Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Biochemical Screening and Determination of Bioactive Components of Commercially Cultured Pacific White Shrimp *Penaeus vannamei*

Jayalakshmi Muniyappan, Vanitha Varadharajan, Pushpabharathi Namadevan

Department of Biochemistry, School of Life Sciences, Vels Institute of Science, Technology, and Advanced Studies, Chennai, Tamil Nadu, India

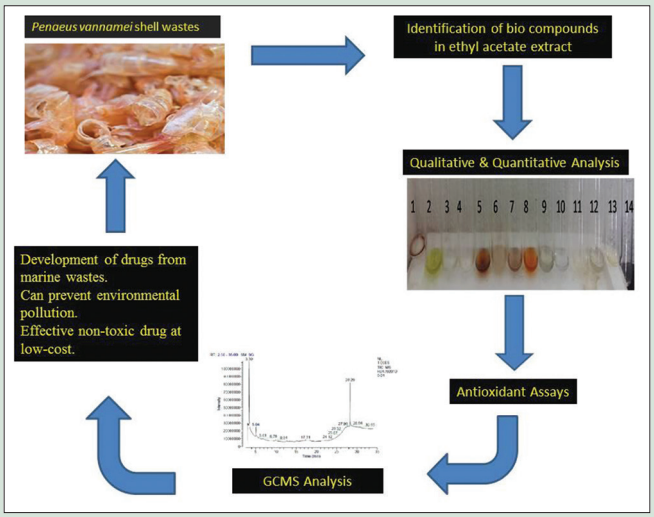
ABSTRACT

Background: Marine waste is an extraordinarily renewable aid for the restoration of several valued metabolites with potential biological applications. **Objectives:** The investigation is planned to detect the biochemical components present in the prawn shell waste by qualitative, quantitative methods, to assess the antioxidant potential, and also to find the bioactive compounds existing in the prawn shell waste by gas chromatography-mass spectrometry (GC-MS) analysis. **Materials and Methods:** *Penaeus vannamei* shell wastes are collected, cleaned, dried, and powdered well. The bioactive compounds present in crude ethyl acetate extract of *P. vannamei* shell was determined by qualitative, quantitative, and GC-MS analysis. The free radical scavenging activity of the extract was studied by different *in vitro* antioxidant assays. **Results:** The bio-compounds such as carbohydrates, saponins, flavonoids, tannins, and quinones show the positive result by qualitative analysis. The higher tannin content 49.2 ± 0.084 mg/g was observed in the ethyl acetate extract of *P. vannamei* shell and the flavonoid was found to be 5.994 ± 0.044 mg/g. The GC-MS analysis of the *P. vannamei* shell shows the various numbers of bio-compounds. Some of the identified compounds are Timonacic which has the powerful antioxidant property, Octadecane, 3ethyl5(2ethylbutyl) is a good antifungal agent, Acetamide possesses antioxidant and anti-inflammatory property. The results of the *in vitro* assays revealed that *P. vannamei* shell extract possess significant antioxidant activity. **Conclusion:** The present study suggests that the effective utilization of prawn shell waste enhances biomedical research field for the development of the natural drug for many chronic diseases with no side effects and at the same time can reduce environmental pollution.

Key words: Antioxidant assays, flavonoids, gas chromatography-mass spectrometry analysis, *Penaeus vannamei,* shell wastes, tannins

SUMMARY

- The ethyl acetate extract of *Penaeus vannamei* shells was subjected to qualitative and quantitative analysis for the identification of secondary metabolites
- The antioxidant potential of the crude extract was determined by the different antioxidant assays
- The active bio-compounds were identified by the gas chromatography-mass spectrometry analysis
- The reports of this study state that the *P. vannamei* shells contain more bioactive components
- It reflects a hope for the development of many more novel antitherapeutic agents from these shell wastes which in the future may serve for the production of biologically improved therapeutic agents
- Moreover, by the utilization of these bio-wastes, we can reduce the environmental problems and can afford inexpensive nontoxic drugs without any side effects.



