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Biochemical Screening of *Penaeus vannamei* Shell Waste by Liquid Chromatography-Tandem Mass Spectrometry

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Marine environment is a progressive route for the attainment of natural novel drugs. Substantially they are of secondary metabolites. This study is about the utilization of marine shrimp shell waste for the identification of unrevealed bioactive compounds with its pharmacological activity by LC-MS method. Biochemical screening of *Penaeus vannamei* shell waste revealed the presence of various bioactive compounds like arecoline which possess antibacterial activity, oleamide which has potent anti-inflammatory activity, desethylchloroquine which reports the antiplasmodial activity, dodecanamide which shows antibacterial property, C17 sphinganine which exhibits antimicrobial function and also the identification of 11-α-acetoxykhivorin which is a rare limonoid khivorin compound. This work is the first report concerned with the screening of biochemical components present in the ethanolic extract of *Penaeus vannamei* shell wastes by LC-MS method. It was concluded that *Penaeus vannamei* shrimp shell contains numerous bio compounds with high medicinal values which can be isolated and utilized in rational methods in future for the discovery of innovative drugs.

Keywords: *Penaeus vannamei*, Shell waste, Arecoline, Oleamide, Dodecanamide, Desethylchloroquine, C17 Sphinganine.

INTRODUCTION

Oceans are of immense value to the world economy. Over 70 % of the earth's surface is covered by the ocean [1]. The ocean contains a radiant diversification of life and a boundless reward of marine resources. Marine environment is a significant store house of unique bioactive compounds with structural and chemical features. The marine species acts as a abundant origin of nutraceuticals and potential candidates for the treatment for various dreadful diseases [2]. In general marine natural products are of secondary metabolites.

Generally the head, shell of body and tail portions of prawn are discarded during cleaning process which is about 50 % of volume of the raw materials [3,4]. Increased production of these discards results in an environmental pollution. But these biowastes contain high valuable bio-compounds like chitin, carotenoids, glycosaminoglycans, pigments and various amino acids. These active bio-compounds have an immense area of approaches in the field of medicine, cosmetics, food applications, biotechnology, paper and pulp industries. Currently, there are more possibilities for the extraction and utilization of these products and more to come [5].

Prawns are easily available species from ocean and are delicious to eat more than that it has health benefits to humans. Prawns are enriched with high proteins and low in fats and calories [6]. It also contains essential fatty acids which provide health benefits for human such as eye and brain development and its function [7]. Guillou *et al*. [8] reported that the fatty acid profile of shrimp waste and the silage will acts as the best source of lipids in aqua-culture diets. Among the different varieties of prawn, *Penaeus vannamei* is the most common farming species in India. In this study, *Penaeus vannamei* shell wastes were collected and used for analysis. It is commonly known as white legged shrimp or Mexican white shrimp. *Penaeus vannamei* is one of the major species of shrimp aqua-culture industry [9].

Liquid chromatography combined with mass spectroscopy is a powerful tool for analysis of the organic molecules from various matrices. LC-MS technique has become the most widely-used and proven to be very reliable method in analyzing the metabolites of the sample [10]. In this study, we used electrospray ionization (ESI) method of LC-MS technique which is suitable for almost all drug like molecules also for steroids and other less polar compounds. This study was planned to identify the bio compounds present in the shell wastes of *Penaeus vannamei* by the analytical method liquid chromatography combined with mass spectroscopy.

EXPERIMENTAL

Collection and preparation of the sample: The shell wastes of *Penaeus vannamei* was collected from the Kasimedu market

in Chennai, India. The shell wastes contain the head part, body shells and other unwanted materials. The body shells were alone collected, the remaining head parts and other contaminants were removed. The collected shells were cleaned and rinsed well without any flesh part and other contaminants and it was dried well.

Extraction of *Penaeus vannamei*: About 10 g of shell powder was taken in a 50 mL centrifuge tube. Added 10 mL of ethanol and vortex for 10 min. The sample was centrifuged at 10000 rpm for 5 min in 4 ºC. The organic layer was filtered and subjected into LC-MS analysis.

