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Determination of bioactive compounds in Hydrophila auriculata leaf extract using GC MS technique

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Abstract
The phytocomponents of Hydrophila auriculata leaves x rays were irradiated evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. The mass spectrum 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 via Octadecanoic acid, 2-oxo-, methyl, 2-Hexadecen-1-ol, 3,7,11,15-tetram, Heptadecanoic acid, Ethyl ester, Phytol isomer, Phytol, acetate and Squalene in the methanolic extract of Hydrophila auriculata. These findings support the traditional use of Hydrophila auriculata for various disorders.

Keyword: Gas chromatography Mass spectroscopy, Hydrophila auriculata, Phytocompounds

1. Introduction
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-Fátima *et al*., 2006). Different medicinal plants and their medicinal values are widely used for various ailments throughout the world. Various chemical compounds isolated and characterized from Boraginaceous plant species are described. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi, 2000 and Shahidi, *et al*., 2008). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes (Meurer-Grimes *et al*., 1996; Koduru *et al*., 2006). It has been shown that in vitro screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).
Within a decade, there were a number of dramatic advances in analytical techniques including FTIR, UV, NMR and GC- MS that were powerful tools for separation, identification and structural determination of phytochemicals. Gas Chromatography Mass Spectroscopy is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites, 1997). The aim of this study is to determine the bioactive compounds present in *Hydrophila auriculata* (Family: Acanthaceae) leaf extract with the aid of GC- MS techniques which may provide an insight in its use as traditional medicine.

Materials and Methods
Plant materials
The *Hydrophila auriculata* leaves were collected in January 2015 from Kurungalam Village, Thanjavur district, Tamil Nadu from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts

The collected *Hydrophila auriculata* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted (Maceration) with methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

Exposure to X- ray radiation

The methanolic extract of the *Hygrophila auriculata* was poured into a separate China dish. China dish contained approximately 15ml of extract. They were placed one by one the x-ray table. The China dish was exposed to x-rays at a dosage of 82 KVP 120MAS. The duration of x-ray exposure to plant extract was around 15minutes and after that they were used to screen for GC MS analysis.

GC–MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min 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 36min. 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).

Results and Discussion

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, inorganic, biochemistry and identification of unknown samples. Additionally, it can identify trace materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely used as a "gold standard" for forensic substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system (Ronald Hites, 1997).

Identification of components

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. The biological activities listed (Table 2) are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA (Duke's, 2013). The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound can be identified from NIST library database.

GC-MS Analysis

Twenty one compounds were identified in *Hydrophila auriculata*by GC-MS analysis. The active principles with their Retention Time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1and Fig 1). The prevailing compounds were Octadecanoic acid, 2-oxo-, methyl, 2-Hexadecen-1-ol, 3,7,11,15-tetram, Heptadecanoic acid, Ethyl ester, Phytol isomer, Phytol, acetate and Squalene present in the extract. The pharmacological activity of *Hydrophila auriculata* is represented in table 2. This study explores the goodness of the leaf of the plant *Hydrophila auriculata* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

The investigation concluded that the stronger extraction capacity of methanol have produced number of active constituents responsible for many biological activities. So these 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 including cancer.

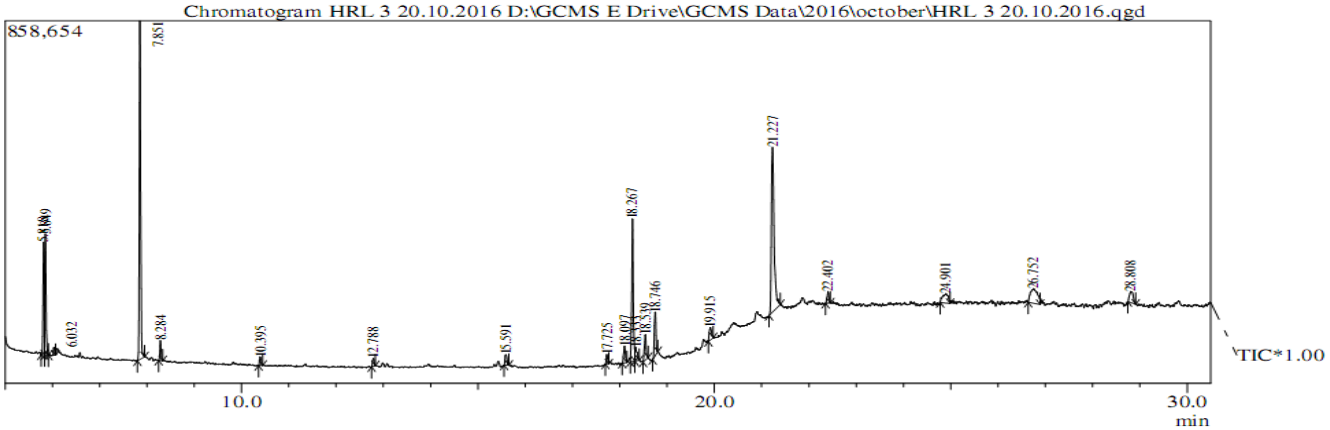

Fig 1: GC MS chromatogram of *Hydrophila auriculata* leaf extract

Phytol is reported to have antioxidant, antiallergic (Santos *et al*., 2013) antinociceptive and anti-inflammatory activities (Ryu *et al*., 2011). Recent studies have revealed that phytol is an excellent immunostimulant. It is superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity (Lim *et al*., 2006). Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Saikia *et al*., 2010). 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 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.