Abbreviations Used: GC-MS: Gas chromatography mass spectrometry; NIST: National Institute Standard and Technology; H_2O_2: Hydrogen peroxide; DPPH: 1,1-diphenyl-2-picrylhydrazyl; NO: Nitric oxide; SO: Superoxide.

Correspondence:
Dr. Vanitha Varadharajan,
Department of Biochemistry, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai - 600 039, Tamil Nadu, India.
E-mail: vanitha.sls@velsuniv.ac.in

DOI: 10.4103/pr.pr_60_18

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

The marine condition is a solitary rooftop where we can recognize the broad number of living creatures with various qualities. Above 80% of different plant and creature species originate in the sea. As of now, the medical industry is in the pivotal circumstance in the finding of novel particles which can be utilized for the progress of unique curative agents. The restorative parts got from the marine premise are sorted into the distinctive classes such as terpenes and terpenoids (40.5%), peptides (19%), macrolides (14.3%), and alkaloids (12%) which were reported by Sawadogo *et al.* in 2011. The majority of these compounds are chemotherapeutic agents (92.7%), and just 7.3% are chemopreventives.[1]

Prawns are effectively accessible species from the sea. Prawns are enhanced with high proteins and low in fats and calories.[2,3] It likewise

Cite this article as: Muniyappan J, Varadharajan V, Namadevan P. Biochemical screening and determination of bioactive components of commercially cultured pacific white shrimp *Penaeus vannamei*. Phcog Res 2019;11:140-6.

contains basic unsaturated fats, which give medical advantages to human, for example, eye and mental health and its capacity.[4] Shrimp industry is a rapidly growing industry in India and all over the world. Shrimp enterprises produce enormous measures of shrimp bio-waste during processing, roughly 45%–55% by weight of raw shrimp. The disposal of these wastes in our surroundings is one of the most important problems, contributing to significant environmental and health hazards. The prawn shell wastes mainly composed of protein (40%), minerals (35%), and chitin (14%–30%), and carotenoids. The most important environment-friendly and profitable option for utilization of shellwaste for the recovery of marketable by-products and production of value-added products through bioconversion.[5] The previous reports showed that they contain useful components such as proteins, lipids, astaxanthin, and of course chitin, which are well known as a marketable product.[6]

There are distinctive assortments of prawn. Among them, *Penaeus vannamei* is the widely recognized species in India. It is generally known as white-legged shrimp or Mexican white shrimp. *P. vannamei* is one of the real types of shrimp aquaculture industry.[7] The bio-remediation of shell waste is potentially the most practical and eco-friendly method for waste usage. The drugs from the bio-resources are of a great necessity for the handling of various human ailments.[8] This study therefore is an effort to minimize pollution caused due to ignorant generation of such wastes and at the same time utilize them for the benefit of humankind. The current work was intended to analyze the lead components of the prawn shells by qualitative and quantitative examination and using the gas chromatography-mass spectrometry (GC-MS) technique and determination of its antioxidant activity by different assays.

MATERIALS AND METHODS

Sample preparation

Shell wastes of prawn species *P. vannamei* were collected from the Kasimedu market in Chennai, Tamil Nadu, India. The wastes contain the shells of head, the cephalothorax, and the tail parts. The head portion and the adhering meat from the abdominal and tail portions of the shell were removed. The shell wastes were washed under the running water and dried well. The dried body shells of *P. vannamei* were powdered well and stored at −200°C until use.

Sample extraction

The sample was extracted three times by hot percolation method with 1:5 ratio volume of ethyl acetate solvent at room temperature for 72 h. The filtrates were utilized for subsequent experiment.