Liquid chromatography-mass spectrometry: The chromatographic screening of all analyte was achieved on a ZORBAX Eclipse plus C18 column (2.1 × 100 mm, 5.0 µm particle size; Agilent Technologies) with 0.5 mL/min flow rate and an injection volume of 5 µL. The mobile phase was composed of 0.1 % formic acid with 10 mM ammonium formate in water and 0.1 % formic acid with 10 mM ammonium formate in methanol (A: 80:20, B:10:90). The elution gradient started with 10 % of B, then 0-7 min 10-50 % methanol, 7-12 min 50-80 % of B, 12-15 min 80-100 % methanol, 15-18 min 100 % of B, 18-18.1 min 100-20 % and 18.1-20 min 20 % B. The oven temperature was maintained at 45 ºC. The MS parameters included drying gas temperature, 325 ºC; gas flow rate, 11 L/min; Nebulizer pressure, 40 psi; sheath gas temperature, 350 ºC; sheath gas flow rate, 8 L/min; delta EMV, 500 V and capillary voltage, 4000 V.

RESULTS AND DISCUSSION

Marine environment paves the route for the finding of novel drugs to treat the several chronic diseases [11]. Scientists reported that the methanolic and lipidic extracts of shrimp muscle and waste have the capability of modifying the many biological processes [12]. Previous study [13] reports proved the presence of pigments such as astaxanthin and its esters, β-carotene in the shrimp wastes. The bio-chemical screening was done by using LC-MS method which provides a window for the identification of compounds present and also the analyte-related searchable information like accurate mass measurement and fragmentation patterns.

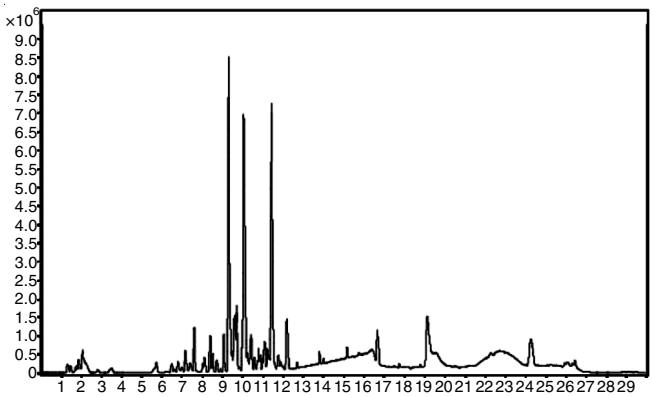


Fig. 1. LC-MS chromatogram of ethanolic extract of *P. vannamei* shells

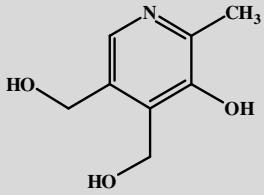
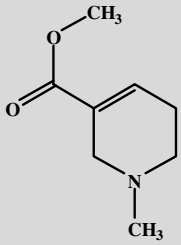
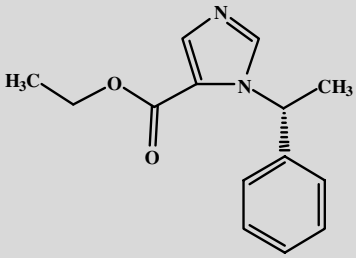
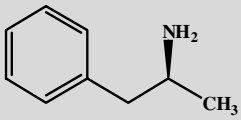
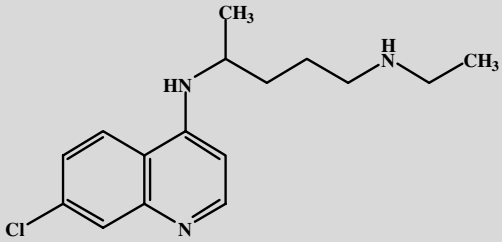
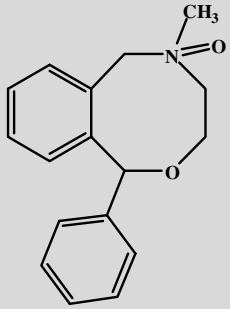
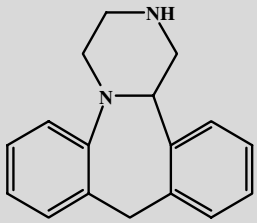
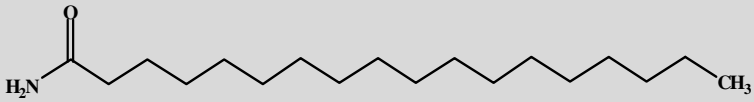
The LC-MS chromatogram of ethanolic extract of *Penaeus vannamei* shells are shown in Fig. 1 and the detected compounds were tabulated with their molecular formula, mass and the retention time in Table-1. The biological activities of the identified compound are tabulated in Table-2.

