
**ISSN: 2347-5129**
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(1): 257-259

www.fisheriesjournal.com
Received: 15-07-2015
Accepted: 18-08-2015

**Tamil Muthu P**
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629001, Tamil Nadu, India.

**Selvaraj D**
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629001, Tamil Nadu, India.

**Correspondence**
**Tamil Muthu P**
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629001, Tamil Nadu, India.

# Analysis of Bioactive Constituents from the Flesh of *Turbo brunneus* (Roding, 1798) By GCMS

**Tamil Muthu P, Selvaraj D**

**Abstract**
*Turbo brunneus* is one of the predatory gastropod occurring tropical and subtropical coastal areas. In recent years, a significant number of novel metabolites with potent pharmacological properties have been discovered from the marine organisms. In the present study bioactive compounds from methanolic flesh extract of marine gastropod *T. brunneus* was purified by TLC analysis, and the purified TLC fraction was subjected to GCMS analysis. The results revealed the presence of eight compounds. Among them, the dominant bioactive compounds were Cyclononasiloxane, octadecamethyl-, 6-(Diphenylphosphoryl)- 3, 4-bis (diisopropylamino)- 5-pyrrolidino pyridazine and Epinephrine-tetratms. The present study indicated that the flesh extract of *T. brunneus* is a potential source of novel bioactive compounds.

**Keywords:** Marine natural products, Marine organisms, *Turbo brunneus*, Bioactive compounds, Secondary metabolites, Drugs.

## 1. Introduction
Apart from the food the marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products [1]. Many of these organisms are known to possess bioactive compounds as a common means of defense [2] and to maintain homeostasis in the environment. Certain physical conditions in the marine environment as lack of light, low temperature, hyper salinity and extreme pressure, force the marine creatures to produce certain metabolic products namely secondary metabolites or bioactive compounds. The compounds involved in the chemical defense could vary dramatically among habitat, between individuals in a local habitat and even within a single individual.
Isolation of natural products from marine organisms increases rapidly and hundreds of new compounds being discovered every year [3, 4]. So far approximately 6500 bioactive compounds were isolated from marine organisms [5]. These compounds belongs to different structural types such as diterpenoids (37%), steroids/sterols, glycosides (18%), sesquiterpenoids (17%) and the remaining were alkaloids, amino acids, fatty alcohol esters and glycosides [5].
Most of these marine bioactive compounds are derived from marine invertebrates. They are sessile and having soft bodies without spines. To compete for space with other sessile species they produce secondary metabolites [6]. Among the invertebrates the molluscs are very good source of biomedically important products [7] and have developed very effective mechanisms that are part of their innate immunity [8]. They are considered as one of the important source to derive bioactive compounds that exhibit antitumour, antimicrobial, anti-inflammatory and anti-oxidant properties [9].
The marine gastropod, *T. brunneus*, are known as Dwarf turban snail under the family Turbinidae. They are large marine gastropod molluscs having a strong thick calcareous operculum. The strong operculum serves as a passive defensive structure against predators. They are available in plenty on the rocks and dead corals in the coastal areas of the Gulf of Mannar. But analysis on its active compound is extremely low compared to other marine organisms. Hence the present study was designed to identify the bioactive compounds present in the flesh extract of marine gastropod *T. brunneus.*

## 2. Materials and Methods
### 2.1. Sample collection and extraction
Live specimens of marine gastropod *T. brunneus* was randomly collected by hand picking from Vijayapathi coast, Tirunelveli District, Southeast coast of India. The collected fresh

molluscs were preserved with ice and transported to the laboratory and identified by the standard literature of SubbaRao [10].

The methanolic extract of flesh was prepared by the method of Chellaram *et al.* [11]. 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 ℃ 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 vaccum evaporator with reduced pressure. The resultant extract were then kept in airtight container and stored at 4 ℃ for further analysis.

### 2.2. Partial purification of crude extracts by TLC

Partial purification of bioactive compounds was carried out using read made silica gel (60-F 254mm). Crude extract of gastropod were spotted at the bottom of the TLC sheet using capillary tube and placed in TLC chamber. n- Butanol, acetic acid and water (6:2:2) mixture was served as the mobile phase. After running the chromatogram, the TLC plate was air dried and placed in closed iodine chamber to clearly visualize the separated compounds as spots. The spots were labeled and their distance from the baseline was measured. The distance between the baseline and the solvent front was also measured. The $R_f$ value were calculated,

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} \text{ X } 100$$

### 2.3. 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 1ml/min and an injection volume of 2µl was employed (injector temperature 250 ℃; ion source temperature 280 ℃).

