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Identification of bioactive components in ethanolic and aqueous extracts of *Amorphophallus campanulatus* tuber by GC-MS analysis

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Abstract

The uses of herbal medicines worldwide provide an excellent opportunity for India to look into the therapeutic compounds from ancient system of therapy, Ayurveda. Since ancient time, *Amorphophallus campanulatus* has been considered as medicinal plant with multiple protective activities. So, the present study was aimed to identify the various phytochemical constituents in the ethanolic and aqueous extracts of *Amorphophallus campanulatus* tuber by GC-MS analysis. The ethanolic and aqueous extracts of *Amorphophallus campanulatus* were dissolved in absolute ethanol (1mg/ml). 10µl of this sample was then injected for gas chromatography- mass spectrometric (GC-MS) analysis. The results for the first time revealed the presence of several bioactive components both in the ethanolic as well as in the aqueous extracts. However, the ethanolic extract showed quantitatively higher amount of these components than the aqueous extract as is clearly indicated by higher % peak area of the compounds in the ethanolic extract. Some components of biological importance include Hexadecanoic acid and its methyl and ethyl esters, Heptadecanoic acid, Linoleic acid and its ester, Oleic acid, Stigmasterol, 1, 3, 5, benzenetriol, 4H-Pyran-4-one, 2, 3-dihydro-3, 5 -dihydroxy-6-methyl-, Squalene and Vitamin E. Thus the results of the present study is an evidence to support the traditional usage of *A. campanulatus* which possess several known and unknown bioactive components. By identifying and isolating these components, new drugs can be formulated to treat various diseases.

Keywords: Antioxidants, Medicinal plant, Oxidative stress, Phytochemistry

Introduction

India is endowed with a rich wealth of medicinal plants and it is one of the 12 mega-biodiversity centers having more than 45,000 plant species. Knowledge of herbs has been handed down from generation to generation for thousands of years [1]. Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity because plants, above all other agents, have been used for medicine from time immemorial for their fewer side effects, easy accessibility and low cost [2].

Several phytochemical screening studies have been carried out in different parts of the world [3-5] and roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources [6]. Therefore, characterization of medicinal plants is necessary, due to their numerous benefits to science and society.

Amorphophallus campanulatus (Roxb.) commonly known as surana in *Sanskrit*, elephant yam in *English* and ol kachu in *Bengali* is a tuberous, stout, indigenous herb, 1.0-1.5 m in height. *Amorphophallus* is a large genus of some 170 tropical and subtropical tuberous herbaceous plants from the Arum family (Araceae). The plant is widely distributed in Bangladesh, India and Africa. The tuber of this plant forms a staple food among the people of these countries as it is easily available throughout the year and economically affordable. The tuberous roots of the plant have been used traditionally for the treatment of piles, abdominal pain, tumors, enlargement of spleen, asthma and rheumatism [7]. Besides, antibacterial [8], anti-inflammatory [9] and cytotoxic [10] activities of these tuberous root extracts have also been reported. Despite of its innumerable and wide range of application in Ayurveda, very few studies have been conducted to explore the underlying components responsible for its versatile use. Recently, in one of our study, the in vitro antioxidant potential of the ethanolic and aqueous extract of this tuber has been brought into focus by studying their capacity to scavenge various free radicals [11]. Though qualitative phytochemical screening of *Amorphophallus campanulatus* have been reported [12]. no reports are available regarding the characterization of its bioactive components.

In recent time, for analysis of components existing in traditional medicines and medicinal plants, mass spectrometry coupled with chromatographic separations such as gas chromatography (GC/MS) is employed. Gas chromatography, a technique of unsurpassed separation capacity, especially, when combined with mass spectrometry, offers high sensitivity and selectivity.

Correlation of chromatographic and spectroscopic methods is also important in analytical chemistry. Therefore, the following study was aimed to identify the specific phytoconstituents present in the ethanolic and aqueous extract of *Amorphophallus campanulatus* using GC-MS analysis.

Materials and Methods

Procurement of plant materials

The tubers of *Amorphophallus campanulatus* were collected from local vegetable market of Kolkata district, West Bengal (India). Authentication was confirmed by Dr. Krishnendu Sarkar, Associate Professor, Department of Botany, Rammohan College under University of Calcutta, West Bengal, (India).