Qualitative biochemical tests

The bioactive compounds were analyzed by the qualitative tests for ethyl acetate extract of *P. vannamei* shells. The subjective investigation was done by approach depicted by Harborne, 1984.[9]

Quantitative biochemical tests

Determination of flavonoid and tannin content

Total flavonoid content in the extract (ethyl acetate) was resolved to utilize the technique illustrated by Chang *et al.*, 2002.[10] The tannin content in the extract was analyzed by the approach described by Amadi *et al.*, 2004.[11]

Gas chromatography-mass spectrum analysis

GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column of 30m length, 0.25mm diameter and 0.25 µm thickness and composed of 100% Dimethyl poly siloxane. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2µl was employed. Injector temperature was 200°C and Ionsource temperature was 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver. 2.53.[12]

Identification of components

Analysis on GC-MS was directed utilizing the database of National Institute Standard and Technology (NIST) having in excess of 62,000 patterns. The range of the unknown components was contrasted and the range of the known components put away in the NIST library. The name, molecular mass, and structure of the components were identified.

Antioxidant assays

The ideal antioxidant assays include 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity,[13] superoxide anion scavenging activity,[14] nitric oxide scavenging activity,[15] and hydrogen peroxide scavenging activity[16] were achieved by standard methods.

RESULTS

P. vannamei is prevalently known as white-legged shrimp or Mexican white shrimp, which is grayish-white in color. Marine wastes are enriched with valuable by-products, and it attracts attention for progress of innovative therapeutic drugs.

Qualitative analysis of *Penaeus vannamei*

The qualitative analysis of bio-compounds from the ethyl acetate extract has been analyzed in this study. Table 1 and Figure 1 demonstrate the

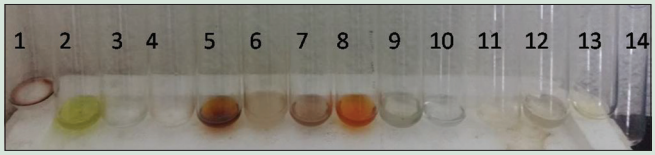


Figure 1: Test tubes showing the results of qualitative analysis shows the positive results for Carbohydrates, tannins, flavonoids, saponins and quinones

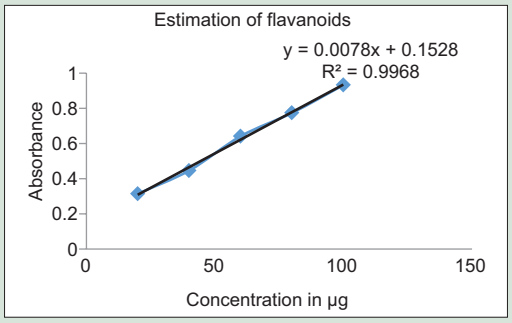


Figure 2: The Quantitative analysis of Flavonoids with standard Gallic acid curve

Table 1: Qualitative analysis of the *Penaeus vannamei* shell extract shows the presence of carbohydrates, tannins, saponins, flavonoids and quinones

Biochemical Tests	Ethyl acetate extract
Carbohydrates test	+
Tannins test	+
Saponins test	+
Flavonoids test	+
Alkaloid test	-
Quinones test	+
Glycosides test	-
Cardiac glycosides test	-
Terpenoids test	-
Phenols test	-
Coumarins test	-
Steroids &Phytosteroids	-
Phlobatannins test	-
Anthraquinones test	-

+ indicates presence; -indicates absence

Table 2: Total Flavonoid and Tannin content of crude extract of *Penaeus vannamei* shell shows 5.994mg/g for flavonoid and for tannin 49.2mg/g

Ethyl acetate extract	Values obtained (mg/g)
Total Flavonoid content	5.994
Total Tannin content	49.2

Table 3: The biochemical composition of ethyl acetate extract of *Penaeus vannamei* shell shows various biocompounds at different retention time

