VOLUME 8 (SPECIAL ISSUE) 2022

ISSN 2454-3055

Manuscripts under Special issue are published with the Theme
"Modern Perspectives of Biological Sciences"

Guest Editor: Dr. S. Mohanasundaram
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INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, Special Issue, 113-118 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Extraction and Identification of Zoochemicals in Marine Sponge *Hyattella intestinalis* (Lamarck, 1814) (Phylum: Porifera) using GC-MS Technique

Karnan R.[1], Sukumaran M.[1*] and Velavan S.[2]

[1]PG and Research Department of Zoology, Rajah Serfoji Govt. College (Autonomous) (Affiliated to Bharathidasan University), Thanjavur 613 005 Tamil Nadu, India
[2]PG and Research Department of Biochemistry, Marudupandiyar College (Affiliated to Bharathidasan University), Thanjavur 613 005 Tamil Nadu, India

**Corresponding Author*

Received: 14th August, 2022; **Accepted:** 18th September, 2022; **Published online:** 3rd October, 2022

https://doi.org/10.33745/ijzi.2022.v08i0s.014

Abstract: Animals provide secondary metabolites with natural bioactive properties, similar to plant secondary metabolites. Most primitive multicellular animals like sponges synthesize secondary metabolites like alkaloids, terpene etc. to be used in ecological aspects. The aim of the present study was to extract and identify Zoochemicals from *Hyattella intestinalis* (Lamarck, 1814), a marine sponge, by using GC-MS techniques. 70% hydroethanolic extract of whole portion of *Hyattella intestinalis* was prepared by using the Soxhlet apparatus with a 3 h duration at 50 to 60°C. At the end of the experiment, a light greenish yellow colour of Zoo-extract was obtained and subjected to qualitatively identified for alkaloids and terpenoids. Sixty (60) compounds were identified by GC-MS analysis. Some of the identified compounds of *Hyattella intestinalis* have naturally reducing agents and biological active properties while using nanoparticle synthesis to reduce heavy metal pollutants in environmental applications. As the *Hyattella intestinalis* involves Zoochemicals, safety can be addressed easily with environmental impacts.

Keywords: *Hyattella intestinalis*, Zoochemicals, GC-MS analysis, Zoo-extraction, Bioactive properties

Citation: Karnan R., Sukumaran M. and Velavan S.: Extraction and identification of zoochemicals in marine sponge *Hyattella intestinalis* (Lamarck, 1814)(Phylum: Porifera) using GC-MS technique. Intern. J. Zool. Invest. 8(Special Issue): 113-118, 2022.
https://doi.org/10.33745/ijzi.2022.v08i0s.014

Introduction

There is a growing interest in utilising marine natural product scaffolds' richness and complexity for rational drug development. Marine-based pharmaceuticals have the potential to identify novel entities that may help in the treatment of biological activities (Khalifa *et al*., 2019). Marine sponges contributed nearly 30 per cent of all natural products, and the initial discoveries from marine sponges led to the belief that it would not be long before true marine-derived drugs were developed; for instance, the discovery and identification of spongothymidine and spongo-

uridine from the sponge of *Tethya crypta*, which are used for antiviral activity, were attributed to marine sponges (Newman *et al*., 2000). The study of marine sponges and bioactive compounds obtained from them is a rapidly increasing area of zoo-chemicals derived from marine organisms (Amudha *et al*., 2017; Muthiyan *et al*., 2020).

From marine algae, sponges, and sponge-associate microorganisms, a number of useful chemical compounds with a wealth of potential bioactivities i.e. medicines, have been identified and shown to have therapeutic utility (Mille *et al*., 2010). Over the last 50 years, natural marine products of sponges have drawn the attention of chemists and biologists across the globe as a source of possible medications (Yalcin, 2007; Mohanasundaram *et al*., 2021). Using GC-MS methods, the present study aimed to extract and identify zoochemicals from *Hyattella intestinalis*.

Materials and Methods

Collection of sponge:

The sponge was collected by hand-picking at the east coast of Mallipattinam village, Thanjavur district, Tamil Nadu, India. The collected sponge was identified by existing literature (Cook and Bergquist, 2002; Srinivasan Balakrishnan, 2007; Sivaleela, 2014; Varsha *et al*., 2020).

Extraction of Hyattella intestinalis and GC-MS analysis:

Hyattella intestinalis was separately cut into pieces and 70% hydroalcoholic extract was prepared by using Soxhlet extraction (3 h and 50 to 60°C) The extract was subjected to zoochemical analysis (Harborne, 1973) and bioactive compounds were identified using GC-MS technique. GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument. Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST).