Preparation of Aqueous and Ethanolic Extract

Soxhlet extraction, the oldest and conventional method of extraction of bioactive substances from natural sources was employed for extract preparation [13]. 15 gm of air dried tuber powders were placed in the Soxhlet thimble and extracted with 200 ml of respective solvents continuously for 30-40 hours. The solution thus extracted was filtered through muslin layer, centrifuged and the collected filtrate was evaporated to dryness on hot plate at constant temperature of 60ºC. The clumpy dry residue obtained was scraped by knife, made into fine powder form and stored in air tight plastic vials.

GC-MS Analysis: Preparation of extract

The dried ethanolic and aqueous extract of *Amorphophallus campanulatus* was dissolved in absolute ethanol (1mg/ml). 10µl of this sample was then injected for gas chromatography- mass spectrometric (GC-MS) analysis.

Instruments and Chromatographic Conditions

GC-MS technique was used to identify the phytoconstituents present in the extract [14]. The plant extract was analyzed using Agilent Technologies 6890 N Network GC system & interfaced to Agilent Technologies 5973 Inert Mass Selective Detector employing the following conditions: column DB-1 ms fused silica capillary column (30X0.25 I.D.X 0.10 Film, composed of 100% Dimethylpolysiloxane) chosen for improved signal to noise ratio for better sensitivity and mass spectral integrity, operating in electron impact mode; helium (5.0) was used as carrier gas at a constant flow of 1ml/min. The injector, MS Source & MS Quadrapole temperature were fixed at 250ºC, 230ºC & 150ºC respectively and turbo Speed of the pump was 100%. The oven temperature was programmed from 50ºC (isothermal for 5 minutes), with an increase of 10ºC/min to 100ºC (isothermal for 2 minutes), then 10ºC/min to 300ºC (isothermal for 5 minutes) For tuning of the MSD in EI mode Perfluorotributylamine (PFTBA) was used as tuning compound. Mass spectra were taken at 2235 EM Volts and fragments from 69 to 502.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST)/National Bureau of Standard (NBS) and Wiley having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST/NBS and Wiley libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

Results

The results pertaining to the GC-MS analysis leads to the identification of thirty two and thirty one pharmacologically important compounds from the GC fractions of the ethanolic and aqueous extracts of *Amorphophallus campanulatus* respectively. These compounds were identified through mass spectrometry attached with GC. The various components present in the ethanolic and aqueous extracts of *Amorphophallus campanulatus* as detected by GC-MS analysis are presented in Table 1 and Table 2, respectively, along with their pharmacological activity. Accordingly, figure 1a and figure 1b represents the *Total Ion Chromatogram* of ethanolic and aqueous extracts of *Amorphophallus campanulatus*. Both the ethanolic and aqueous extracts of *Amorphophallus campanulatus* are found to possess wide range of saturated and unsaturated aliphatic hydrocarbons and their isomers like 1-Nonadecene, 1-Octadecene, Tetradecane, 1-Undecene, 1-Hexadecene, Hexadecane etc; saturated and unsaturated fatty acids and their esters like Tetradecanoic acid, Hexadecanoic acid and its methyl and ethyl esters, Pentadecanoic acid, Heptadecanoic acid, Octadecanoic acid, Dodecanoic acid, Linoleic acid and its ester, Oleic acid, Ethyl oleate etc, and plant sterols like Stigmasterol, β-Sitosterol, Campesterol, Fucosterol etc. Apart from these the extracts are also rich source of -tocopherol, the common and most popular antioxidant vitamin. It is also worth mentioning that the basic difference between the ethanolic and aqueous extracts of *Amorphophallus campanulatus* lies in its polyphenols, flavonoid and triterpene content. The ethanolic extract contains greater variety of phenolic compounds like 1, 3, 5, benzenetriol, dodecanol, Phenol, 2,4-bis(1,1-dimethyleth..., the flavonoid fraction 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- and the triterpene Squalene. Moreover it is also seen that majority of the said compounds showed greater peak area % in the ethanolic extract in comparison to that of the aqueous extract indicating that in the similar volume of 10µl, the pharmacologically active compounds are present in higher amount in the ethanolic extract than that in the aqueous extract.

Table1: Phytoconstituents present in ethanolic extract of *Amorphophallus campanulatus* tuber using GC-MS.