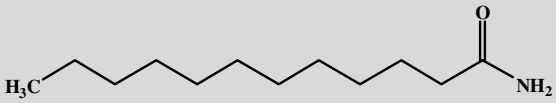
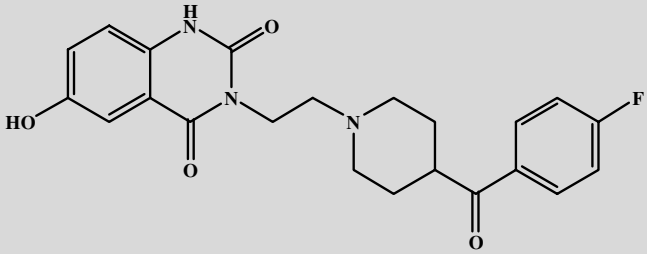
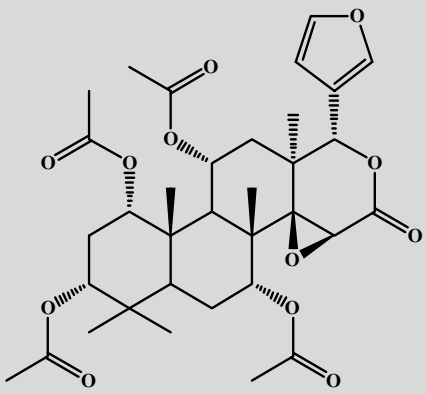
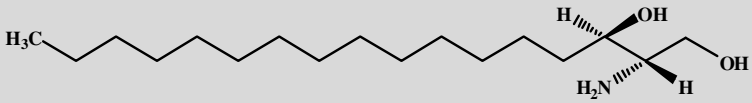
For compound **1** at 1.806 min, the ESI-MS spectrum displayed at *m/z* 169 which could be taken as indication of the presence of pyridoxine (vitamin B6) (Fig. 1). Pyridoxine has the potential to reduce the risk of coronary heart disease, non-fatal myocardial infection and non-multi vitamin supplement users [14]. Compound **2** shows the peak at RT 1.879 with the ESI-MS spectrum at *m/z* 155 confirms the presence of a compound arecoline (Fig. 2), which reports to have the significant antibacterial activities against *Bacillus protease*, *Candida albicans* and *Bacillus anthracis* [15]. Huang *et al*. [16] reported that the arecoline has the ability in preventing BCC tumorigenesis by reducing the levels of IL-6 which is the tumour cell survival factor and increases the level of tumour suppressor factor p53 followed by apoptosis.