RT	Name of the compound	Molecular Formula	Molecular weight	Peak Area %
5.04	Ethyl benzene	C_8H_{10}	106	18.08
6.79	Propanamide 2 hydroxy	$C_3H_7NO_2$	89	1.81
6.79	Acetic acid, cyano	$C_3H_3NO_2$	85	1.81
6.79	Acetic acid, hydroxy, Ethyl ester	$C_4H_8O_3$	104	1.81
6.79	Timonacic	$C_4H_7NO_2S$	133	1.81
9.84	Acetyl iodide	C_2H_3IO	170	4.00
12.61	Octadecane, 3ethyl5 (2ethylbutyl)	$C_{26}H_{54}$	366	4.17
15.06	Heneicosane, 11 (1ethylpropyl)	$C_{26}H_{54}$	366	3.85
15.06	Butane, 3methyl1(methylthio)	$C_6H_{14}S$	118	3.85
15.06	Heptanoic acid, 3methylbutylester	$C_{12}H_{24}O_2$	200	3.85
15.06	Ethosuximide	$C_7H_{11}NO_2$	141	3.85
15.06	Acetic acid, pentyl ester	$C_7H_{14}O_2$	130	3.85
16.21	17Pentatriacontene	$C_{35}H_{70}$	490	2.24
16.21	1Tricosanol	$C_{23}H_{48}O$	340	2.24
16.21	11Tricosene	$C_{23}H_{46}$	322	2.24
16.21	1Hexadecanesulfonyl Chloride	$C_{16}H_{33}ClO_2S$	324	2.24
16.21	Dodecanoic acid, hexadecyl ester	$C_{28}H_{56}O$	424	2.24
17.31	Octadecane, 3ethyl5 (2ethylbutyl)	$C_{26}H_{54}$	366	2.29
17.39	Heptadecane, 9hexyl-	$C_{23}H_{48}$	324	2.29
17.39	Ethanol, 2(octadecyloxy)-	$C_{20}H_{42}O_2$	314	2.29
17.39	Dodecane, 5,8diethyl-	$C_{16}H_{34}$	226	2.29
17.39	Docosane, 11butyl-	$C_{26}H_{54}$	366	2.29
18.38	Silane, trichlorooctadecyl	$C_{18}H_{37}C_{l3}Si$	378	1.88
18.38	Cyclohexane	$C_{27}H_{54}$	378	1.88
18.38	Heptadecane, 9hexyl	$C_{23}H_{48}$	324	1.88
18.38	Clocortolonepivalate	$C_{27}H_{36}ClFO_5$	494	1.88
27.10	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O7Si_7$	516	1.96
27.10	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	532	1.96
28.29	Cholesterol	$C_{27}H_{46}O$	386	58.92
28.29	Cholesteryl hydrogen phthalate	$C_{35}H_{50}O_4$	534	58.92

qualitative results of the sample in the ethyl acetate extract. Qualitative analysis shows the positive outcome of the existence of compounds such as carbohydrates, saponins, flavonoids, tannins, and quinones. Carbohydrates were detected to be available in the ethyl acetate extract of the sample. The carbohydrates are considered to be the first among the organic substances to be utilized for the generation of energy in the cell. Ravichandran *et al.* stated that the carbohydrate content in the exoskeleton (3.62%) of *Macrobrachium macrobrachion* is lesser than the

