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Bioactive Compound Evaluation of Ethanol Extract from *Geodorum densiflorum* (Lam.) Schltr. by GC-MS analysis

Keerthiga M* and Anand S. P

Department of Biotechnology, National College (Autonomous & CPE), Tiruchirappalli – 01, Tamil Nadu, India

Corresponding author*
Keerthiga Manohar,
Research Scholar,
Department of Biotechnology,
National College (Autonomous & CPE), Tiruchirappalli – 01, Tamil Nadu, India
E-mail: keerthigamanohar@gmail.com

Abstract

The phytochemical constituents are responsible for medicinal value of the plant species. The present investigation was carried out to analyze the bioactive components from the whole plant of *Geodorum densiflorum* (Lam.) Schltr. using GC-MS technique. The chemical compositions of the ethanolic extract of *G. densiflorum* were investigated using Perkin - Elmer Gas Chromatography – Mass Spectrometry and about twenty one bioactive phytochemical compounds were identified. The prevailing compounds where Hexadecanoic acid, Ethyl ester (38.884 %), Ionone (7.125 %), 3-Deoxy-d-mannoic lactone (7.4441 %), 2,3-Butanediol (4.725 %) and 2-Piperidinone, N-[4-bromo-n-butyl]- (4.004 %), (E)-9-Octadeconoic acid ethyl ester (3.891 %), 1H-Pyrrole-2-Carbonitrile (3.778 %), Pyridinium, 1-amino-, chloride (3.305 %), 4H- Pyran-4-one, 3,5-dihydroxy-2-methyl- (3.274) and having various biological activities. This was the first report on the identification of bioactive compounds from ethanol extract of *G. densiflorum*.
Keywords: Phytochemical Compounds, GC-MS analysis and *Geodorum densiflorum*.

1. Introduction

From ancient times higher plants are best sources of bioactive compounds, play principal role in the maintenance of human health. Literature studies represent the herbal plants are reservoir of effective chemotherapeutants, these are more systemic, easily biodegradable and non-phytotoxic.[1]-[3] In general, secondary metabolites from plants were having interesting biological activities. These secondary metabolites are act as lead compound for new drugs because of its variety of structural arrangements and properties.[4] Knowledge on the phytoconstituents of plants is desirable for the discovery of therapeutic agents, new sources of economic phytocompounds for the synthesis of complex chemical substances and for disclosing the actual significance of folkloric remedies.[5] The standardization of the natural drugs has emerged as a new branch of science as the phytochemicals have complementary and overlapping mechanism of action; hence a thorough validation of the herbal drugs was emphasized and prioritized. Mass Spectrometry coupled with chromatographic separations, Gas Chromatography (GC/MS) is normally used for direct analysis of chemical components existing in herbal medicines. For the analysis of medicinal plants, GC-MS technique have been proved to be highly commended analysis for non-polar components and volatile essential oil, fatty acids, lipids[6] and alkaloids[7].

Recently there has been tremendous progress in medicinal plants research; however orchids have not been exploited fully for their medicinal application. Recent researches reported the presence of Anthocyanins, Stikbnoids, Triterpenoids, Orchinol, Hircinol, Cyperpedinl, Jibantine, Nidemin, Lorogcossin, Gymopusin in *Bulbophyllum gumpous*; alkyl perulate and β-sistosero D-glucoside in *Vanda tessellate*; Alkanes and Alkanol Sistosterol, Resin, Saponin, Tannins, Fatty Acids, Colouring Agents etc.[8] *Geodorum densiflorum* (Lam.) Schltr. (Family: *Orchidaceae*) is an endangered terrestrial orchid widely distributed in humid tropical forests of south India. Literature studies indicate that no reports on *G. densiflorum* has so far been undertaken to provide enough scientific data in favour of reported traditional uses. Traditional use varies among the local practitioners for menstrual cycle regulation, joint pain and arthritis, diabetic, applied externally to cure Carbuncles, etc.[9] As part of the endeavor for search of therapeutic properties of *G.densiflorum* we herein reported the GC-MS analysis of ethanol extract from the whole plant.

2. Materials and Methods

The plant *Geodorum densiflorum* (Lam.) Schltr. were collected from Periyakombai hill area of Kolli hills in Namakkal District of Tamil Nadu, India. The botanical identity of this plant was confirmed by Dr. G. V. S. Murthy, Scientist – F & Head of Office, Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu. The voucher

specimen was submitted at the Department of Botany, National College (Autonomous), Tiruchirappalli-620 001, Tamil Nadu, India.

