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Bioactive compounds in ethanolic extract of Sansevieria roxburghiana leaves using GC-MS technique

Anuradha G¹, Mani N^{2*}

- ¹ Research Scholar, Department of Chemistry, AVVM Sri Pushpam College (Affiliated to Bharathidasan University), Poondi, Thanjavur, Tamil Nadu, India
 - ² Assistant Professor, PG Research Department of Chemistry, AVVM Sri Pushpam College (Affiliated to Bharathidasan University), Poondi, Thanjavur, Tamil Nadu, India

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 *Sansevieria roxburghiana* 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 Benzaldehyde, 2-nitro, Tetradecanal, 1-Octadecanol, Hexadecanoic acid, Hexadecane, 1-Heptadecanol, 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*, R*-(E)], 9, 12 Octadecadienoic acid, 9-Octadecenoic acid of *Sansevieria roxburghiana* leaves. These findings support the traditional use of *Sansevieria roxburghiana* leaves in various disorders.

Keywords: gas chromatography and mass spectroscopy, sansevieria roxburghiana, 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 Meyer (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, 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) [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]. 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) [7]. The chosen medicinal plant namely as Sansevieria roxburghiana leaves belongs to Agavaceae Family. Sansevieria roxburghiana leaves is widely distributed in southern India and Sri Lanka. The aim of this study is to determine the organic compounds present in the Sansevieria roxburghiana leaves extract with the aid of GC-MS Technique.

Material and methods Collection of plant materials

The whole plant of *Sansevieria roxburghiana* leaves were collected from Ariyalur, 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 Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of leaf extract

The collected Sansevieria roxburghiana leaves were washed several times with distilled water to remove the traces of impurities from the leaves. 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 Sansevieria roxburghiana leaves extract was stored in refrigerator until used.

GC -MS analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32 mm, column length is 30 m, column thickness 0.50 µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 μI was employed (split ratio of 10:1) injector temperature 270 °C; ion-source temperature 200 °C. The oven temperature was programmed from 40 °C (isothermal for 2 min), with an increase of 8°C/min, to 150°C, then 8°C/min to 250°C, ending with a 20min isothermal at 280°C. 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 51.25 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 Srinivasan et al $(2013)^{[8]}$.

Identification of components

Interpretation on GCMS 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 Dr. Dukes (2013) [9].

Result 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) [10]. 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) [11]. 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, antihepatotoxic and anti-ulcer actions (De-Fatima et al 2006) [12]. 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 *Sansevieria roxburghiana* 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 Figure 1). The prevailing compounds were Benzaldehyde, 2-nitro, Tetradecanal, 1-Octadecanol, Hexadecanoic acid, Hexadecane, 1-Heptadecanol, 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*, R*-(E)], 9, 12 Octadecadienoic acid, 9-Octadecenoic acid. 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.

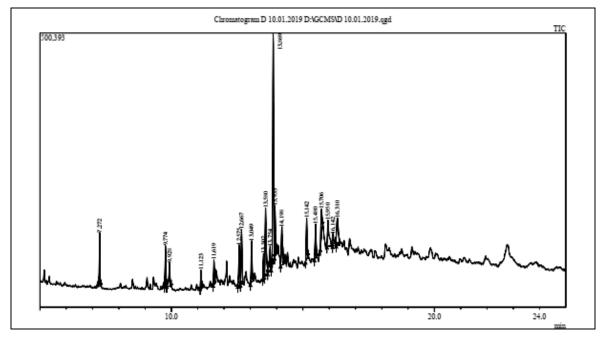


Fig 1: GC MS Chromatogram of Sansevieria roxburghiana leaves extract

Table 1: Identification of bioactive compounds in ethanolic extract of Sansevieria roxburghiana leaves extract using GC MS

Peak #	R. Time	Area%	Molecular formula	Molecular weight	Molecular Name
1	7.272	3.72	C ₁₃ H ₂₈	184	Tridecane
2	9.774	3.32	$C_{15}H_{32}$	212	Pentadecane
3	9.928	3.07	C ₇ H ₅ NO ₃	151	Benzaldehyde, 2-nitro.
4	11.123	1.54	$C_{12}H_{26}$	170	Decane, 3,7-dimethyl
5	11.619	1.87	C_9H_{20}	128	Hexane, 2,4,4-trimethyl
6	12.575	4.56	C ₁₄ H ₂₈ O	212	Tetradecanal
7	12.667	5.18	C ₁₃ H ₂₆ O	198	2-Undecanone, 6,10-dimethyl
8	13.049	4.29	C ₁₈ H ₃₈ O	270	1-Octadecanol
9	13.502	2.03	C ₁₄ H ₃₀	198	Decane, 2,3,5,8-tetramethyl
10	13.580	8.52	$C_{14}H_{22}N_2O$	234	Xycaine
11	13.754	3.43	$C_{20}H_{42}O_2$	314	Octadecane, 1,1-dimethoxy-
12	13.869	25.17	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
13	13.933	4.53	C ₁₆ H ₃₄	226	Hexadecane
14	14.198	5.08	$C_{18}H_{36}O_{2}$	284	Hexadecanoic acid, ethyl ester
15	15.142	3.97	C ₁₇ H ₃₆ O	256	1-Heptadecanol
16	15.480	3.89	$C_{20}H_{40}O$	296	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]
17	15.706	3.77	$C_{18}H_{32}O_2$	280	9,12-Octadecadienoic acid
18	15.950	4.64	C ₁₈ H ₃₄ O ₂	282	9-Octadecenoic acid
19	16.142	2.52	$C_{13}H_{28}$	184	Decane, 2,3,4-Trimethyl
20	16.310	4.91	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, ethyl ester

Table 2: Biological activity of phytocomponents identified in the ethanol leaf extract of Sansevieria roxburghiana leaves

S. No	Compound name	Biological activities
1	Benzaldehyde, 2-nitro	Antimicrobial activity.
2	Tetradecanal	Antioxidant, Lubricant, Hypercholeste rolemia, Cancer-preventive, Cosmetic Antibacterial activity
3	1-Octadecanol	Antibacterial, antioxidant, anticancer activity.
4	Hexadecanoic acid	Antioxidant, hypocholesterolemic, Anti androgenic, hemolytic, Alpha reductase inhibitor.
5	Hexadecane	Antimicrobial and antioxidant activity.
6	1-Heptadecanol	Antimalarial, antifungal, Antioxidant activity.
7	2-Hexadecen-1-ol, 3,7,11,15-	Anti-cancer, Antioxidant, Anti-inflammatory, Diuretic, Cytotoxicity, Anti-microbial, Cancer
	tetramethyl-, $[R-[R*,R*-(E)]$	preventive
8		Anti-inflammatory, Hypocholesterolemic cancer preventive, hepatoprotective, nematicide,
	9,12-Octadecadienoic acid	insectifuge, antihistaminic antieczemic, anticancer, 5-Alpha reeducates inhibitor, anti-androgenic,
		Anti-arthritic, anti-coronary.
9	9-Octadecenoic acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol.

^{**}Duke's. Phytochemical and Ethnobotanical Databases, www.ars-gov/cgi-bin/duke/, 2013.

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; Falodun *et al* 2009) [13, 14]. 1, 2- benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent (Heinonen *et al* 1998) [15]

Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic acid, 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) [16]. Likewise, hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2benzenedicarboxylic acid and 9-octadecenoic acid (Z)-ethyl ester were reported in Clerodendrum inerme and C. phlomidis leaves (Anandhi and Ushadevi 2013; Balaji and Kilimozhi 2014) [17, 18].

Conclusion

The investigation concluded that the stronger extraction capacity of ethanol 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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