Results and Discussion

Zoochemical extraction technique and preliminary zoo chemical identified:

Marine sponges are preliminary invertebrates that can be found in temperate, polar and tropical regions. They are major contributors of bioactive compounds (Varijakzhan *et al*., 2021). In the present study screening of Zoochemicals was performed from *Hyattella intestinalis* using extraction and GC-MS techniques. Zoochemicals are animal equivalent of phytochemicals in plants (http://lpi.oregonstate.edu/infocenter/phytochemicals.html). The Soxhlet method involves boiling the *Hyattella intestinalis* whole portion with 70% ethanol, with a duration of 3 h and temperature from 50 to 60°C. *Hyattella intestinalis* 20 g sample extract with 200 ml and 70% ethanol after 3 h produced light greenish yellow colour extract which was concentrated to 20 ml (Fig. 1) and further qualified for alkaloids, terpenoids (Table 1; Fig. 2) and GC-MS analysis. Abubakar and Haque (2020) extracted sponge compound which are involved in secondary metabolites (Mohanasundaram *et al*., 2021). Muthiyan *et al*. (2020) reported the major zoo-chemical constituent of the sponge present as alkaloids. The present study derives support from observations of Gupta (2019) who has extracted terpenes and alkaloid as bioactive secondary metabolites from the genus of *Haliclona*.

GC-MS analysis of Hyattella intestinalis Zooextract:

The present study evaluated the presence of 60 Zoochemicals from 70% ethanolic (hydro-alcoholic) extract of *Hyattella intestinalis* by using GC-MS techniques (Table 2; Fig. 2). Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST). Table 2 illustrates the active principles with their RT (Retention time), name of the compound, molecular formulae, molecular weight (g/mol) by comparing a query mass spectrum with reference data in a library of spectrum matching (Wei *et al*., 2014).

The potential of marine sponges producing bioactive compounds having biological active properties has been reported by Perdicaris *et al*. (2013). Marine sponges are considered as rich

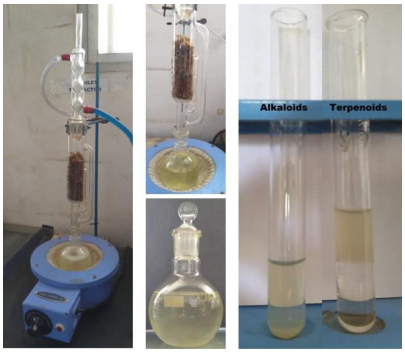

Fig. 1: Extraction of Zoochemicals using Soxhlet method and qualified.

Table 1: Preliminary qualified in Zoochemicals of *Hyattella intestinalis*

Zoochemicals	Observation	Result (figure 2)
Alkaloids	White precipitate	Higher concentration (++)
Terpenoids	Appearance of two layer in reddish brown colour	Presence (+)

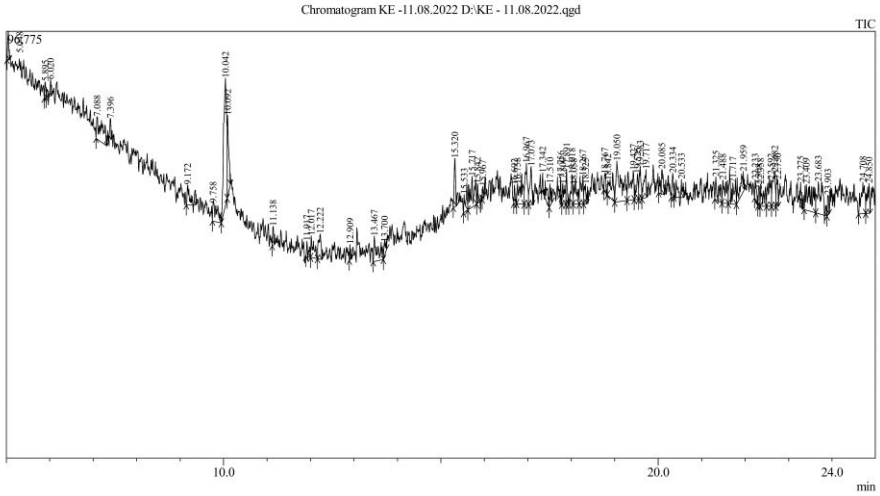

Fig. 2: GC-MS chromatogram of *Hyattella intestinalis* Zooextract.

sources of natural products and metabolites like alkaloids (Shen *et al*. 2012). Alkaloids were present in *Hyattella intestinalis*. In marine sponge *Callyspongia diffusa* Rachanamol *et al*. (2016) reported the presence of long chain fatty acids such as 9, 12-Octadecadienoic acid (Z, Z)-2, 3-di contributing significantly to bioactivities using GC-MS analysis. In this study we identified more than 60 compounds including Trimethylsilyl-di(timethylsiloxy)-silane which was previously reported to possess biological activity by Nagati et al. (2012) and Adebayo-Tayo *et al.* (2021).