TABLE-1
COMPOUNDS IDENTIFIED IN THE ETHANOLIC EXTRACT OF *P. vannamei* SHELLS

S. No.	Compound name	Retention time	Molecular formula	Molecular mass
1	Pyridoxine (Vitamin B6)	1.806	$C_8H_{11}NO_3$	169.00
2	Arecoline	1.879	$C_8H_{13}NO_2$	155.00
3	Dextroamphetamine	1.920	$C_9H_{13}N$	135.10
4	Desethylchloroquine	6.486	$C_{16}H_{22}N_3Cl$	291.15
5	4,7,10,13-Docosatetraynoic acid	7.402	$C_{22}H_{28}O_2$	324.20
6	Nefopam N-oxide	8.011	$C_{17}H_{19}NO_2$	269.14
7	8-cyclopentyltheophyllin	8.079	$C_{12}H_{16}N_4O_2$	248.12
8	7,8-Diaminononanoate	8.386	$C_9H_{20}N_2O_2$	188.15
9	Etomidate	8.712	$C_{14}H_{16}N_2O_2$	244.12
10	10,11-Dihydro-10,11-dihydroxyprotriptyline	9.062	$C_{19}H_{23}NO_2$	297.17
11	2-Amino-tetradecanoic acid	9.296	$C_{14}H_{29}NO_2$	243.22
12	Oleamide	10.786	$C_{18}H_{35}NO$	281.27
13	Dihydrodeoxystreptomycin	11.425	$C_{21}H_{41}N_7O_{11}$	567.29
14	11-alpha-acetoxykhivorin	11.750	$C_{34}H_{44}O_{12}$	645.27
15	Desmethylmianserin	13.799	$C_{17}H_{18}N_2$	250.14
16	Dodecanamide	15.175	$C_{12}H_{25}NO$	199.19
17	6-HydroxyKetanserin	15.238	$C_{22}H_{22}N_3O_4F$	411.15
18	3-*n*-Decyl acrylic acid	15.879	$C_{13}H_{24}O_2$	212.18
19	Phthalic acid Mono-2-ethylhexyl Ester	16.666	$C_{16}H_{22}O_4$	278.15
20	2-Oxo-4-methylthiobutanoic acid	16.668	$C_5H_8O_3S$	148.00
21	Linoleamide	17.746	$C_{18}H_{33}NO$	279.25

TABLE-2
BIO-ACTIVITY OF THE COMPOUNDS IDENTIFIED IN THE ETHANOL EXTRACT OF *Penaeus vannamei* SHELL BY LC-MS

S. No.	Compound name	Structure	Biological activity
1	Pyridoxine (vitamin B6)		Reduce the risk of Coronary heart disease [14]
2	Arecoline		Antibacterial activity [15,16]
3	Etomidate		No therapeutic purposes
4	Dextroamphetamine		In the treatment of narcolepsy [17]
5	Desethylchloroquine		Antiplasmodic activity [18,19]
6	Nefopam N-oxide		Pain killing medication [20]
7	Desmethylmianserin		No therapeutic purposes
8	Oleamide		Anti-inflammatory activity [21]

9	Dodecanamide		Antibacterial property [22]
10	6-Hydroxy ketanserin		No therapeutic purposes
11	11 alpha acetoxykhivorin		Limonoid Khivorin compound. May possess anticancer activity [23,24]
12	C17 Sphinganine		Antimicrobial activity [25]