2.1 Plant Sample Extraction

The whole plant material was shaded dried and made into fine powder. 20 g powdered plant material is soaked in 50 ml of absolute ethanol overnight and then filtered through whatmann filter paper No.41 along with 2gm Sodium Sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with Sodium Sulfate is wetted with ethanol the filtrate is then concentrated and reduces the volume to 1ml the extract contains both polar and non-polar Phytocomponents.

2.2 GC-MS Analysis

GC MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and Gas Chromatography interfaced to a Mass Spectrometer (GC-MS) instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30 m and 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 µl was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8ºC/min, to 150 ºC, then 8 ºC/min to 250 ºC, ending with a 20 minutes 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 26 minutes. 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 [10].

2.3 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. Duke's. Phytochemical and Ethnobotanical Databases).

3. Results

Twenty one compounds were identified in ethanol extract of *G. densiflorum* by GC-MS analysis. The bioactive compounds with their Retention Time (RT), Molecular structure, Molecular Formula, Molecular Weight, Concentration (peak area %), nature of compounds and biological activity are presented in (Table 1 & 2 and Fig 1). The prevailing compounds where Hexadecanoic acid, ethyl ester (38.884 %), Ionone (7.125 %), 3-Deoxy-d-mannoic lactone (7.4441 %), 2,3-Butanediol (4.725 %) and 2-Piperidinone, N-[4-bromo-n-butyl]- (4.004 %), (E)-9-Octadeconoic acid ethyl ester (3.891 %), 1H-Pyrrole-2-Carbonitrile (3.778 %), Pyridinium, 1-amino-, chloride (3.305 %), 4H- Pyran-4-one, 3,5-dihydroxy-2-methyl- (3.274), Undecanal, 2-methyl- (2.128 %), 5-Hydroxymethylfurfural (2.005 %), Propanoic acid, 2-hydroxyl-, 2-methylpropyl ester (1.628 %) and p-Ethoxybenzyl alcohol (1.457 %). The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting to biological activity will definitely give fruitful results.

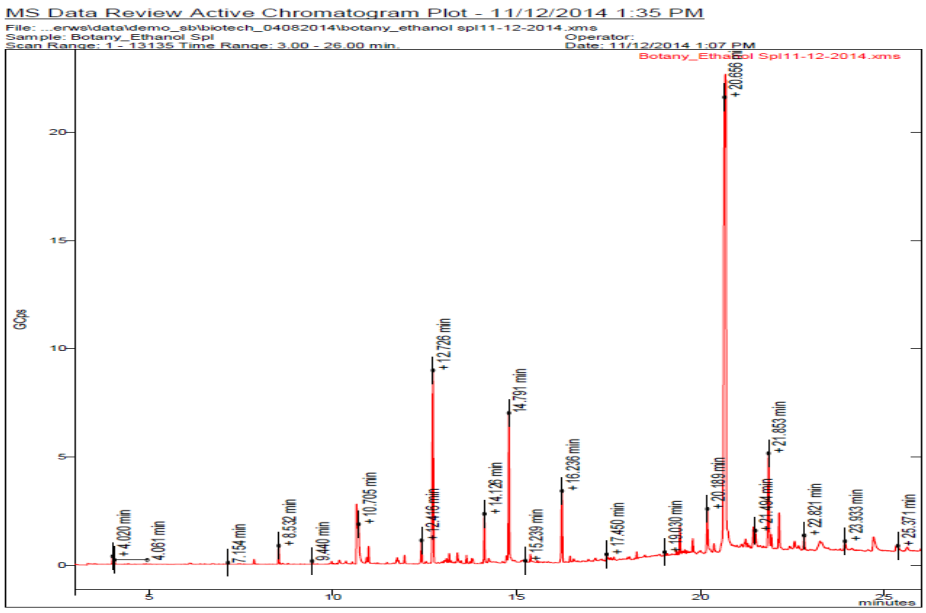
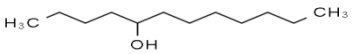
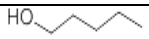
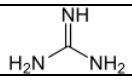
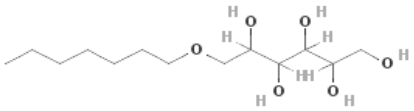
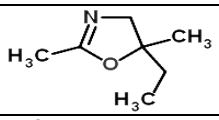
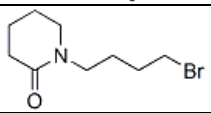
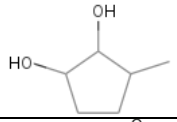
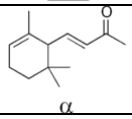
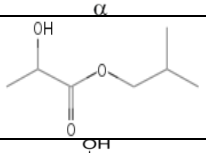
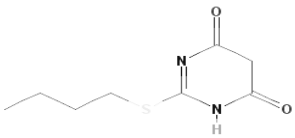
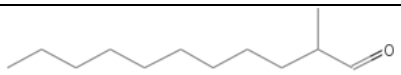
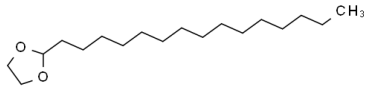
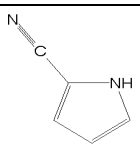
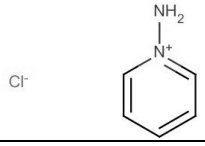


