contained in a thermostatically controlled oven.

5) Detector – integral detector or link to a mass spectrometer

Mass spectrometry is the most sensitive and selective method for molecular analysis and can yield information on the molecular weight as well as the structure of the molecule. Combining chromatography with mass spectrometry (GC-MS) provides the advantage of both chromatography as a separation method and mass spectrometry as an identification method. In mass spectrometry, there is a range of methods to ionize compounds and then separate the ions.

Used in the analysis of the herbal medicines, there are at least two significant advantages for GC–MS, that is: (1) with the capillary column, GC– MS has in general very good separation ability, which can produce a chemical fingerprint of high quality; (2) with the coupled mass spectroscopy and the corresponding mass spectral database, the qualitative and relatively quantitative composition information of the herb investigated could be provided by GC–MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in herbal medicine and its pharmacology in further research. Thus, in our opinion, GC–MS should be the most preferable tool for the analysis of the volatile chemical compounds in herbal medicines.

Applications in Phyto chemistry

There are three different stages in GC analysis:

1. The preparation of the sample.

2. The development of the separation and the production of the chromatogram.

3. The processing of the data and the production of the results.

Phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and root that work with and fibers to act as a defense system against diseases of more accurately, to protect against diseases (Krishnaiah *et al*., 2009). *Cassia* is the major genus of the family Caesalpiniaceae and possesses about 600 species distributed worldwide (Viega *et al*., 2004). *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses (Hennebelle *et al*., 2009). Skin diseases such as ringworm, scabies, eczema and gastro-intestinal disorders like ulcers (Jacob *et al*., 2002). *Cassia italica* leaves are used in the treatment of gas troubles and skin diseases.

The aim of the present study is to identify the phytocomponents of this plant and subjecting the methanol extract of the plant leaves to Gas chromatography – Mass Spectrum analysis. In the present study, volatile organic matter of the leaf sample of plant was analyzed for the first time. This work will help to identify the compounds, which may be used in body products or of therapeutic value.

MATERIALS AND METHODS

Gas chromatography analysis was carried out at Food Testing Laboratory, Indian Institute of Crop Processing Technology, Thanjavur. It is one of the key techniques, generally used for screening/identification of different groups of plant phytochemicals. The high attainable separation power in combination with wide range of the detectors employing various detection principles to which it can be coupled makes GC an important, often irreplaceable tool in the analysis at trace level of plant phytochemical compounds. Gas chromatograph study includes the important optimization process such as,

i) introduction of sample extract onto the GC Column

ii) separation of components on an analytical column and

iii) detection of target analysis using Mass Spectrometric (MS) detector

Fresh leaves of the selected plant *C. italica* having medicinal value were collected from Sathiyanathapuram, Theni District, Tamilnadu, India. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India (BSI), Coimbatore (Certificate No.: BSI/SC/5/23/08-09/Tech.241)

Principle

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. 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. 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 is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Applications of GC

1) Very minute amounts of a substance can be measured.

2) Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process.

3) Gas Chromatography is used in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents.

4) Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations.

5) The most important application area is in pollution studies, forensic work and general trace analysis.

Preparation of extract

25gm of the powdered leaves were soaked in 95% methanol for 12hrs. The extracts were then filtered through Whatman filter No.41 along with 2gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95% ethanol along with sodium sulphate. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phyto-components in the plant material. 2µl of this solution was employed for GC-MS analysis (Merlin *et al*., 2009).

The plant 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 ethanol solution of the sample was injected into the column with the injector temperature at 250ºC. GC oven temperature started at 110ºC and holding for 2min and it was raised to 200ºC at the rate of 10ºC/min, without holding. Holding was allowed at 280ºC for 9 min with program rate of 5ºC/min. The injector and detector temperatures were set at 250ºC and 280ºC respectively. Ion source temperature was maintained at 200ºC. The mass spectrum of compounds in samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 45-450amu (atomic mass units). A scan interval of 0.5seconds and fragments from 45 to 450 Da was maintained. The total running time was 36minutes.

Identification of components

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. 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 or group of compounds, 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.

RESULTS

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of *C. italica* leaves revealed the presence of seventeen compounds (phytochemical constituents) that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table and Fig 1 & 2. The first compound identified with less retention time (5.08min) was 1-Butanol, 3-methyl, formate, whereas Squalene was the last compound which took longest retention time (29.84min) to identify. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table 2. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.