Hexadecanoic acid, ethyl ester is recommended to be a saturated fatty acid and it might act as an Antioxidant, hypocholesterolemic, anti-androgenic, hemolytic and alpha reductase inhibitor (Sermakkani, 2012). 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 cidand Squalene were Identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen kumar *et al.,* 2010). Squalene has earlier been reported as antimicrobial, antioxidant, anticancer , Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer (Ponnamma and Manjunath, 2012). Devi *et al.* (2009) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Uraku (2015) investigated the Chemical Compositions of *Cymbopogon citrates* Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) Method. Six compounds were identified in the methanol leaf extract and they include; hexadecanoic acid (8.11%), hepta-9,10,11-trienoic acid (17.43%), octadecenoic acid (8.41%), 2-ethenyltetradecan-1-ol (13.28%), eicosane aldehyde (37.56%) and 1-ethoxyoctadecane (15.20%) as the major chemical constituents.

Das and Sudhakar Swamy (2016) determined the bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia* species from south India. Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid was the major compound identified. In *A. racemosa* also 27 compounds were identified and n- Hexadecanoic acid was the major compound.

Uraku (2016) examined the Bioactive Constituents of Methanol Fraction of *Spilanthes uliginosa* (Sw) Leaves. The major phytocompounds identified in the leaf extract are hexadecanoic acid (8.68%), hepta-9, 10, 11-trienoic acid (19.36%), octadecenoic acid (8.14%), 5-hydroxylmethyl heptadecane (14.02%), docosane aldehyde (41.72%) and 1-ethoxyoctadecane (8.08%).

Table-1: GC-MS analysis revealed the presence of Phytochemical component in leaf of *Hydrophila auriculata*

Peak	R. Time	Area %	Name of the compound(s)	Molecular Formula	Mol. Weight
1	5.818	8.23	Butane, 1,1-diethoxy-3-methyl-	$C_9H_{20}O_2$	160
2	5.849	7.51	Pentane, 1,1-diethoxy-	$C_9H_{20}O_2$	160
3	6.032	0.50	3,3-Diethoxy-2-Butanone	$C_8H_{16}O_3$	160
4	7.851	23.59	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176
5	8.284	1.29	1,1,3-Triethoxybutane	$C_{10}H_{22}O_3$	190
6	10.395	0.68	Benzene, [Ethoxy(1-Propenyloxy	$C_{12}H_{16}O_2$	192
7	12.788	0.67	Nonane, 3,7-Dimethyl-	$C_{11}H_{24}$	156
8	15.591	1.03	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
9	17.725	0.68	Octadecanoic acid, 2-oxo-, methyl ester	$C_{19}H_{36}O_3$	312
10	18.097	1.57	Isopropyl myristate	$C_{17}H_{34}O_2$	270
11	18.267	11.37	2-Hexadecen-1-ol, 3,7,11,15-tetram	$C_{20}H_{40}O$	296
12	18.333	1.37	5-Undecene, 3-methyl-, (E)	$C_{12}H_{24}$	168
13	18.539	2.28	2,6,10-trimethyl,14-Ethylene-14-Pe	$C_{20}H_{38}$	278
14	18.746	3.60	7-Octadecyne, 2-methyl-	$C_{19}H_{36}$	264
15	19.915	1.49	Heptadecanoic acid, Ethyl ester	$C_{19}H_{38}O_2$	298
16	21.227	22.65	Phytol Isomer	$C_{20}H_{40}O$	296
17	22.402	1.10	Phytol, acetat	$C_{22}H_{42}O_2$	338
18	24.901	2.72	Tridecanol, 2-ethyl-2-methyl-	$C_{16}H_{34}O$	242
19	26.752	5.46	Squalene	$C_{30}H_{50}$	410
20	28.808	2.21	1,2-Benzenedicarboxylic	$C_{24}H_{38}O_4$	390

Table-2: GC-MS analysis revealed the presence of phytochemical component in leaf of *Hydrophila auriculata* and their pharmacological activities

Peak#	R. Time	Area%	Name of the compound	Nature of the compound	Pharmacological Activity**
1.	17.725	0.68	Octadecanoic acid, 2-oxo-, methyl ester	Ester	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propecic, Suppository
2.	18.267	11.37	2-Hexadecen-1-ol, 3,7,11,15-tetram	Acyclic diterpene alcohol	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents
3.	19.915	1.49	Heptadecanoic acid, Ethyl ester	Fatty acid ester	Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor Antimicrobial,
4.	21.227	22.65	Phytol isomer	Diterpene	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1
5.	22.402	1.10	Phytol, acetate	Diterpene	Anti cancer, Anti-inflammatory Hypocholesterolemic, Nematicide, Anticoronary, Antiarthritic, Hepatoprotective, Anti -androgenic,
6.	26.752	5.46	Squalene	Triterpene	Antibacterial, Antioxidant,Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor

Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

Conclusion

The present study characterized the phytochemical profile of the *Hydrophila auriculata* leaf extract using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a fraction of retention time. The heights of the different peaks indicates the relative concentration of the compounds exist in the methanolic extract of *Hydrophila auriculata* leaf. The mass spectrometer analyses of the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the NIST library. The identification of various bioactive compounds confirms the therapeutic application of *Hydrophila auriculata* leaf for a variety of diseases.

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