The oven temperature was programmed from 100 ℃ (isothermal for 5 min) with a temperature of 4 ℃ /min to 240 ℃ with column flow rate of 1.21ml/min. The sample was run for 40 mins with solvent out time of 9.50 mins. Mass spectra were taken with scan interval of 0.6 seconds. Interpretation on mass spectrum was achieved by using data base of Wiley 22a LIB and MIST05S LIB for different bioactive compounds.

## 3. Result and Discussion

Marine 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 qualitative separation of bioactive compounds was carried out by TLC analysis. The TLC profiling of *T. brunneus* flesh methanolic extract indicated the presence of single spot. The compounds present in that fraction were identified by GCMS (Figure 1 and Table 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) were observed.

Eight compounds were detected in *T. brunneus* flesh extract. Among them the heterocyclic organic compound 6-(Diphenylphosphoryl)-3, 4-bis (diisopropylamino)-5-pyrrolidino pyridazine (RT-6.99) is a stimulant drug. It produces stimulant effects in animals and humans [12] and also has herbicidal, anti- inflammatory, acaricidal, antiparasitic, insecticidal, antioxidant, analgesic and antimicrobial properties. The aroma compound 3, 3, 4, 4-Tetracyano-5, 6-diphenyl-2-(cyclohexylimino)-2, 3, 4, 5-tetrahydropyridine (RT-12.47) is a neuroprotective agent used to prepare medicines for treating disease causing demyelination. The tetrahydropyridines were prepared and tested for its antioxidant and antimicrobials properties [13].

1H-Purin-6-amine, [(2-fluorophenyl) methyl]-(CAS) is a cell division and growth regulation factor found in various plant parts and in yeast. This compound is reported for its antimicrobial and antifungal activities and is subsequently highlighted as a potent mechanism-based inhibitor of several enzymes like acyl coenzyme A, cholesterol acyltransferase, monoamine oxidase, heat shock protein 90, cathepsin D, and c-Jun N-terminal kinases. Its derivatives are also known to possess antitubercular, anti-inflammatory, antitumor, amoebic, antiparkinsonian, anthelmintic, antihypertensive, antihyperlipidemic, antiulcer, chemoprotective and selective CCR3 receptor antagonist activity. The bioactive constituent 3-(4-chlorophenyl)-4, 6-dimethoxy-1-(prop-2'-enyl) indole-7 carbaldehyde is an aldehyde. Normally carbaldehyde derivatives are used as a fragrance and making ingredient in many skin care products. It exhibits potential anti androgen, antiplatelet agent, antitubulin and antimicrobial properties [14, 15].

