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Research Article

GC-MS Analysis of Chloroform Extract of *Solanum Nigrum* Leaf

Sivakamasundari[1], Ravishankar[1], Mariajancyrani[2*], Chandramohan[2]
[1]Department of chemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613 005, Tamil Nadu, India.
[2]Department of chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur-613 503, Tamil Nadu, India.

***Corresponding author**
J.Mariajancyrani
Email: **mariajancyrani@yahoo.in**

Abstract: Plants are the almost exclusive 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. The chemical compounds in solanum nigrum help in treating aliments like liver diseases, cancer, diabetes, and kidney diseases by functioning as antioxidants. The present study focuses on the analysis of the chloroform extract of solanum nigrum leaves by GC-MS. The study revealed the presence of 23 phyto components such as palmitic acid, phytol, hexacontan, Ethyl linoleolate. The mass spectra of these compounds were matched with the National Institute of Standards and Technology (NIST) Library.
Keywords: Antioxidants, Hexacontan, Kidney diseases, Palmitic acid, phytol, solanum nigrum

INTRODUCTION

Solanum nigrum is a fairly common herb or short-lived perennial shrub known as 'Black Nightshade. The plant native to India and America but found in almost all parts of Africa [1]. Extract of S. nigrum have shown anti- tumour and neuro-pharmacological properties as well as antioxidant and cancer chemo-protective matter [2-4]. The leaves and seed (berries) are used in India as vegetables in soup. Besides being used for human consumption, the leaves serve as fodder and browse for domestic herbivorous animals. The plant is used for the treatment of boils and gonorrhea but toxic to man. The berries especially when unripe were reported to contain poisonous solanocapsine and other alkaloids, that are fatal to man and animals. S. nigrum and its varieties have shown properties [5-7].

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 voltaic essential oil, fatty acid, lipids and alkaloids [8]. 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 selectivity absorbent stationary phase. It also plays a fundamental role as an analytical technique for quality control and standardization of phyto therapeuticals. There are a number of different kinds of chromatography, which differ in the mobile and the stationary phases used [9].

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. 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 had become one of the most important tools for the separation of volatile compounds [10].

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) direct identification of unknown compounds is easy to establish [11]. The objective of the present study is to identify the possible phytoconstituents present in the chloroform extract of Solanum nigrum using GC-MS study.

MATERIALS AND METHODS

Plant material

The leaves of the plant Solanum nigrum collected from Thanjavur district of Tamil Nadu. The botanical identify of the plant was confirmed by Dr. John Britto, Rapinet Herbarium, St .Joseph's College,

Tiruchirappalli. The leaves were cleaned, dried in shadow and crushed into powder.

Preparation of extract

The powder (1kg) was extracted with petroleum ether followed by chloroform at room temperature for 48h. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The chloroform extract were subjected to GC-MS analysis.

GC-MS analysis

Instruments and chromatographic conditions

GC-MS analysis was carried out on GC-MS-QP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions : column VF-5MS fussed silica capillary column (30.0m x 0.25mm x 0.25µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70ev; helium (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 240 ^{0}C ion-source temperature 200 ^{0}C. The oven temperature was programmed from 70 ^{0}C (isothermal for 3 min), with an increase of 10 ^{0}C/min, to 240 ^{0}C, ending with a 9min isothermal at 280 ^{0}C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 440Da. Total GC running time is 40min.

Identification of compounds

Interpretation of 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.

RESULTS AND DISCUSSION

The GC-MS chromatogram (Figure-1) showed 23 peaks indicating the presence of twenty three phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the 23 phytoconstituents were characterized and identified, which are listed with their retention time (RT), molecular formula, molecular weight, structure and biological activity in Table-1 and 2.