Fig-1: Chromatogram obtained from GC-MS with the ethanolic extract of *G.densiflorum* (Lam.) Schltr.

Table 1: Components detected in ethanolic extract of *G.densiflorum* (Lam.) Schltr.

S. No	RT	Compound Name	Molecular Structure	Molecular Formula	Molecular Weight	Peak Area %
1	4.020	5-Dodecanol		$C_{12}H_{26}O$	186.334	0.132
2	4.061	1-Pentanol		$C_5H_{12}O$	88.15	0.437
3	7.154	Guanidine		CH_5N_3	59.07	0.261
4	8.532	D-Mannitol, 1-o-heptyl		$C_{13}H_{28}O_6$	280.357	0.275
5	9.440	2,5-Dimethyl-5-ethyl-2-oxazoline		$C_7H_{13}NO$	127.184	0.346
6	10.705	2-Piperidinone, N-[4-bromo-n-butyl]-		$C_9H_{16}BrNO$	234.136	4.004
7	12.416	1,2-Cyclopentanediol, 3-methyl-		$C_6H_{12}O_2$	116.158	0.305
8	12.726	Ionone		$C_{13}H_{20}O$	192.30	7.125
9	14.126	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester		$C_7H_{14}O_3$	146.184	1.628
10	14.791	2,3-Butanediol		$C_4H_{10}O_2$	90.121	4.725
11	15.239	Pyrimidine-4,6(3H, 5H)-dione, 2-buttylthio-		$C_8H_{12}N_2O_2S$	200.258	0.401
12	16.236	Undecanal, 2-methyl-		$C_{12}H_{24}O$	184.318	2.128
13	17.450	1,3-Dioxolane, 2-pentadecyl-		$C_{18}H_{36}O_2$	284.477	0.553
14	19.030	1H-pyrrole-2-Carbonitrile		$C_5H_4N_2$	92.1	3.778
15	20.189	Pyridinium, 1-amino-, chloride		$C_5H_7N_2.Cl$	138	3.305
16	20.656	Hexadecanoic acid, ethyl ester		$C_{18}H_{36}O_2$	284.477	38.884

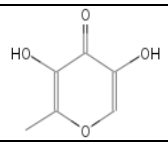
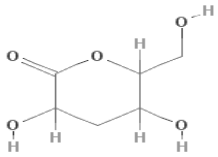
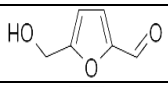
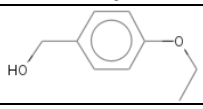
17	21.494	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-		$C_6H_6O_4$	142.109	3.274
18	21.853	3-Deoxy-d-mannoic lactone		$C_6H_{10}O_5$	162.140	7.444
19	22.821	(E)-9-Octadeconoic acid ethyl ester		$C_{20}H_{38}O_2$	310.514	3.891
20	23.933	5-Hydroxymethylfurfural		$C_6H_6O_3$	126.11	2.005
21	25.371	P- Ethoxybenzyl alcohol		$C_9H_{12}O_2$	152.190	1.457

Table 2: GC-MS analysis showed phytochemical compounds, their nature and their biological activities of ethanol extract of *G. densiflorum*.