Table 1: GC-MS analysis revealed the presence of phytochemical components in methanol leaf extract of *C. italica*

S. No	R/T	Name of the Compound	Molecular Formula	MW	Peak Area %
1	5.08	1-Butanol, 3-methyl, formate	$C_6H_{12}O_2$	116	4.38
2	10.97	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.44
3	12.99	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	48.37
4	13.69	11-Dodecenoic acid, 10-hydroxy-, methyl ester	$C_{13}H_{24}O_3$	228	1.46
5	14.42	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.37
6	15.51	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.14
7	16.20	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.70
8	16.48	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.28
9	18.14	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.31
10	18.47	Phytol	$C_{20}H_{40}O$	296	1.64
11	18.79	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.47
12	18.92	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	16.30
13	19.22	Octadecanoic acid	$C_{18}H_{36}O_2$	284	4.25
14	20.96	S-[2-[N,N-Dimethylamino]N,N-dimethylcarbamoyl thiocarbohydroximate	$C_8H_{17}N_3O_2S$	219	0.26
15	21.82	Ricinoleic acid	$C_{18}H_{34}O_3$	298	2.26
16	24.97	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.46
17	29.84	Squalene	$C_{30}H_{50}$	410	2.92

Table 2: GC-MS analysis showed phytochemical compounds, their nature and their biological activities of methanol leaf extract of *C. italica*.

S. No	R/T	Peak Area	Name of the Compound	Compound Nature	Activity
1	12.99	48.37	3-O-Methyl-d-glucose	Sugar moiety	Preservative
2	18.92	16.30	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid	Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary,
3	16.20	11.70	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
4	5.08	4.38	1-Butanol, 3-methyl, formate	Alcoholic compound	Antimicrobial
5	19.22	4.25	Octadecanoic acid	Stearic acid	No activity reported
6	29.84	2.92	Squalene	Triterpene	Antibacterial, Antioxidant,Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor
7	14.42	2.37	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial
8	21.82	2.26	Ricinoleic acid	Ricinoleic acid	Antimicrobial Anti-inflammatory
9	18.47	1.64	Phytol	Diterpene	Antimicrobial, Anticancer, Cancer preventive, Diuretic Antiinflammatory,
10	18.79	1.47	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic,

					5-Alpha reductase inhibitor, Antiandrogenic, Anticoronary,
11	13.69	1.46	11-Dodecenoic acid, 10-hydroxy-, methyl ester	Unsaturated compound	No activity reported
12	16.48	1.28	Hexadecanoic acid, ethyl ester	Ester compound	Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor
13	24.97	0.46	1,2- Benzenedicarboxylic acid, diisooctyl ester	Plasticizer compound	Anti fouling Antimicrobial
14	10.97	0.44	Undecanoic acid	Fatty acid	No activity reported
15	18.14	0.31	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid ester compound	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic,
16	20.96	0.26	S-[2-[N,N-Dimethylamino]N,N-dimethylcarbamoyl thiocarbohydroxim-ate	Amino compound	Antimicrobial
17	15.51	0.14	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic Pesticide, 5-Alpha reductase inhibitor

DISCUSSION

The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) (Cong *et al*., 2007). For quantitative determination, gas-chromatography with flame ionization detector (GC-FID) and GC-MS are preferred (Lee *et al*., 2005; Lampronti *et al*., 2006; Haznagy-Radnal *et al*., 2007).

The GC-MS analysis of *C. italica* leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid (R/T 20.06) possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid (R/T 17.25) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol-Diterpene (R/T 19.67) is an antimicrobial, anticancer, anti-inflammatory and diuretic agent (Praveen kumar *et al*., 2010). 9, 12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and di-isooctyl ester were present in *Caesalpinia sappan* ethanol extract (Sarumathy *et al*., 2011). Similar types of compounds were identified among the seventeen compounds of this present study.

Phytol is one among the seventeen compounds of the present study. Similarly Maria Jancy Rani *et al.* (2011) observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (2011) in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* (Sathish kumar and Manimegalai, 2008). Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al*., 2005). Phytol, Phenol, 2, 4-bis (1-phenylethyl) - which are all have medicinal properties. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K_1. It is used along with simple sugar or corn syrup as a hardener in candies.Mangunwidjaja *et al*. (2006) reported the main components of 9, 12 octadecadienoic acid, Octadec- 9enoic acid and 9,12-actadecadienoic acid present in *Croton tiglium* seed. These compounds were found to have potential antioxidant and anticancer activities.

Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al*., 2002) and *Melissa officinalis* (Sharafzadeh *et al*., 2011). Parasuraman *et al*. (2009) identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid (Siddig Ibrahim *et al.,* 2009). n-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15-Octadecatrienoic acid and Squalene were identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen kumar *et al*., 2010). Squalene is used in cosmetics as a natural moisturizer**.** Devi *et al*. (2009) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9, 12-Octadecadienoic acid. These reports are in accordance with the result of this study.

CONCLUSION

The source of many plants (herbs and spices) can often be identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many alcoholic beverages. The technique of fingerprint could really identify the false herbal products. The construction of chromatographic fingerprints aims at evaluating the quality of Herbal Medicines **(**Yi-Zeng et al 2004**)**. The fundamental reason of quality control of herbal medicines is based on the concept of phytoequivalence of herbs, and then to use this conception to identify the real herbal medicine and the false one, and further to do quality control.

Therefore, GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *Cassia italica* suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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