Figure.1 GC-MS chromatogram of the chloroform extract of the *Solanum nigrum*

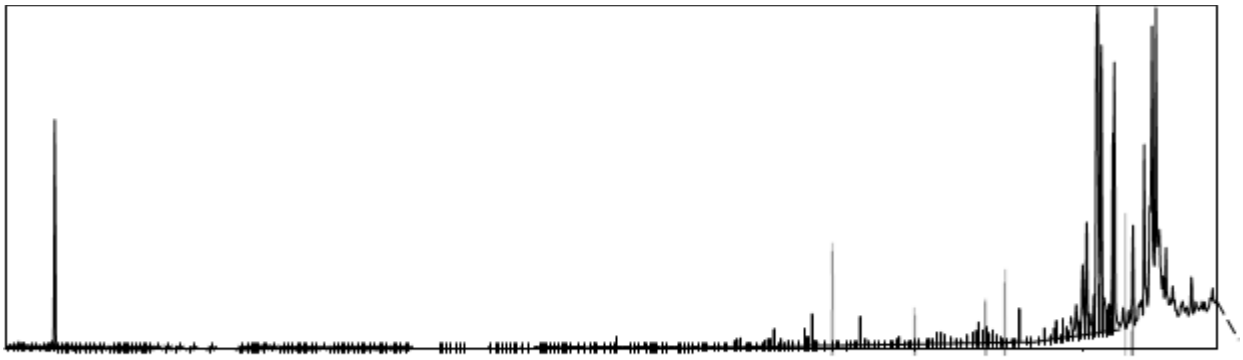
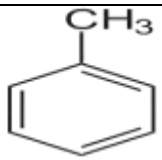
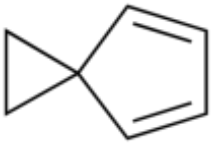


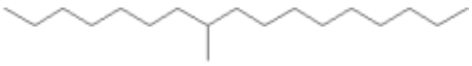
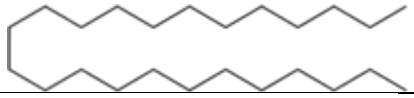
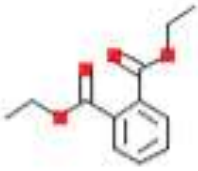
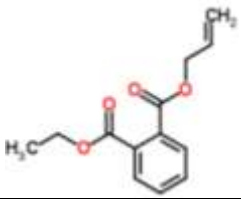
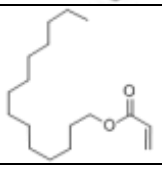
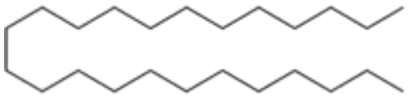
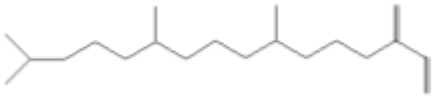
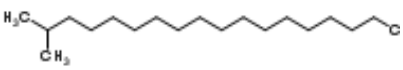
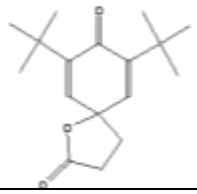
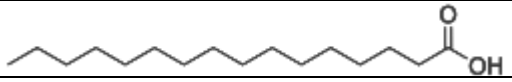
Table.1 Phyto-components identified for Solanum nigrum.

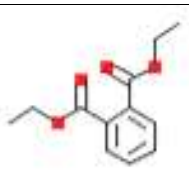
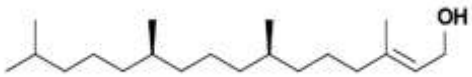
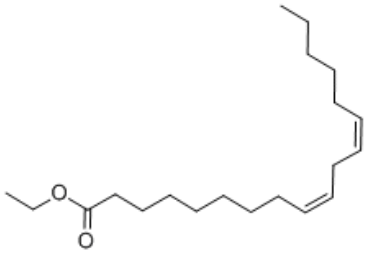
Compound Name	RT	Peak Area %	Molecular Formula	MolecularWeight	Compound nature
Toluene	4.447	2.55	C7 H8	92	Aromatic compound
1,3,5-Cycloheptatriene	4.447	2.55	C7 H8	92	Hydrocarbon
Spiro[2.4]hepta-4,6-diene	4.447	2.55	C7 H8	92	Hydrocarbon
Heptadecane, 8-methyl-	25.785	1.09	C18 H38	254	Hydrocarbon
Octadecane	25.785	1.09	C18 H38	254	alkane hydrocarbon
Tetracosane	25.785	1.09	C24 H50	338	alkane hydrocarbon