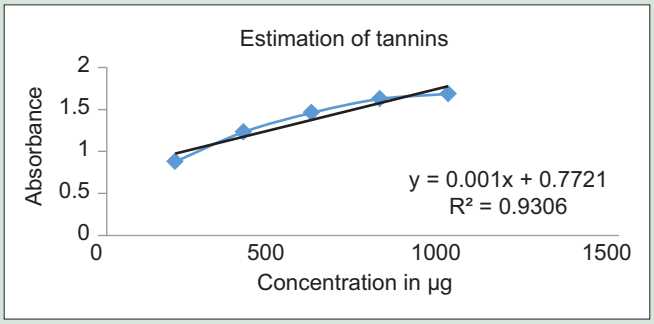


Figure 3: The Quantitative analysis of Tannins with standard Gallic acid curve

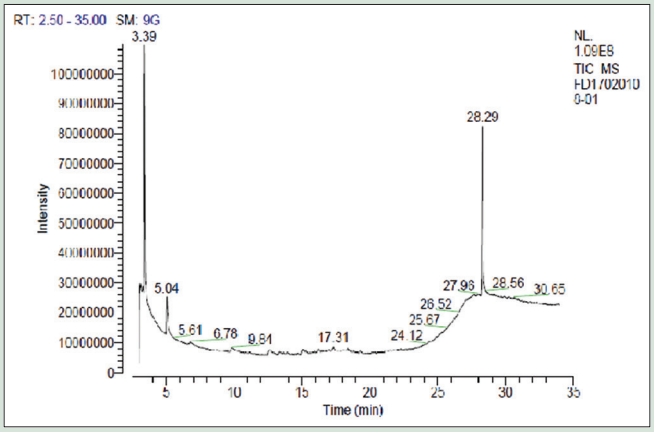


Figure 4: Chromatogram obtained for ethyl acetate fraction of *Penaeus vannamei* shell powder

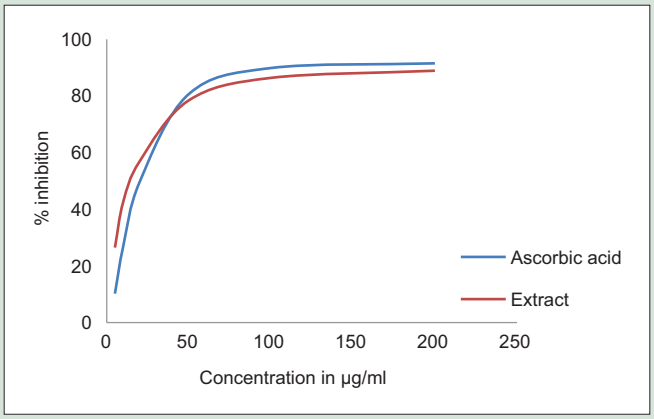
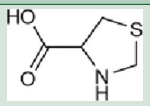
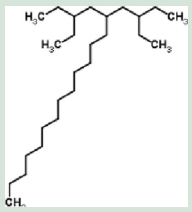
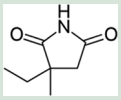
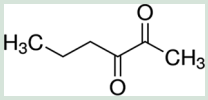
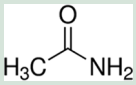
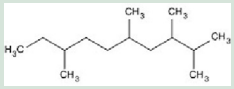
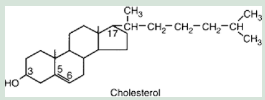
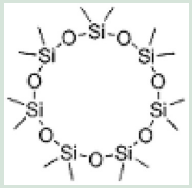
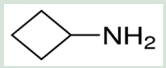
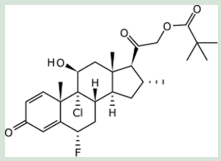
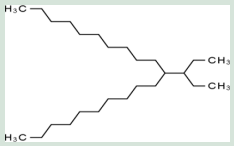
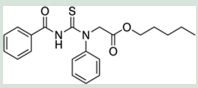


Figure 5: 1,1-diphenyl-2-picrylhydrazyl scavenging effect of different concentrations (10-200µg/ml) of Ethyl acetate extract of *P. vannamei*

Table 4: Bioactivity of components identified in the ethyl acetate extract of *Penaeus vannamei* shell by GC-MS shows the presence of various biologically active compound

Compound Name	Structure	Biological Activity
Timonacic		Antitumor activity
Octadecane, 3ethyl5 (2ethylbutyl		Antifungal and antimicrobial agent
Ethosuximide		Used in the treatment of congenital adrenal hyperplasia
2,3 hexane dione		Apoptotic and necrotic effects against neuroblastoma cells
Acetamide		Antioxidant Activity and Anti-Inflammatory activity
1-Tricosanol		Antibacterial, Antifungal activity
Do decane, 5,8 -diethyl		Used for Tetany, Pulmonary edema and Muscle weakness.
Cholesterol		Dietary sources, Plasma transport and regulation of absorption and metabolism, recycling and excretion
Cycloheptasiloxane, tetradecamethyl		Antimicrobial activity
Cyclobutylamine		No Therapeutic purposes
Clocortolone pivalate		It is used to treat a variety of skin conditions like eczema, dermatitis, allergies and rashes. Anti-inflammatory agent
Propanamide 2 hydroxy		Anti-oxidant and anti-bacterial activity.
Acetyl iodide		No Therapeutic purposes
Heneicosane, 11-(1-ethylpropyl)		No Therapeutic purposes
Acetic acid, pentyl ester		No Therapeutic purposes
1Hexadecane sulfonyl Chloride		No Therapeutic purposes