Kasitowati *et al.* (2019) reported 39 compounds from *Fascaplysinopsis* sp. by using GC-MS analysis. Alkaloid and steroid have been isolated from genus *Haliclona*. Mudianta *et al.* (2012) have identified 21 new psammaplysin from marine sponge *Aplysinella strongylata*. In the present study 60 Zoochemicals were identified from *Hyattella intestinalis* using GC-MS analysis

Table 2: Identification of Zoochemicals using GC-MS techniques in *Hyattella intestinalis* Zooextract

Peak #	R. Time	A/H ratio	Molecular weight (g/mol)	Molecular formula	Molecular name
1	5.048	1.73	99	$C_6H_{13}N$	1,2,2-Trimethylcyclopropylamine
2	5.895	1.63	262	$C_{17}H_{26}O_2$	[1,1'-Bibicyclo[2.2.2]Octane]-4-carboxylic acid
3	6.020	2.14	41	C_2H_3N	Acetonitrile
4	7.088	8.79	42	CH_3BO	Borane carbonyl
5	7.396	1.62	40	Ar	Argon
6	9.172	4.75	208	$C_{13}H_{20}O_2$	3-Buten-2-one, 4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-YL)-
7	9.758	7.02	85	$C_3H_3NO_2$	Acetic acid, cyano-
8	10.042	4.94	222	$C_{12}H_{14}O_4$	Diethyl Phthalate
9	10.092	2.11	339	$C_{17}H_9NO_3S_2$	3-Phenyl-5-phthalidylidenerhodanine
10	11.138	1.73	85	$C_3H_3NO_2$	2-Cyanoacetic acid
11	11.917	2.35	251	$C_{16}H_{13}NO_2$	Pyrano[3,4-B]Indol-3(9H)-One, 1-(4-Pentynyl)
12	12.017	3.98	84	$C_4H_4O_2$	But-3-Ynoic acid
13	12.222	3.98	206	$C_7H_{11}BrO_2$	1,3-Dioxolane, 2-(3-bromo-3-buten-1-yl)-
14	12.909	0.99	112	$C_4H_4N_2O_2$	1H-Imidazole-4-Carboxylic acid
15	13.467	5.56	114	$C_7H_{14}O$	2-Methyl-5-hexen-3-ol
16	13.700	2.99	104	$C_4H_8O_3$	2-Methoxy-1,3-dioxolane
17	15.320	2.23	458	$C_{20}H_{42}O_4Si_4$	Silane, [[4-[1,2-Bis[(Trimethylsilyl)Oxy]Ethyl]-1,2-Phenylene]Bis(Oxy)]BIS[Trimethyl
18	15.533	4.03	237	$C_{12}H_{25}B_2NO_2$	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-
19	15.717	5.93	361	$C_{22}H_{35}NO_3$	Glycine, N-(4-ethylbenzoyl)-, undecyl ester
20	15.842	1.34	224	C_3H_9AlCsF	Cesium trimethylfluoro) Aluminate
21	15.967	1.11	206	$C_{13}H_{18}O_2$	Ethanone, 1-[4-[(1,1-Dimethylethoxy)Methyl]Phenyl]
22	16.692	1.46	355	$C_{22}H_{13}NO_4$	3-(3-OXO-3H-Benzo[F]Chromen-2-YL)-2,4(1H,3H)-Quinolinedione
23	16.758	8.23	153	$C_9H_{15}NO$	Hexanenitrile, 2-(2-Methoxyethylidene)-, (E)
24	16.967	3.62	458	$C_{20}H_{42}O_4S_{i4}$	4-(3,4-Bis[(Trimethylsilyl)OX
25	17.073	3.07	353	$C_{16}H_{23}NO_4SSi$	3-(4-Hydroxy-3-methoxyphenyl)-2-isothiocyanatopropionic acid, ethyl ester, TMS
26	17.342	3.91	222	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, Hexamethyl-
27	17.510	2.08	206	$C_{10}H_{22}S_2$	t-Butyl n-hexyl disulfide
28	17.756	0.89	153	$C_9H_{15}NO$	3-Cyano-1-Methoxyhept-2-ENE
29	17.800	3.76	254	$C_{15}H_{14}N_2O_2$	4-(Methoxymethyl)-6-Methyl-2-Phenoxynicotinonitrile
30	17.891	1.60	297	$C_9H_9Cl_2NO_4S$	2,4-Dichloro-5-Dimethylsulfamoyl-benzoic acid
31	18.018	2.49	131	$C_4H_2ClNO_2$	Isoxazole-5-carbonyl chloride
32	18.058	5.84	111	$C_7H_{13}N$	N-(1,1-Dimethyl-2-propynyl)-N,N-dimethylamine
33	18.267	3.96	134	$C_4H_6O_5$	Methyltartronic acid
34	18.325	2.71	237	$C_{12}H_{25}B_2NO_2$	Caprolactimether, (NB)-O-[(Diethylboryloxy)(ETHYL)BORYL]