Sl.No	Retention Time	% Peak Area	Compound	Pharmacological Activity
1.	4.32	0.14	2-Furanmethanol	Not Reported
2.	9.67	1.29	4H-Pyran-4-one, 2, 3-dihydro-3, 5...	Antimicrobial, antiinflammatory
3.	11.37	2.39	2-Furancarboxaldehyde, 5-(hydro...	Antimicrobial, Preservative
4.	12.53	1.12	1,3,5-Benzenetriol	Antiseptic, Antioxidant, Antidermatitic, Fungicide Insecticide, Candidicide
5.	13.79	0.22	Vanillin	Antianaemic, anticancer, antibacterial, insectifuge
6.	14.05	0.16	3-Octadecene, (E)-	Not Reported
7.	14.69	0.50	5, 9-Undecadien-2-one, 6, 10-dime...	Not Reported
8.	15.03	2.37	Phenol, 2,4-bis(1,1-dimethyleth...	Antioxidant
9.	16.40	0.45	Dodecanoic acid	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide, anti-atherosclerotic.
10.	16.48	0.11	3-Tetradecene, (Z)-	Not Reported
11.	16.71	0.45	1-Hexadecene	Not Reported
12.	17.20	0.70	Benzaldehyde, 4-hydroxy-3,5-dim...	Antimicrobial
13.	17.96	0.36	Hexadecanal	Not Reported
14.	18.04	0.39	Tetradecanoic acid	Antioxidant,Cancer preventive, Nematicide, Lubricant, Hypocholesterolemic
15.	18.91	0.80	5-Octadecene, (E)-	Not Reported
16.	18.97	0.43	1-Heptadecene	Not Reported
17.	19.44	0.82	1,2-Benzenedicarboxylic acid, b...	Antimicrobial,Antifouling
18.	19.69	0.53	Pentadecanoic acid	Antioxidant
19.	19.90	0.38	Cyclopropanenonanoic acid, 2-[(...	Not Reported
20.	20.37	1.35	Dibutyl phthalate	Antimicrobial Antifouling
21.	20.89	19.18	Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
22.	21.62	0.69	Heptadecanoic acid	Anticancer
23.	22.52	26.72	9,12-Octadecadienoic acid (Z,Z)-	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
24.	22.69	4.02	Octadecanoic acid	5-alpha reductase inhibitor, hypocholesterolemic, suppository.
25.	24.45	0.47	Cyclotetracosane	Not Reported
26.	25.45	0.69	Bis(2-ethylhexyl) phthalate	Sedative
27.	27.58	1.25	Squalene	Anticancer, antimicrobial, antioxidant, chemopreventive pesticide, anti- tumor, sunscreen

28.	29.57	2.83	Vitamin E	Antiageing, analgesic, antidiabatic antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
29.	30.47	1.78	Campesterol	Antioxidant, Hypocholesterolemic
30.	30.76	2.85	Stigmasterol	Antioesteoartheritic, Hypocholesterolemic, hypoglycemic, thyroid inhibitory and antiperoxidative, anti-inflammatory, hepatoprotective, antifungal
31.	31.28	2.00	beta.-Sitosterol	Anti-bacterial, anticancer (breast, cervix and lung), anti-inflammatory, antioxidant, hepatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic
32.	31.37	0.35	Fucosterol	Hypocholesterolemic, anti-inflammatory, hepatoprotective, anticancer

Table2: Phytoconstituents present in aqueous extract of *Amorphophallus campanulatus* tuber using GC-MS.

Sl.No	Retention Time	% Peak Area	Compound	Pharmacological Activity
1.	10.58	0.45	9-Octadecene, (E)-	Not Reported
2.	14.05	2.29	1-Tetradecene	Not Reported
3.	14.21	0.39	Tetradecane	Not Reported
4.	15.58	1.37	Phenol, 2, 4-bis (1, 1-dimethyleth...	Antioxidant
5.	16.48	0.29	1-Undecene	Not Reported
6.	16.71	3.45	1-Hexadecene	Not Reported
7.	16.82	0.70	Hexadecane	Not Reported
8.	18.54	3.39	Tetradecanoic acid	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
9.	18.91	1.80	5-Octadecene, (E)-	Not Reported
10.	18.97	4.43	1-Heptadecene	Not Reported
11.	19.44	0.62	1, 2-Benzenedicarboxylic acid, b...	Antimicrobial,Antifouling
12.	19.69	0.43	Pentadecanoic acid	Antioxidant
13.	20.19	0.58	Hexadecanoic acid, methyl ester	Antioxidant, Flavor, Hypocholesterolemic Pesticide, 5-Alpha reductase inhibitor
14.	20.37	3.35	Dibutyl phthalate	Antimicrobial Antifouling
15.	20.89	14.18	Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti

				androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
16.	20.85	7.69	Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
17.	20.92	1.05	9-Eicosene, (E)-	Not Reported
18.	20.96	4.23	1-Octadecene	Not Reported
19.	21.52	0.32	Heptadecanoic acid	Anticancer
20.	22.08	0.12	Heptadecanoic acid, 16-methyl-,...	Anticancer
21.	22.16	4.90	9,12-Octadecadienoic acid (Z,Z)-	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
22.	22.22	3.34	Oleic Acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, AnemiagenicInsectifuge, Flavor
23.	22.36	2.70	Linoleic acid ethyl ester	holesterolemic, Cancerpreventive Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
25.	22.48	0.89	Ethyl Oleate	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
26.	22.68	0.80	Octadecanoic acid, ethyl ester	Anti inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, anti histaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, anti androgenic, anti arthritic, anti coronary, insectifuge
27.	22.77	2.93	1-Nonadecene	Anticancer
28.	29.55	1.08	Vitamin E acetate	Antiageing, analgesic, antidiabatic antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor,anticancer, hepatoprotective,hypocholesterolemic,antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
29.	30.42	1.18	Ergost-5-en-3-ol, (3.beta.)-	Antioxidant, Hypocholesterolemic
30.	30.68	3.85	Stigmasterol	Antioesteoartheritic, Hypocholesterolemic, hypoglycemic, thyroid inhibitory and antiperoxidative, anti-inflammatory, hepatoprotective, antifungal
31.	31.28	1.27	beta.-Sitosterol	Anti-bacterial, anticancer (breast, cervix and lung), anti-inflammatory, antioxidant, hepatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic

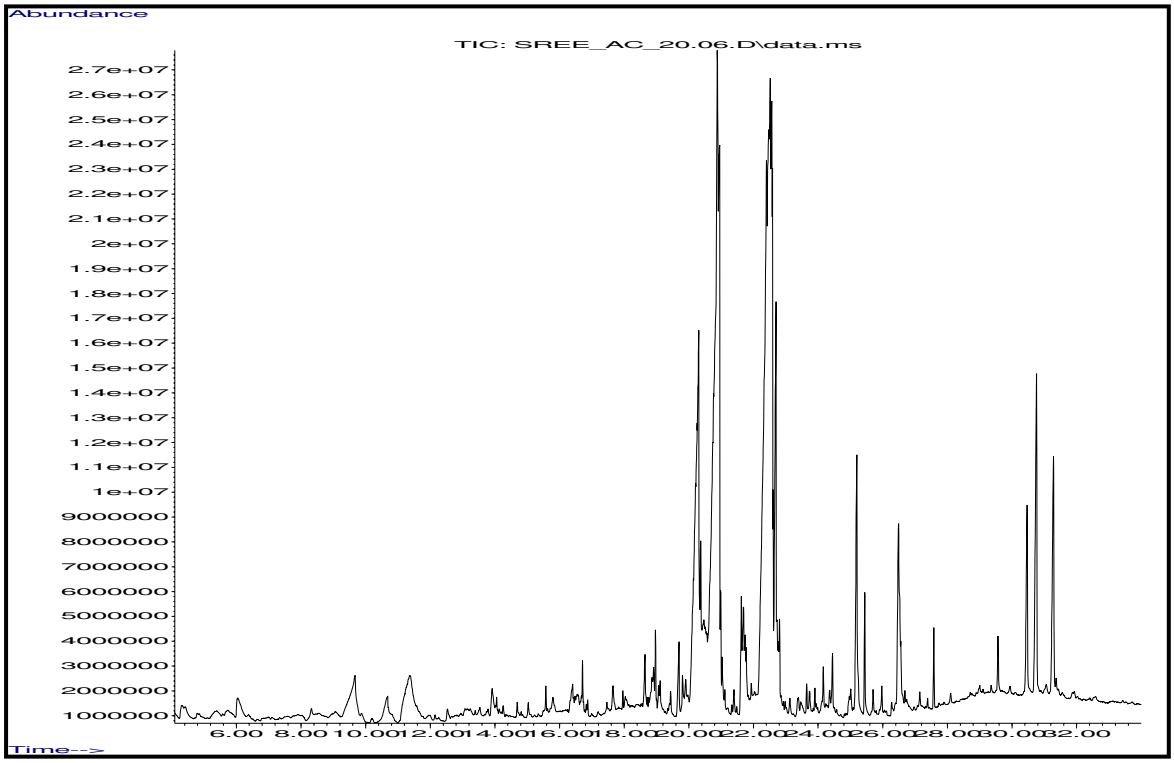


Figure1: Total Ion Current Chromatogram of ethanolic extract of *Amorphophallus campanulatus*