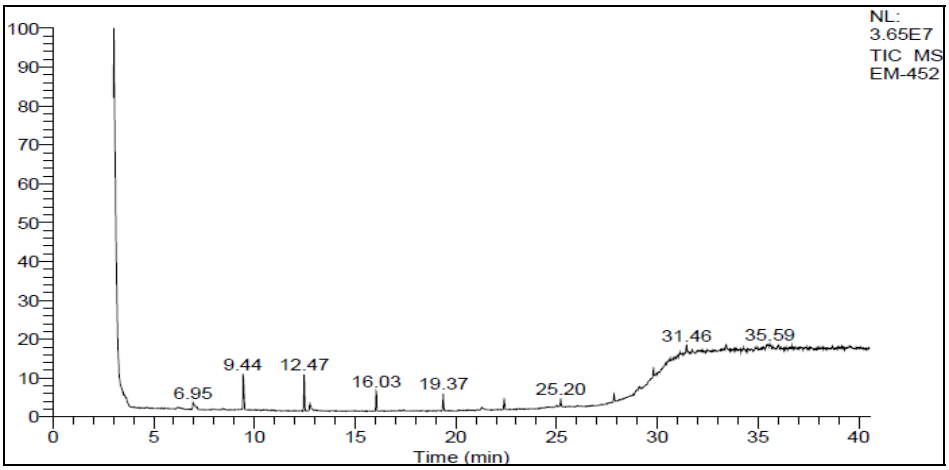


**Fig 1:** GCMS Spectrum of *T. brunneus* flesh extract

**Table1:** Bioactive compounds identified in the *T. brunneus* flesh extract

| S.No | RT | Compound name | Molecular formula | Molecular weight | Area (%) |
|---|---|---|---|---|---|
| 1 | 6.99 | 6-(Diphenylphosphoryl)-3,4-bis(diisopropylamino)-5-pyrrolidino pyridazine | $C_{32}H_{46}N_5OP$ | 547 | 3.30 |
| 2 | 9.46 | Cyclohexasiloxane dodecamethyl-(CAS) | $C_{12}H_{36}O_6Si_6$ | 444 | 8.04 |
| 3 | 12.47 | 3,3,4,4-Tetracyano-5,6-diphenyl-2-(cyclohexylimino)-2,3,4,5-tetrahydropyridine | $C_{27}H_{22}N_6$ | 430 | 6.43 |
| 4 | 16.03 | 2,4-bis(trimethylsilyl)-1,3 dithietane-1,3,3-tetraoxide | $C_{14}H_{32}O_4S_2Si_2$ | 384 | 3.78 |
| 5 | 19.37 | Cyclononasiloxane, octadecamethyl- | $C_{18}H_{54}O_9Si_9$ | 666 | 2.69 |
| 6 | 25.20 | 1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS) | $C_{12}H_{10}FN_5$ | 243 | 2.48 |
| 7 | 31.44 | 3-(4-chlorophenyl)-4,6-dimethoxy-1-(prop-2'-enyl) indole-7 carbaldehyde | $C_{20}H_{18}CINO_3$ | 355 | 6.02 |
| 8 | 36.00 | Epinephrine-tetratms | $C_{21}H_{45}NO_3Si_4$ | 471 | 1.09 |

## 4. Conclusion

The presence of various bioactive compounds justifies the use of *T. brunneus* flesh for various ailments. Further investigation on the purification and chemical elucidation of the active principle present in the extract shall pave the way for the development of either the base or a new drug itself in future.


## 5. Acknowledgement

The authors would like to thank Dr. S. Sam Manohar Dhas, HOD, Department of Zoology, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu, India, for providing the lab Facility.


## 6. References


1. Ireland CM, Roll DM, Molinski TF, McKee TC, Zabriske TM, Swersey JC. Uniqueness of the marine chemical environment categories of marine natural products from invertebrates. In: Fautin, (Eds) D. G. Biomedical importance of marine organisms. Mem Calif Acad Sci 1988; 13:41-57.
2. Indap MM, Pithare SP. Cytotoxicity and bioactivity of some marine animals. Indian J Mar Sci. 1998; 27:433-437.
3. Faulkner DJ. Marine natural products. Natural Product Reports 2002; 19(1):1-48.
4. Proksch P, Muller WEG. Frontiers in Marine Biotechnology. Horizon Bioscience: Norfolk, UK, 2006.
5. Kamboj VP. Bioactive agents from the Ocean biota. In: Ocean science trends and future direction, Somayajalu, B. L. K. (Ed). Indian National Science Academy, New Delhi, India, 1999, 97- 227.
6. Pawlik JR, McFall G, Zea S. Does the odor from sponges of the genus *Ircinia* protect them from fish predators? J Chem Ecol. 2002; 28:1103-1115.
7. Shenoy AS. Octopus a delicacy in Japan. Sea Food Exp J. 1988; 20:21-25.
8. Tincu JA, Taylor SW. Antimicrobial peptides from marine invertebrates. Antimicrobial Agents Chemother 2004; 48:3645-3654.
9. Nagash YS, Nazeer RA, Kumar NSS. *In vitro* antioxidant activity of solvent extracts of molluscs (*Loligo duvauceli* and *Donax strateus*) from India. World Journal of Fish and Marine Sciences. 2010; 2:240-245.
10. Subha Rao NV. Indian seashells (Part: 1) Polyplacoptera and Gastropoda. Zoological Survey of India, Occasional 2003; 192:426.
11. Chellaram C, Gnanambal KM, Edward JKP. Antibacterial activity of the winged oyster, *Pteria chinensis* (Pterioida: Pteridae). Indian J. Mar. Sci. 2004; 33:369-372.
12. Staack RF, Maurer HH. Metabolism of designer drugs of abuse. Curr Drug Metab 2005; 6:259-274.
13. Prachayasittikul S, Worachartcheewan A, Lawung R, Ruchirawat R, Prachayasittikul V. Activities of thiotetrahydropyridines as antioxidant and antimicrobial agents. EXCLI Journal. 2009; 8:107-114.
14. Abdel-Wahab BF, Khidre RE, Farahat AA, El-Ahle AAS. 2-Chloroquinoline-3-carbaldehydes: synthesis, reactions and applications. ARKIVOC 2012; 1:211-276.
15. Naik N, Kumar HV, Roopashree NM. Novel indole-3-carboxaldehyde analogues: Synthesis and their antioxidant evaluation. Der Pharma Chemica 2012; 4(2):783-790.