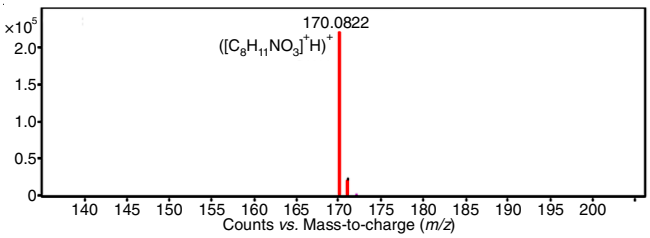


Fig. 2. Mass spectrum of pyridoxine

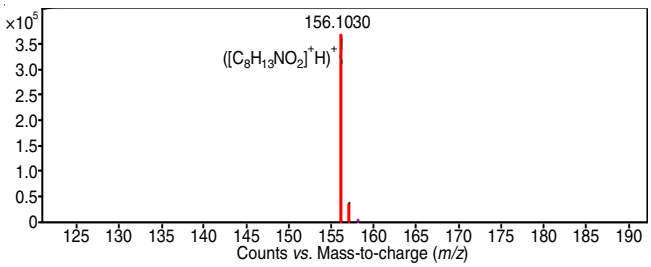


Fig. 3. Mass spectrum of arecoline

Compound **3** exhibited a base peak at the retention time 1.92 ESI-MS spectrum displayed at *m/z* 135.10 (Fig. 3) indicates the presence of compound dextroamphetamine with its structure. It is used to treat attention deficit hyperactivity disorder (ADHD) and narcolepsy [17]. Compound **4** displays the ESI-MS spectrum at *m/z* 291.15 and exhibits a peak at the retention time 6.486 (Fig. 4), which predicts the presence of a compound desethylchloroquine. Ogubona *et al.* [18] reported that desethylchloroquine drug and its metabolites are secreted in the human saliva. The research findings of Dorn *et al.* [19] proves that desethylchloroquine possess antiplasmodic activity [19]. Nefopam N-oxide was confirmed by the base peak found at the retention time 8.011 with the ESI-MS spectrum at *m/z* 269.14 (Fig. 6). Nefopam is used in the treatment of moderate, acute or chronic pain medication [20].

The presence of a compound oleamide was confirmed by the ESI-MS spectrum at *m/z* 281.27 (Fig. 7) and the base peak obtained at the retention time 10.786. It is reported that oleamide is a potent anti-inflammatory compound and reduces inflammation in nerve cells and in the brain [21]. The displayed ESI-MS spectrum at *m/z* 199.19 (Fig. 8) and the base peak obtained