Nonadecane	26.927	1.16	C19 H40	268	alkane hydrocarbon
1,2-Benzenedicarboxylic acid, diethyl ester	28.458	2.37	C12 H14 O4	222	phthalate ester
Ethylallylphthalate	28.458	2.37	C13 H14 O4	234	Ester compound -
acrylic acid tetradecanyl ester	30.625	1.09	C17 H32 O2	268	Ester compound -
Tetracosane	31.980	1.32	C24 H50	338	alkane hydrocarbon
Docosane	31.980	1.32	C22 H46	310	l'alcane
Neophytadiene	33.799	1.55	C20 H38	278	Alkene compound
2-Methyl-octadecyne	33.799	1.55	C19 H40	268	Hydrocarbon
n-Tetratriacontane	35.020	1.23	C34 H70	479	Hydrocarbon
7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	35.141	1.51	C17 H24 O3	276	Ketone compound -
Palmitic acid	35.464	8.10	C16 H32 O2	256	fatty *acid*
1,2-Benzenedicarboxylic acid, dibutyl ester	35.573	3.85	C16 H22 O4	278	phthalate ester
Phytol	36.850	2.57	C20 H40 O	296	diterpene alcohol
Phytol isomer	36.850	2.57	C20 H40 O	296	diterpene alcohol
Hexacontan	37.020	1.72	C60 H122	843	Hydrocarbon
9,12,15-Octadecatrien-1-ol	37.085	5.20	C18 H32 O	264	Alcoholic compound
Ethyl linoleolate	37.085	5.20	C20 H36 O2	308	Ester compound-

Table.2 Biological activity of chloroform extract of *Solanum nigrum*

S. No	Compound Name	Structure	Function
1	Toluene		Encephalopathic
2	1,3,5-Cycloheptatriene		No activity reported
3	Spiro[2.4]hepta-4,6-diene		No activity reported -
4	Heptadecane, 8-methyl-		

			No activity reported -
5	n-Octadecane		No activity reported -
6	Tetracosane		No activity reported -
7	Nonadecane		No activity reported -
8	1,2-Benzenedicarboxylic acid, diethyl ester		Antimicobial
9	Ethyl allyl phthalate		Antimicrobial, Antioxidant -
10	acrylic acid tetradecanyl ester		No activity reported -
11	n-Tetracosane		No activity reported -
12	Docosane		No activity reported -
13	Neophytadiene		No activity reported
14	2-Methyl-octadecyne		No activity reported -
15	Tetratriacontane		No activity reported -
16	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione		No activity reported -
17	Palmitic acid		Antioxidant, pesticide, antimicrobial, lubricant

18	1,2-Benzenedicarboxylic acid, dibutyl ester		Antioxidant, nematicide, hyperchloesteromic, lubricant, anticancer
19	Phytol		Antimicrobial, anticancer, cancer preventive diuretic, anti-inflammatory
20	Phytol isomer		Antimicrobial, anticancer, cancer preventive diuretic, anti-inflammatory
21	Hexacontan	-	No activity reported-
22	9,12,15-Octadecatrien-1-ol		Antimicrobial -
1. 23	2. Ethyl linoleolate	 **3.**	4. Antimicrobial **5.** **6.** **7.** -

Four major phytochemical constituents were obtained from mass spectra, identified as Palmitic acid (8.1%), Phytol (2.57 %), 9, 12, 15-octadecatrien-1-ol (5.2%) and Ethyl linoleolate (5.2%) respectively. The identified compounds possess many biological properties. For instance, palmitic acid can e an antioxidant hypocholesterolemic, nematicide pesticide, lubricant activities and hemolytic 5-alpha is reductase inhibitors. Phytol Diterpene is an antimicrobial, anticancer, anti inflammatory and diuretic agent. The biological activities listed are based on Dr. Dukes phytochemical and Ethanobotanical Databases by Dr. Jim Duke of the Agricultural Research Service, USDA [12].

Phytol is one among the twenty three compounds of the present study. Similarly Mariajancyrani et al observed the presence of phytol in the leaves of Lantana camara and Sridharan et al in Mimosa pudica leaves. Similar result was also observed in the leaves of Lantana camara [12-14]. 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 [9-15]. These reports are in accordance with the result of this study.

CONCLUSION

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 Solanum nigrum suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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