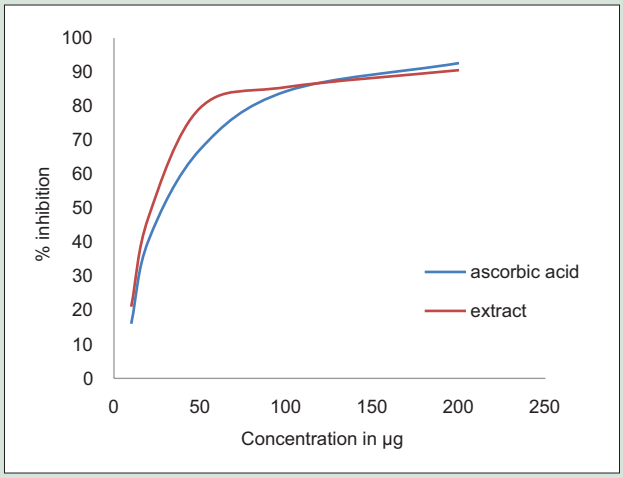


Figure 6: Nitric oxide scavenging effect of different concentrations (10-200µg/ml) of Ethyl acetate extract of *P. vannamei*

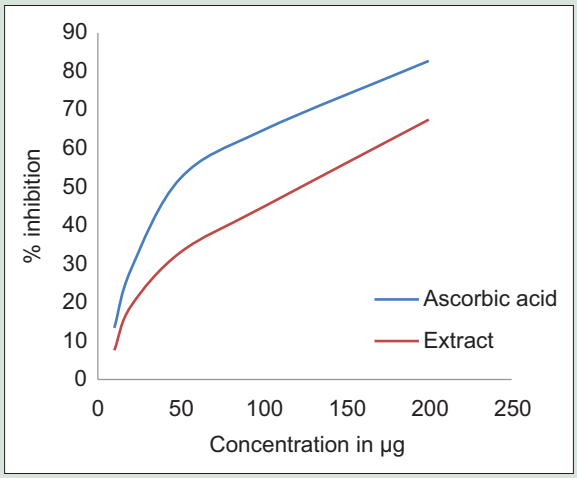


Figure 7: Superoxide scavenging effect of different concentrations (10-200µg/ml) of Ethyl acetate extract of *P. vannamei*

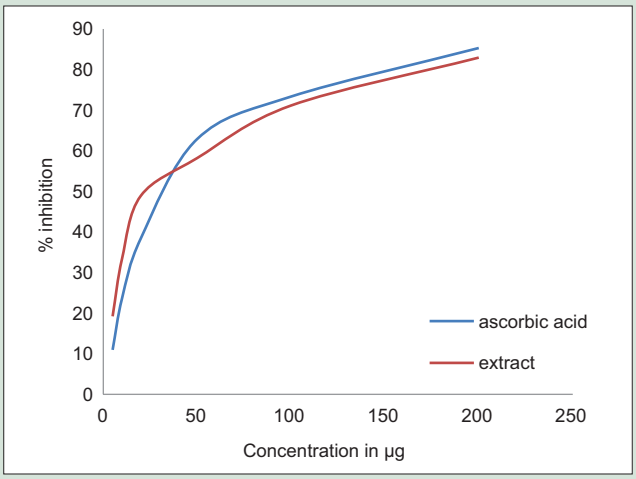


Figure 8: Hydrogen peroxide scavenging effect of different concentrations (10-200µg/ml) of Ethyl acetate extract of *P. vannamei*