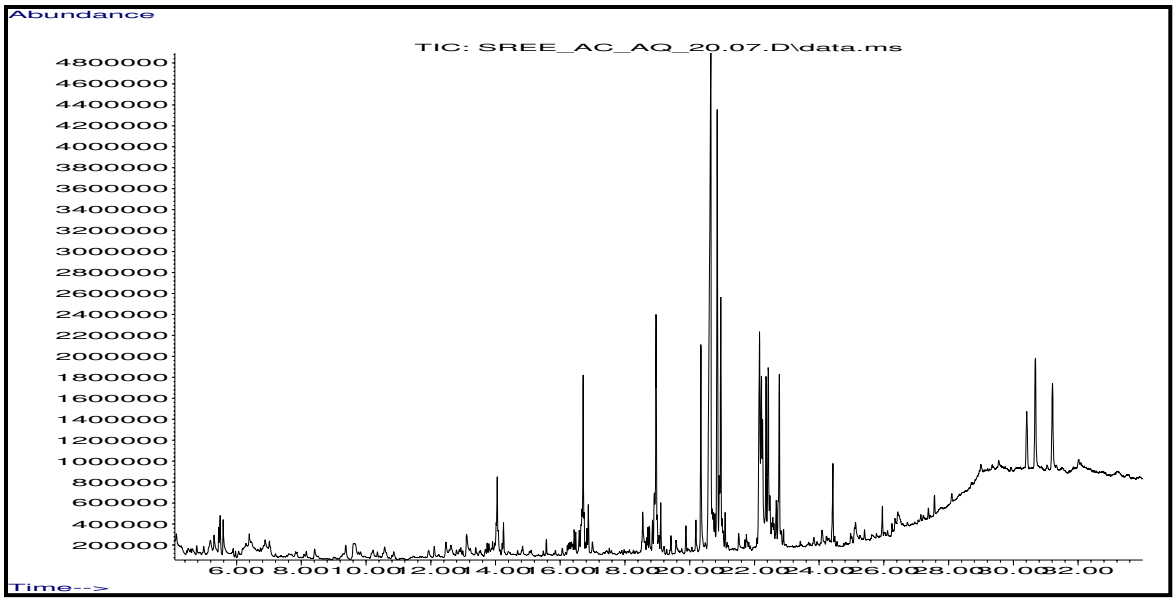
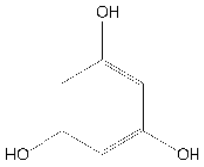
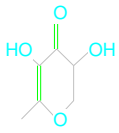
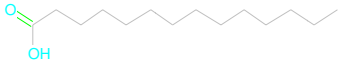
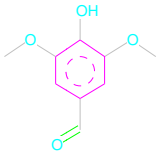
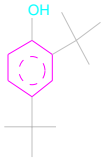
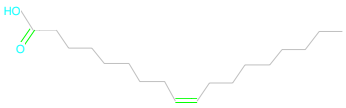
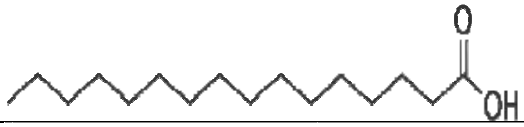
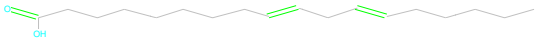
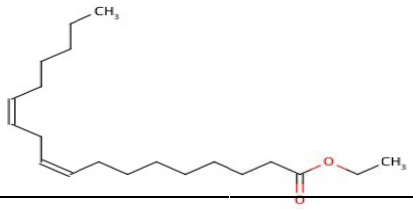
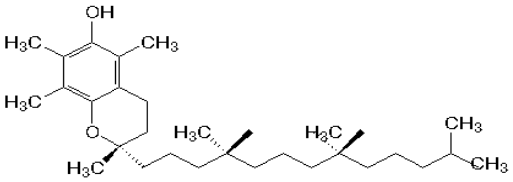
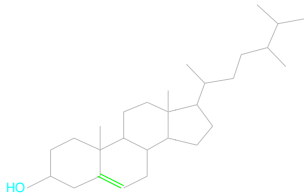
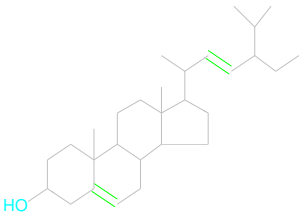
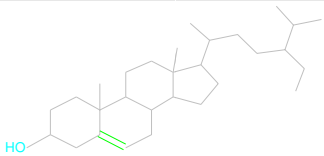


