# GC-MS DETERMINATION OF BIOACTIVE CONSTITUENTS OF *H*YDRILLA *VERTICILLATA* (L.f.) Royle. COLLECTED FROM UNPOLLUTED AND POLLUTED WATER SOURCES.


## ABSTRACT

The investigation was carried out to determine the chemical components of *H*ydrilla *verticillata* using 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. GC/MS analysis of ethanolic extract of *H*ydrilla *verticillata* revealed the existence of five compounds in samples collected from polluted water and five compounds in samples collected from unpolluted water. Out of ten compounds, sesquiterpene compound (Coryan-17-ol, 18,19-di dehydro-10-methoxy-acetate), Steroid compound (Ergost -5-en-ol, 22, 23-dimethyl acetate), plasticizer compound (1,2 Benzene dicarboxylic acid butyl octylester), Linoleic compound (10-Octadecenoic acid, methyl ester), Stearic acid (Pentadecanoic acid, 14-methyl, methyl ester) and Phytol (Diterpene compound) have anti-fungal, anti-bacterial, anti-arthritic, anti-inflammatory, anti-cancer, anti-oxidant, anti-diabetic and enhances the immunity. The results of this study offer a platform of using *H*ydrilla *verticillata* as herbal alternative for various diseases.


*Keyword:* Anti-bacterial, Chromatography, immunity, sesquiterpene, plasticizer and phytol.

## 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[1]. The aim of this study is to determine the organic compounds present in the active fraction of *H*ydrilla *verticillata* plant extract with the aid of GC-MS technique, which may provide an insight in its use in traditional medicine. In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [2, 3]. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug [4, 5]. Turger and Usta[6] screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer [6] and Alzhemir's diseases[8]. However, few reports are available with respect to the pharmacological properties of the plant. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC/MS can also be used in airport security to detect substances in luggage or on human beings. However, fewer reports are available with respect to the pharmacological properties. Keeping this in view, the present study has been undertaken to identify the phyto constituents present in ethanolic leaf extracts of *H*ydrilla *verticillata* using GC-MS analysis.

## Methodology

**Plant Collection and Authentication:** The fresh leaves of *H*ydrilla *verticillata* of Hydrocharitaceae family were collected from Asaripallam, Kanyakumari district of Tamilnadu, India and authenticated by BSI, Southern circle, Kovai, India.

**Preparation of Extracts:** Five hundred grams of coarse powder of shade dried leaves of *H. verticillata* was extracted successively with ethanolic in soxhlet extractor for 48 hours. Dark green residues were obtained after concentrating the extract under reduced pressure. The obtained extracts were stored in desiccators for further GC-MS.

## GC-MS Analysis of Phytocomponents

GC-MS analysis in the ethanol extracts of *H. verticillata* was performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite – 5MS (5 % Diphenyl/95 % Dimethyl Poly Siloxane) fused capillary column (30 x 0.25 µm IDx0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999 %) was used as carrier gas at a constant flow rate of 1.491 ml/min, and an injection volume of 2 µl was employed (split ratio of 10:1). The injector temperature was maintained at 250$^{o}$C, the ion-source temperature was 200$^{o}$C, and the oven temperature was programmed from 110$^{o}$C with an increase of 10$^{o}$C/min to 200$^{o}$C, then 5$^{o}$C/min to 280$^{o}$C, ending with a 9 min isothermal at 280$^{o}$C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45-450 Da. The solvent delay was 0 to 2 min and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass-Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a GC-MS solution ver-2.53.

### Identification of Phytocomponents

The relative percentage amount of each component was calculated by comparing its average peak area to the total peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver. 25.3 – year 2005 library. The compound prediction is based on Dr. Duke's phytochemical and Ethno botanical Database [9] by Dr. Jim Duke of the Agricultural Research Service. Interpretation of GC-MS was conducted using the database of 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.

## GC-MS analysis of *Hydrilla verticillata*

GC-MS analysis was carried out to determine the phytocomponents in the whole plant ethanolic extracts of *H. verticillata* collected from unpolluted and polluted water sources, and the chromatogram, mass spectrum and structure of phytocomponents were identified by GC-MS. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) were determined in the whole plant ethanolic extracts of *H.verticillata* (Fig –1*)* collected from unpolluted and polluted water sources, respectively, are presented in Table-1. The GC-MS analysis for the active principles in the whole plant ethanolic extracts of *H.verticillata* indicated the presence of 19 compounds and the structure of the identified compounds are shown in Figure- 1.

