



# GC-MS Determination of Bioactive compounds of *Pila virens* (Lamarck, 1822)

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Article Info: Received 14 March 2017; Revised: 27 May 2017; Accepted 11 June 2017.

## ABSTRACT

In this study, the bioactive compounds of *Pila virens* have been evaluated using GC-MS. The experimental approach consists of a systematic investigation about the action of crude or purified extract on physiological preparations or whole animals to outline the profile of their biological activities and mechanism of action. The present work has been undertaken for isolation and purification of bioactive compounds and action of the extracts of the snail for its biological activity. The chemical compositions of the whole animal methanol extract of *Pila virens* were investigated using GC SHIMADZU QP2010 Gas chromatography-mass spectrometry. GC-MS analysis of *Pila virens* whole animal methanol extract revealed the existence of the compound Benzaldehyde, Benzene, 1,3-Bis[1,1-Dimethylethyl]- [14.573], Phenol, 2,4-Bis[1,1-Dimethylethyl]- [21.337] Hexadecanoic Acid and Methyl Ester [30.613]. The present study indicated that, *Pila virens* extract is a potential source of novel bioactive compounds. Studies on the freshwater snail *P. virens* of lower anaicut reservoir is scanty and the present study on *P. virens* is the first GC- MS work on Indian Ampullariidae.

**Keywords:** GC-MS analysis, Bioactive compounds, *Pila virens*, Benzaldehyde, 4-Methyl.

## 1. INTRODUCTION

India is the birth place of renewed system of Indigenous medicine such as Siddha, Ayurvedha and Unani. Natural products have served as an important source of drugs since ancient times and about half of the useful drugs today are derived from natural sources. The freshwater molluscs form an important part of the aquatic ecosystems. These sentinel organisms are used in aquatic pollution monitoring programs as they accumulate toxic environmental contaminants to levels well above those present in the surrounding environment thus providing

information on the spatio-temporal pollution trends [1,2]. Natural products, whether based on microbial, plant, terrestrial animals, marine organisms or other origins have prone to be a rich source of lead compounds in drug discovery [3]. Venomous animals offer an enormous additional potential. Currently, more than 100000 venomous animal species are reported, covering almost all phyla (snakes, scorpions, spiders, cone snails, hymepters, sea anemones, jellyfishes and even some lizards, birds and fishes) each of them is characterized by a unique venom compound of hundreds of bioactive molecules [4,5].

The use of environmentally clean advanced extraction techniques allows for the attainment of the target compounds of interest with more efficient extraction procedures, while, at the same time, minimizing the use of organic toxic solvents. Depending on the extraction techniques selected, diverse extraction parameters should be tested in order to study the influence of solvents, temperatures, pressures, and other important parameters that might have a significant influence on the outcome of the extraction process employed. The different extracts, obtained using diverse conditions, must then be tested for biological bioactivities by performing the appropriate functional activity assay[s] [6].

The coupling of chromatographic methods such as high pressure liquid chromatography [HPLC] with diode array detection, mass spectrometry [MS] or nuclear magnetic resonance spectroscopy [NMR] or, and with, on-line bioactivity assays, is an important tool for high throughput screening of natural product mixtures. The introduction of a dereplication step after extraction by using a reproducible pre-separation method would enable the rapid elimination of false positives [7]. The effective use of automated procedures and databases in the isolation, identification and biological profiling of bioactive compounds from natural sources will be the best guarantee to the continued discovery of novel chemotypes from nature [8].

The freshwater gastropod, *Pila virens* are known as snail under the family Ampullariidae. They are large freshwater gastropod molluscs having a strong thick calcareous operculum. Among the invertebrates the molluscs are very good source of biomedically important products [9] and have developed very effective mechanisms that are part of their innate immunity [10]. They are considered as one of the important source to derive bioactive compounds that exhibit antitumour, antimicrobial, anti-inflammatory and antioxidant properties [11]. Hence the present study was designed to identify the bioactive compounds present in the extract of freshwater gastropod, *Pila virens*.

## 2. MATERIALS AND METHODS

### 2.1.1. Sample collection and extraction

Live specimens of freshwater gastropod *Pila virens* was randomly collected by hand picking from In the Coleroon river, Lower anicut is one of the major fresh water fishery resource areas in Thanjavur District of Tamil Nadu [11° 15' N latitude and 79° 30'

E longitude] which is selected for the present study. The collected fresh molluscs were preserved with ice and transported to the laboratory and identified by the standard literature of [12]. The methanolic extract of flesh was prepared by the method of [13]. The specimen was brought to the laboratory and their soft bodies were removed by breaking the shell. The flesh sample was dried using hot air oven at 60°C and powdered. 25grams sample was soaked in methanol and maintained for 3 days. The extract was filtered through Whatman No.1 filter paper. The resultant extract was concentrated by using rotary vacuum evaporator with reduced pressure. The resultant extract were then kept in airtight container and stored at 4°C for further analysis. The mass spectrum of the compounds, found in the extract was matched with characterization and measurement of the Sathyabama University, Centre for Ocean Research Institute.