35	18.767	2.41	234	$C_5H_9CsO_2$	Propanoic acid, 2,2-dimethyl-, cesium salt
36	18.842	9.01	268	$C_{17}H_{16}O_3$	2-(4-Benzoylphenyl)-2-Methyl-1,3-Dioxole
37	19.050	8.10	384	$C_{12}H_{36}O_4Si_5$	Pentasiloxane, Dodecamethyl
38	19.427	6.22	251	$C_8H_7Cl_2NO_4$	2-(2,4-Dichloro-6-Nitrophenoxy)Ethanol
39	19.525	2.98	253	$C_{11}H_7N_7O$	2-Propenenitrile, 2-Methyl-3-(9-OXO-9H-[1,2,4]Triazolo[4',3':2,3]Pyr idazino[6,1-C][1,2,4]Triazin-8-YL)-
40	19.583	2.68	223	$C_{12}H_{21}NOSi$	Paredrine tms
41	19.717	3.62	209	$C_7H_{16}NO_2PS$	5,5-Dimethyl-2-(Dimethylamino)-1,3,2-Dioxaphosphorinane 2-Sulphide
42	20.085	3.71	296	$C_8H_{24}O_4Si_4$	Cyclotetrasiloxane, octamethyl-
43	20.334	2.13	253	$C_{11}H_7N_7O$	9H-[1,2,4]Triazolo[4',3':2,3]Pyridazin
44	20.533	4.84	320	$C_{19}H_{28}O_4$	Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester
45	21.325	4.49	368	$C_{13}H_{36}O_4Si_4$	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
46	21.488	5.65	353	$C_{20}H_{23}N_3O_3$	Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5H-dibenzo[b,f]azepinyl]-, ethyl ester
47	21.717	7.46	296	$C_{15}H_{32}Si_3$	Cyclohexa-1,4-Diene, 1,3,6-Tris(Trimethylsilyl)-
48	21.959	6.37	396	$C_{22}H_{19}N_5O$	1-(4-Acetamidoanilino)-3,7-dimethylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile
49	22.233	2.32	206	$C_4H_9F_3O_2SSi$	Methanesulfinic acid, Trifluoro-, trimethylsilyl ester
50	22.308	2.48	253	$C_{12}H_{15}NO_5$	Glycine, N-[[(4-Methoxyphenyl)Methoxy]Carbonyl]-N-Methyl
51	22.358	4.42	291	$C_{20}H_{21}NO$	1(2H)-Naphthalenone, 2-(3,3-Dimethyl-2-Phenyl-2-Aziridinyl)-3,4-Dihydro
52	22.592	4.92	234	$C_{11}H_{22}OS_2$	1,3-Dithiane-2-Ethanol, .Alpha.-Ethyl-2-(1-Methylethyl)
53	22.682	4.24	460	$C_{29}H_{48}O_4$	Phthalic acid, propyl octadecyl ester
54	22.750	2.58	364	$C_{18}H_{12}F_3O_3P$	Tris(4-fluorophenyl) phosphite
55	23.275	1.63	417	$C_{26}H_{43}NO_3$	Glycine, N-(4-ethylbenzoyl)-, pentadecyl ester
56	23.409	9.70	253	$C_{13}H_{19}NO_2S$	Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-ethyl-, ethyl ester
57	23.683	7.77	282	$C_{16}H_{14}N_2O_3$	Benzofuro[3,2-B]Pyridine-1(2H)-Carboxylic acid, 3-Cyano-4-Methyl-, Ethyl ester
58	23.903	1.94	280	$C_9H_{28}O_2Si_4$	Trimethylsilyl-di(timethylsiloxy)-silane
59	24.708	5.55	234	$C_5H_9CsO_2$	Cesium pivalate
60	24.850	3.59	374	$C_{24}H_{22}O_4$	Phthalic acid, 3,4-dimethylphenyl 3,5-dimethylphenyl ester

and supported by studies *in silico, in vitro* and *in vivo* studies to be recommended as eco-friendly (Mohanasundaram *et al.*, 2017).

Conclusion

Hyattella intestinalis was positive for alkaloids and terpenoids while the conformation of 60 Zoochemical compounds was identified using GC-MS techniques. *Hyattella intestinalis* naturally reduces agents, the exhibits stimulating pharmacological activity in vitro, in vivo, in silico studies, and has social relevance because society requires harmless but effective drug/reducing agents that can offer advancements to chemical drugs.

Because *Hyattella intestinalis* involves Zoochemicals, safety can be easily addressed with environmental impacts and scientifically with the results of the current study.

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