Five compounds were reported in the whole plant ethanolic extract of *H.verticillata* collected from polluted water and five compounds from unpolluted water (Table-1). Out of the ten compounds, nine compounds were reported to have anti-cancer, anti-microbial, anti-oxidant, anti-fungal, anti-bacterial, anti-diabetic, sedative, anti-fouling, anti-diabetic, and enhances the immunity and wound healing activity. One compound was reported to have no activity.

The GC-MS analysis for the active principles in the whole plant ethanol extract of *H. verticillata* showed the presence of five compounds in samples collected from polluted water and five compounds in samples collected from unpolluted water. Out of ten compounds, sesquiterpene compound (Coryan-17-ol, 18, 19-di dehydro-10-methoxy-acetate), Steroid compound (Ergost -5-en-ol, 22, 23-dimethyl acetate), plasticizer compound (1,2 Benzenedicarboxylic acid butyl octylester), Linoleic compound (10-Octadecenoic acid, methyl ester), Stearic acid (Pentadecanoic acid, 14-methyl, methyl ester) and phytol (Diterpene compound) have anti-fungal, anti-bacterial, anti-arthritic, anti-inflammatory, anti-cancer, anti-oxidant, anti-diabetic and enhances the immunity.

In general, the identified compounds showed antimicrobial and antioxidant activities. Among these nine compounds, plasticizer compounds (1, 2 Benzenedicarboxylic acid, butyl cyclohexyl ester), Linoleic compound (10, Octadecenoic acid, 2-hydroxyl -1 – (Hydroxymethyl) ethyl ester), Palmitic compound (Hexadecanoic acid ethyl ester), ester compound (Desycarpindan-1-methanol, acetate (ester) have anti inflammatory, antioxidant, antifouling, antimicrobial, antiarthritic, hepatoprotective, nematicide, anti-coronary and cancer preventive activities.

A large number of therapeutic agents in use today have been isolated or derived from plant sources [10]. It is found to give good as well as preventive and therapeutic results against inflammation. The results show that, reactive oxygen species-promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [11]. 9-12 Octadecenoic acid has the property of anti-inflammation and anti-arthritis as reported by the earlier workers [12, 13]. Phytol is recommended to be a di-terpene compound and it might act as an antimicrobial, anticancer, anti-inflammatory and diuretic [14, 15, 16, 17, 18.]

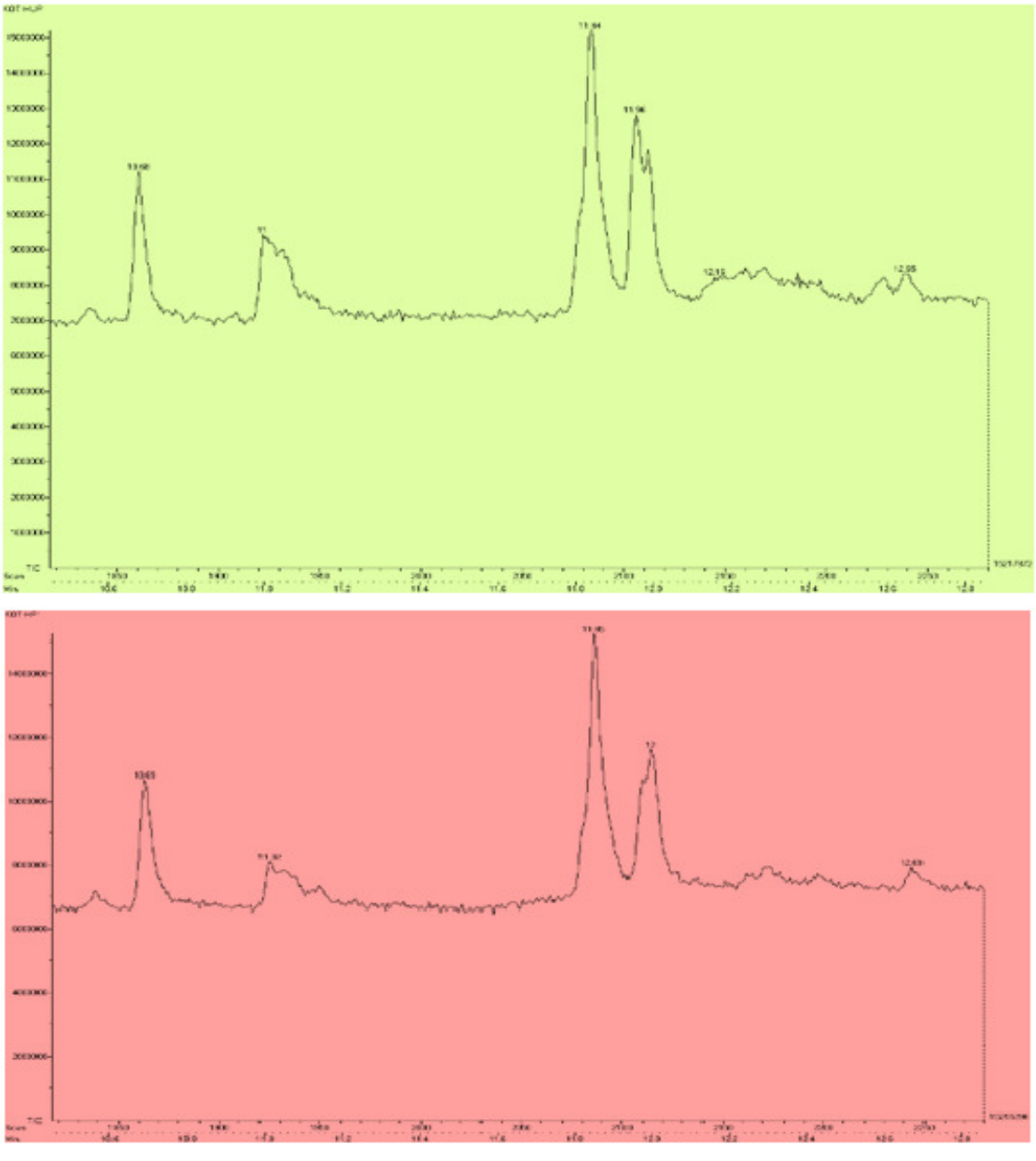
Phytol and 9-12 Octadecenoic acid were found in the ethanol extract of *H. verticillata* whole plant samples and which are being used for the pharmacological work. The biological activities of the phyto compounds identified in the whole plant ethanol extracts of *H. verticillata was* based on Dr. Duke's Phytochemical and Ethno-botanical databases. The present investigation may be used to authenticate the scientific reason of free radical scavenging with the use of plants in the treatment or prevention of the onset of deadly disorders like arthritis, cancer, inflammatory, diabetic and coronary diseases etc. and also it is a right step in the direction of searching for novel and more effective gas chromatography and mass spectroscopy analysis which showed the existence of various compounds with variable chemical structure. At end point, it is conclude that the *in vivo* studies on biological systems can open up new way for natural anti-oxidants that can also be employed for clinical traits which may generate successful results in future.