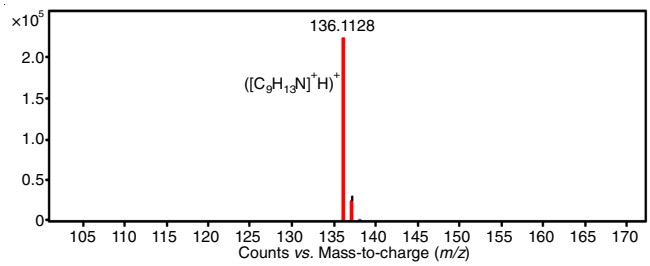


Fig. 4. Mass spectrum of dextroamphetamine

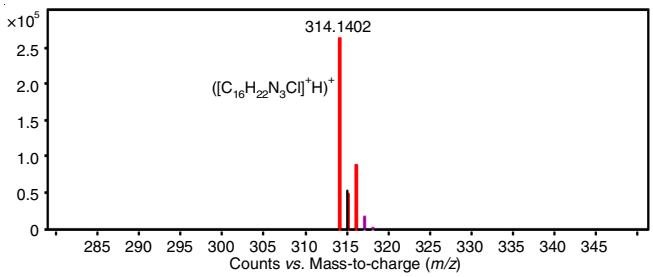


Fig. 5. Mass spectrum of desethylchloroquine

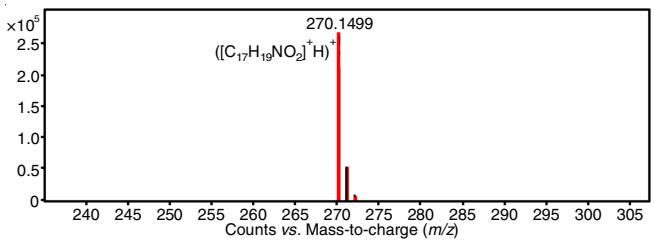


Fig. 6. Mass spectrum of nefopam N-oxide

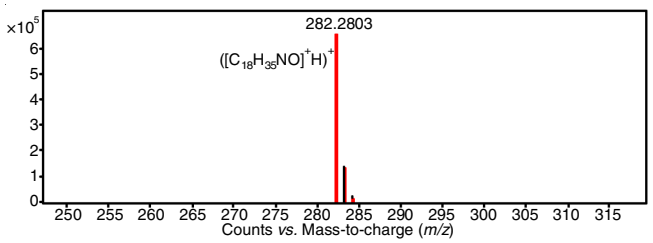


Fig. 7. Mass spectrum of oleamide

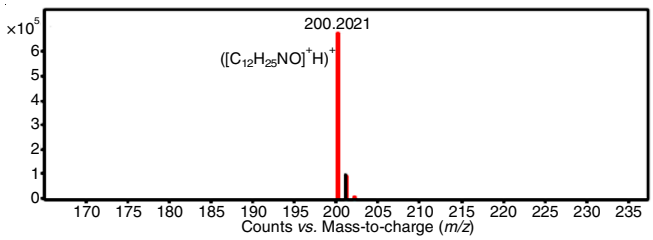


Fig. 8. Mass spectrum of dodecanamide

at the retention time 15.175 predicts the presence of the compound dodecanamide. Beltagy [22] reported the presence of dodecanamide in tea leaves and found its antibacterial activity against *Candida albicans*, *Pseudomonas aeruginosa*, S*taphylococcus aureus* and *Salmonella typhii* [22].

The presence of a compound 11-α-acetoxykhivorin was confirmed by the ESI-MS spectrum at *m/z* 645.27 (Fig. 9) and the base peak obtained at the retention time 11.75 which is a rare compound identified after the long period of time. This compound was first identified in the timber of *Khaya madagascariensis* [23]. Several GC-MS analyses of plants shows its presence but this is the preliminary finding of its presence in the marine wastes. This compound is a khivorin group of limonoid triterpenoid in nature hence possesses an anticancer activity [24]. The compound C17 sphinganine is confirmed by the base peak displayed at *m/z* 287.28 (Fig. 10) with the retention time 10.115. From the literature it was proved that the compound has antimicrobial properties, which can also serve as a precursor for the biosynthesis of barrier lipids. It is a potent inducer of keratinocyte differentiation [25]. Thus, the LC-MS report of ethanolic extract of *Penaeus vannamei* shell wastes shows the presence of various bioactive compounds.

Conclusion

The recovery of bioactive compounds from marine wastes is a potential and innovative area of research and development for the utilization of those by-products. This work is the first report dealing with the screening of biochemical components present in the ethanolic extract of *Penaeus vannamei* shell wastes by LC-MS method. The active bio-compounds present in the shell wastes will acts as the potential source for the drug development. The result of this study concludes that ethanolic extract of *Penaeus vannamei* shell wastes contains pharmacologically valued substances with antibacterial, anti-inflammatory and antiplasmodic activity. Therefore, it could be the new source of the development of new therapy for the management of several chronic diseases.

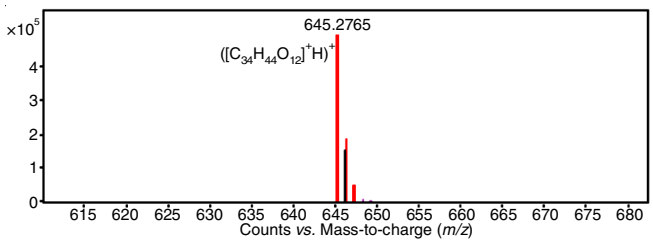


Fig. 9. Mass spectrum of 11-alpha acetoxy khivorin

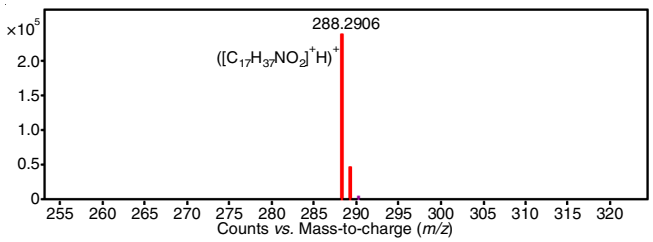


Fig. 10. Mass spectrum of C17 sphinganine

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