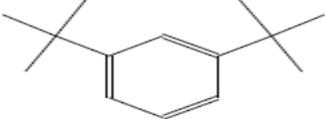
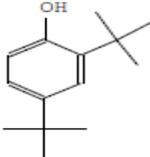
### 2.2. Identification of bioactive compounds by GCMS

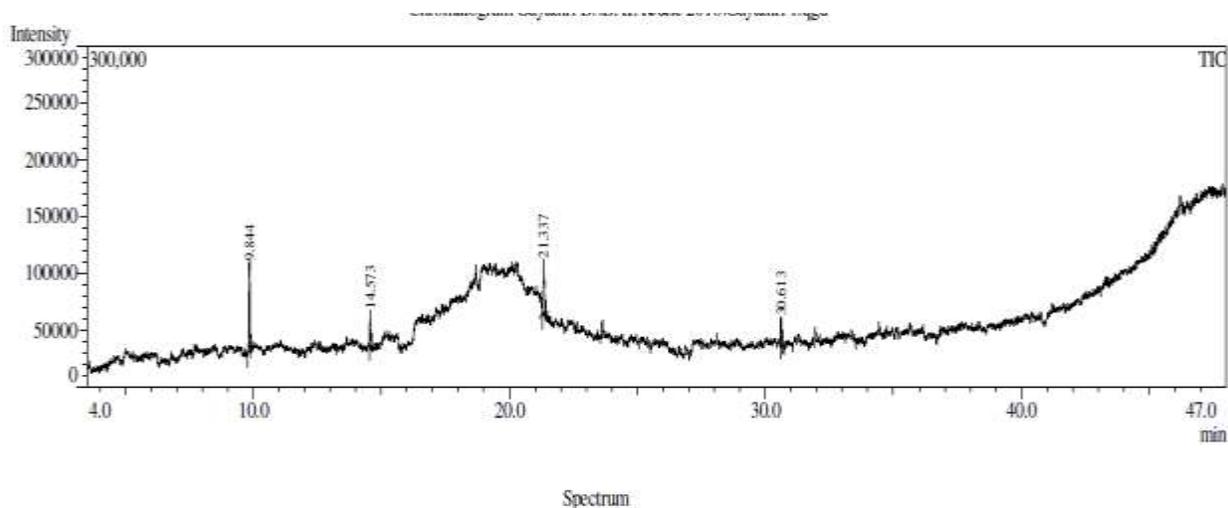
The purified gastropod fractions were individually examined using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a mass spectrometer equipped with Elite-1 fused silica capillary column. For GCMS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium was used as carrier gas at constant flow rate 4 ml/min and an injection volume of 2µl was employed [injector temperature 250°C: ion source temperature 280 °C]. The oven temperature was programmed from 100 °C [isothermal for 5 min] with a temperature of 4 °C /min to 240 °C with column flow rate of 1.21ml/min. The sample was run for 47 mins with solvent out time of 9.50 mins. Mass spectra were taken with scan interval of 10 mins. Interpretation on mass spectrum was achieved by using data base of WILEY8.LIB and NIST11S LIB for different bioactive compounds.

### 2.3. Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute of Standard and Techniques [NIST11s] and WILEY8 having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST11s and WILEY8 library. The name, molecular weight, molecular formula and structure of the component of the test material were identified.

**Table 1.** Bioactive compounds identified in the *Pila virens*

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	Name	Compound Structure
1	9.844	40.29	C <sub>8</sub> H <sub>8</sub> O	120	Benzaldehyde, 4-Methyl	
2	14.573	17.83	C <sub>14</sub> H <sub>22</sub>	190	Benzene, 1,3-Bis[1,1-Dimethylethyl]	
3	21.337	27.21	C <sub>14</sub> H <sub>22</sub> O	206	Phenol, 2,4-bis[1,1-Dimethylethyl]	
4	30.613	14.68	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	Hexadecanoic Acid, Methyl Ester	

**Figure 1.** GC-MS chromatogram of *Pila virens* methanol extract

### 3. RESULTS AND DISCUSSION

Animal venoms are usually complex mixtures of bioactive molecules, including proteins and peptides. These combinations confer a formidable array of toxic properties on the venom, the peptides and the -

polypeptides being responsible for a variety of biological activities. Freshwater gastropods are found to be a vital source of useful bioactive substances. These bioactive compounds are involved in various

biological functions such as communication, infection, reproduction and self- defense.

The studies on the active principles in the *Pila virens* whole animal methanol extract by GC-MS analysis clearly showed the presence of four compounds [Table-1]. The active principles with their retention time [RT], molecular Formula, Molecular weight [MW], and concentration [Peak area %] are presented in Table-1. The GC-MS chromatogram of the four peaks of the compounds detected was shown in figure-1. The mass spectrum and structure of the compounds identified were presented. The total number of compounds identified in methanol extracts. The results revealed that Benzaldehyde, 4-Methyl [9.844], Benzene, 1,3-Bis[1,1-Dimethylethyl]- [14.573], Phenol, 2,4-Bis[1,1-Dimethylethyl]- [21.337] Hexadecanoic Acid, Methyl Ester [30.613].

#### 4. CONCLUSION

An attempt has been made to outline the most important aspects of the empirical approach to find new lead compounds from natural product mixtures such as animals. In the present study sixteen chemical constituents have been identified from methanol extract of the whole animal of *Pila virens* by Gas chromatogram Mass spectrometry [GC-MS] analysis. The presence of various bioactive compounds justifies the use of whole animal for various ailments by traditional practitioners.

#### Conflicts of Interest

There are no conflicts of interest.

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