Figure 2: Total Ion Current Chromatogram of aqueous extract of *Amorphophallus campanulatus*

Table3: Molecular Formula, Molecular Weight and Structure of some major Phytochemical Compounds Present in Ethanolic and Aqueous Extracts of *Amorphophallus campanulatus.*

Sl. No.	Compound	Structure
1.	1,3,5-Benzenetriol Molecular Formula: $C_6H_6O_3$ Molecular Weight: 126.03 CAS No: 000108-73-6	
2.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Molecular Formula: $C_6H_8O_4$ Molecular Weight: 144.04 CAS No: 028564-83-2	
3.	Tetradecanoic acid Molecular Formula:$C_{14}H_{28}O_2$ Molecular Weight: 228.21 CAS No: 000544-63-8	
4.	Benzaldehyde, 4-hydroxy-3,5-dimethoxy- Molecular Formula:$C_9H_{10}O_4$ Molecular Weight:182.06 CAS No: 000134-96-3	
5.	Phenol, 2,4-bis(1,1-dimethylethyl)- Molecular Formula:$C_{14}H_{22}O$ Molecular Weight: 206.17 CAS No:	
6.	Oleic Acid Molecular Formula: $C_{18}H_{34}O_2$ Molecular Weight: 282.26 CAS No: 000112-80-1	
7.	Hexadecanoic acid Molecular Formula:$C_{16}H_{32}O_2$ Molecular Weight: 256.24 CAS No: 000057-10-3	
8.	9,12-Octadecadienoic acid (Z,Z)- Molecular Formula:$C_{18}H_{32}O_2$ Molecular Weight: 280.24 CAS No: 000060-33-3	
9.	Linoleic acid ethyl ester MolecularFormula:$C_{20}H_{36}O_2$ Molecular Weight: 308.27 CAS No: 000544-35-4	

10.	Squalene Molecular Formula:$C_{30}H_{50}$ Molecular Weight: 410.39 CAS No: 007683-64-9	
11.	Vitamin E Molecular Formula:$C_{29}H_{50}O_2$ Molecular Weight: 430.38 CAS No: 000059-02-9	
12.	Campesterol Molecular Formula: $C_{28}H_{48}O$ Molecular Weight: 400.37 CAS No: 000474-62-4	
13.	Stigmasterol Molecular Formula:$C_{29}H_{48}O$ Molecular Weight: 412.37 CAS No: 000083-48-7	
14.	beta.-Sitosterol Molecular Formula:$C_{29}H_{50}O$ Molecular Weight: 414.38 CAS No: 000083-46-5	

The biological activities listed are based on Dr. Duke's phytochemical and ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA [15]. The molecular structure and molecular weight of some major biologically active compounds present both in ethanolic and aqueous extract are presented in Table 3.

Discussion

Plants have formed the basis for traditional medicinal system for thousands of years. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge system have given clues to the discovery of valuable drugs [16].

Here in this study we have actually focused on the presence of the phytochemical constituents that contribute to the plant's versatile use. The different components identified possess diverse medicinal value and are also important constituents of other medicinal plants. Among the major components identified, the PUFA, saturated fatty acids and their esters, phenolic compounds, squalene, -tocopherol and the phytosterols are of immense medicinal value. The phenol and flavanoid fractions are responsible for the free radical scavenging activity due to their redox properties and can thus attribute to the antioxidant property of the tuber [17]. Besides, the study also reveals the fact that ethanol serves a more potent solvent extractor than water, as the potentially important compounds are quantitatively more in the ethanolic extract than that of the aqueous extract.

Conclusion

Therapeutic mechanism of a plant can be better understood with a proper investigation of its active ingredients. The compounds identified by the GC-MS analysis of ethanolic and aqueous extract of *Amorphophallus campanulatus* tubers relate their applications in folklore medicine. These active principles provide inspiration for further investigation to achieve lead molecules in the discovery of novel herbal drugs. Furthermore, as this tuber is very popular among a certain geographical location, more insight into isolation of active components may lead to low cost drug development

against oxidative stress as well as other killer diseases. It can turn out to be a promising nutraceutical in future.

Authors Contribution

SB and URC carried out the total GC-MS analysis. GPD and URC participated in the designing of the study and co-ordination. SB and MD helped to draft the manuscript. All authors read and approved the final manuscript.

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