126 **Table –1: GC-MS analysis of phytocomponents and their activities in the whole plant ethanolic**
127 **extracts of *Hydrilla verticillata* collected from unpolluted and polluted water sources.**

| Sl. No. | Nature, Name & Molecular formula of the Components | RT | MW | Peak Area % | Activity* |
|---|---|---|---|---|---|
| **I.** | ***H. verticillata* from unpolluted water source:** | | | | |
| 1 | **Stearic acid**–[ Pentadecanoic acid, 14-methyl-methyl ester] –($C_{17}H_{34}O_2$) | 10.68 | 270.46 | 7.10 | Anticancer, Antimicrobial, Antioxidant |
| 2 | **Plasticizer compound**–[1,2 Benzenedicarboxylic acid butyl octyl ester] –($C_{20}H_{30}O_4$) | 11.00 | 334.45 | 10.72 | Antimicrobial, Anti fouling |
| 3 | **Linoleic acid**–[10-Octadecenoic acid, methyl ester] –($C_{19}H_{36}O_2$) | 11.84 | 296.49 | 6.01 | Enhances the immunity |
| 4 | **Diterpene**–[Phytol]–($C_{20}H_{40}O$) | 11.96 | 296.00 | 12.21 | Antioxidant, antimicrobial, anti-arthritic, anti-inflammatory |
| 5 | **Steroid**–[Ergost – 5-en-3-ol, 22,23-dimethyl – acetate] –($C_{23}H_{54}O_2$) | 12.65 | 470.77 | 4.01 | Wound healing, Anti diabetic, Anti Oxidant |
| **II.** | ***H. verticillata* from polluted water source:** | | | | |
| 1 | **Stearic acid**–[Pentadecanoic acid, 14-methyl, methyl ester] –($C_{17}H_{34}O_2$) | 10.69 | 270.46 | 2.01 | Anticancer, Antimicrobial, Antioxidant |
| 2 | **Aromatic fatty compound**–[Ribitol, Pentaacetate] –($C15_5H_{21}O_{10}$) | 11.02 | 482.43 | 6.02 | No activity |
| 3 | **Linoleic acid**–[10 – Octadecenoic acid, Methyl ester] –($C_{19}H_{36}O_2$) | 11.85 | 296.49 | 19.21 | Enhances the Immunity |
| 4 | **Sesquiterpene**–[Coryon – 17-ol, 18,19-didehydro-10-methoxy-acetate] –($C_9H_{31}O_{41}$) | 12.68 | 317.21 | 5.09 | Antifungal, Antibacterial, Anti diabetic Sedative |

* Source: Dr. Duke's phytochemical and ethnobotanical databases.