S. No	Compound Name	Nature of Compound	Biological Activity	References
1	5-Dodecanol	Fatty alcohol	Flavour	[11]
2	1-Pentanol	Alcohol	Antimicrobial	[11]
3	Guanidine	Nitrogenous Compound	Anticancer, Antidiabetic, Antiviral, Anti-inflammatory,Antibiotic,Antileishmenial, Antiprotozoal, Antihistaminic and Antihypertensive	[12]
4	d-mannitol, 1-o-heptyl	Sugar alcohol	Osmotic diuretic agent, Renal vasodilator	[13]
5	2,5-Dimethyl-5-ethyl-2-oxazoline	Heterocyclic Compound	Antibacterial, Anti-inflammatory, Anti-tumour and Antioxidant	[14]
6	2-Piperidinone, N-[4-bromo-n-butyl]-	Alkaloid	Antimicrobial, Antioxidant, Anti-inflammatory	[15]
7	1,2-Cyclopentanediol, 3-methyl-	Alcoholic Compound	Antimicrobial	[16]
8	Ionone	Monoterpenes	Antibacterial, Sedative, Antitumor, Cytotoxic, Anti-inflammatory, Insecticidal and Molluscidal activity	[17]
9	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester	Alkene Compound	Anticancer activity	[18]
10	2,3-Butanediol	Volatile Compound	Pesticides	[19]
11	Pyrimidine-4,6(3H, 5H)-dione, 2-buttylthio-	Heterocyclic Compound	Hypoglycemic, Antimalarial, Analegesic, Anti-inflammatory, PDE_4 inhibitor and Spasmolytic activity	[20]
12	Undecanal, 2-methyl-	Aldehydes	Pesticides, Flavors and Fragrances	[21]
13	1,3-Dioxolane, 2-pentadecyl-	Aromatic Compound	Antibacterial and Antifungal activity	[22]
14	1H-pyrrole-2-Carbonitrile	Heterocyclic aromatic Compound	Hypolipidemic, Antimicrobial, Anti-inflammatory and Antitumor activity	[23]
15	Pyridinium, 1-amino-, chloride	Nitrogen Compound	Antimicrobial and Anti-inflammatory activity	[24]
16	Hexadecanoic acid, ethyl ester	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anliandrogenic, Flavor, Hemolytic, 5 Alpha reductase inhibitor	[25]
17	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	Flavonoid Fraction	Antimicrobial, Anti-inflammatory and Antioxidant	[16]
18	3-Deoxy-d-mannoic lactone	Cyclic ester	Antimicrobial activity	[26]
19	(E)-9-Octadeconoic acid ethyl ester	Oleic acid ester	Anti-inflammatory, Anticancer, hypocholesterolemic, 5-Alpha reductase inhibitor and antiandrogenic activity	[27]
20	5-Hydroxymethylfurfural	Sugar Compound	Antioxidant, Antiproliferative activity	[28]
21	P- Ethoxybenzyl alcohol	Alcoholic Compound	Antimalarial	[29]

3. Discussion

The identified compounds with more percentage like Hexadecanoic acid, ethyl ester (Palmitic acid ester) (38.884 %), Ionone (Monoterpenes) (7.125 %), 3-Deoxy-d-mannoic lactone (Cyclic ester) (7.4441 %), 2,3-Butanediol (Volatile Compound) (4.725 %) and 2-Piperidinone, N-[4-bromo-n-butyl]- (Alkaloid) (4.004 %),showed a wide range of potent bioactivity. Among the twenty one identified compounds 10 showed Anti-microbial activity, 9 showed Anti-inflammatory, 6 showed Anti-cancer and 4 showed anti-oxidant and also showed activities such as hypocholesterolemic, antiandrogenic, Anliandrogenic, antiproliferative, Hemolytic, 5 Alpha reductase inhibitor, Hypolipidemic, Antimalarial, Nematicide, Pesticide, Insecticidal and Molluscidal activity and also having compounds for Lubricant and Flavor. Unsaturated fatty acids are important to every cell for normal growth, to support the lubricating quality of skin [30] and to lower cholesterol levels of the blood.[31] Pure isolated plant alkaloids are used as basic medicinal agent for analgesic, anti-spasmodic, anti-bacterial properties, etc. For example Quinine (alkaloid) extracted from Cinchona is used to treat malaria.[32] The previous study on *G. densiflorum* showed antibacterial, antioxidant, cytotoxic, thrombolytic, sedative, analgesic and anxiolytic activities.[33] The presences of these phytocompound are responsible for the properties of plant.

4. Conclusion

The GC-MS studies carried out on ethanol extract of *Geodorum densiflorum* (Lam.) Schltr. showed the presence of bioactive compounds responsible for potent activity of medicinal orchid. Further work regarding specific activity of the identified compound will provide more insight about the role of plant.

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