**Figure 1: GC-MS chromatogram of the ethanolic extract of *Hydrilla verticillata* collected from unpolluted and polluted water sources.**



**REFERENCES**

1. De-Fatima, A., Modolo, L.V., Conegero, L.S., Pilli, R.A., Ferreira, C.V., Kohn, L.K., de-Carvalho, J.E., Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design, Curr. Med. Chem; **2006**, **13**:3371-3384.

2. Robertson DG. Metabonomics in toxicology: A review.1995, Toxicol Sci; **85**: 809 - 822.

3. Fernie, A.R., Trethewey, R.N., Krotzky, A.J., Willmitzer, L., Innovation - Metabolite profiling: from diagnostics to system biology. Nat Rev Mol Cell Biol., **2004**, **5**: 763 - 769.

4. Vinoth Kumar, D., Balaji, G., Geetha, M., Manivachakam, P., Sumathi, R. and Murugesan, S., Phytochemical screening of *Phyllanthus emblica* in different agroclimatic zones of Tamil Nadu. *Pestology*, 2010, **33(3)**: 15-20.

5. Savithramma, N., Venkateswarlu, P., Suhrulatha, D., Basha, S.K.M. and Venkataramanadevi, C.H., Studies of *Boswellia ovalifoliolata* Bal. and Herny – An endemic and endangered medicinal plant. The Biosc., 2010**, 5**: 359-362.

6. Turker, A.U., Usta, C., Biological screening of some Turlish medicinal plants for antimicrobial and toxicity studies. Nat. Prod, 2008, **22**: 136-146.

7. Sheeja, K., Kuttan, G., Activation of cytotoxic Tlymphocyte responses and attenuation of tumor growth in vivo by *Andrographis paniculata* extract and andrographolide. Immunopharmacol Immunotoxicol., 2007, **29**: 81-93.

8. Mukherjee, P, K., Kumar, V., Houghton, P. J., Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytother Res; 2007, **21**: 1142-1145.

9. Dukes, H. H., The physiology of domestic animals. 7th edition, 1955, Bailers Tindal and Co. London.

10. Simlai, X. and Roy, A., Analysis and correlation between phytochemical and antimicrobial constituents of *Ceriops decandra*, a medicinal mangrove plant, from Indian Sundarban estuary. *Journal of Medicinal Plants Research*; 2012, **6(32)**:4755-4765.

11. Ogunlesi, G.K., Singh, R.P. and Sakariah, K.K,. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. *Food Chem*., 2009, **73**: 285-290.

12. Lalitharani, J., Stushnoff, C., Locke, E. and Vivanco, J.M., Antioxidant activity and total phenolic content of Iranian *Ocimum accessions*. *Food Chem*., 2003, **83**: 547-550.

13. Kala, A., Dagila, M., Aceti, C., Quaglia, M., Gregotti, C. and Grazzani, G., Isolation of and *in vitro* and *in vivo* antiradical melanoidin from roasted barely. *J. Agric. Food Chem*., 2006, **54**: 1209-1216.

14. Praveen, P.A., Okwuasaba, F.K. and Binda, L.G. Antidiarrhoeal and antiulcerogenic effects of methanolic extracts of *Asparagus pubescens* root in rats. *Journal of Ethnopharmacolgoy*, 2000 , **72**: 421-427.

15. Sermakkani, M., Thangapandian, V., Asian J Pharm Clin Res, 2012, **5 (2)**: 90-94.

16. Alagammal., M., Tresina, P.S., and Mohan, V.R., GC-MS determination of bioactive components of *Polygala javanica* dc. *Int J of Curr Pharm Res.,* 2012., **4 (2)**: 42-4.

17. Gopinath, S., Sakthidevi, G., Muthukumaraswamy, S. and Mohan, V.R., GC-MS analysis of bioactive constituents of *Hypericum mysorense* (Hypericaceae). *J. Curr. Chem. Pharm. Sci .,* 2013., **3(1)**: 6-15.

18. Prabhadevi, V., Sathish, S., Johnson, M., Venkatramani, B., Janakiraman, N., Phytochemical studies on *Allamanda cathartica* L. using GC-MS. *Asian Pac J Trop Biomed.,* 2012., **2 (2)**: 550-4.