Table 5: The antioxidant activity of *Penaeus vannamei* shells determined by 1,1-diphenyl-2-picrylhydrazyl assay shows the activity is dose dependent

Concentration (µg/ml)	Standard ascorbic acid	Sample extract
10	26.74±0.77	42.47±1.38
20	49.50±1.34	56.82±0.72
50	80.60±1.21	78.38±0.77
100	89.80±1.24	86.32±0.78
200	91.46±0.31	88.85±0.11

Table 6: The antioxidant activity of *Penaeus vannamei* shells determined by Nitric oxide scavenging Assay shows as the concentration increases activity also increases

Concentration (µg/ml)	Standard Ascorbic acid	Sample extract
10	16.03±0.91	21.05±0.88
20	40.46±1.03	47.29±1.17
50	67.27±0.53	79.46±1.10
100	84.34±0.52	85.55±0.78
200	92.6±0.77	90.53±0.12

Table 7: The antioxidant activity of *Penaeus vannamei* shells determined by Superoxide anion scavenging assay shows the good scavenging activity

Concentration (µg/ml)	Standard Ascorbic acid	Sample extract
10	13.42±1.05	7.61±0.98
20	28.50±1.91	19.02±1.93
50	52.27±1.89	33.04±1.32
100	64.73±1.38	44.80±1.86
200	82.69±1.23	67.45±1.66

Table 8: The antioxidant activity of *Penaeus vannamei* shells determined by Hydrogen peroxide scavenging Assay shows the good scavenging activity near to the standard

Concentration (µg/ml)	Standard Ascorbic acid	Sample extract
10	23.57±0.71	33.33±1.84
20	38.37±1.36	48.87±1.84
50	62.84±1.11	58.16±0.89
100	73.31±1.12	78.11±1.11
200	85.28±1.71	82.92±0.83

whole prawn content (10.18%) and in the flesh (8.41%).[17] The maximum level of carbohydrate was detected in the shell + head of *Penaeus notialis*. Quinones likewise show its quality by the positive outcome. Quinones confer cytotoxic activity through the impedance of DNA and RNA replication and mitochondrial oxidative pathways.[18] Flavonoids have antimicrobial, antiviral, antioxidant, and spasmolytic activity. Flavonoids and tannins additionally demonstrate the progressive results for their presence. Qualitative analysis shows the lack of alkaloids, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, steroids and phytosteroids, phlobatannins, and anthraquinones.

Quantitative analysis of flavonoids

Flavonoids have created an incredible passion since it has promising profitable consequences for human health in invading diseases. Some of the major advantageous purposes of flavonoids are anti-inflammatory, antioxidant, anticancer, antiviral, and antibacterial. Flavonoids have been recognized to possess a cyto-protective effect on coronary and vascular systems, liver, and pancreas. These distinct features of flavonoids put them as favored biochemical among the natural products.[19] The flavonoid content in the extract was found to be 5.994 ± 0.044 mg/g. Quantitative analysis of flavonoid in the extract was predicted in Table 2 and Figure 2.

Quantitative analysis of tannin

Tannins have additionally demonstrated potent antibacterial and antiviral impacts.[20] Tannins are the polyphenolic compound happens naturally with high mass to form complexes with the proteins and they are common among the natural sources. It varies from other phenolic compounds by their capacity to precipitate proteins such as gelatin from solution. Tannins mainly act as antiviral, antibacterial, antiulcer, and antioxidant agents. The tannin content in the extract was 49.2 ± 0.084 mg/g which was revealed in Table 2 and Figure 3.

Gas chromatography-mass spectrometry analysis

In the present study, 40 compounds have been identified from ethyl acetate extract of the shells of *P. vannamei* by GC-MS analysis. The chromatogram obtained is shown in Figure 4. The active principle, area of the peak, concentration (%), and retention time (RT) are presented in Table 3. The bioactivity of identifying components with their structure is presented in Table 4. The predominant compounds were cholesterol (58.92%), cholesteryl hydrogen phthalate (58.92), ethyl benzene (18.08%), octadecane, 3-ethyl-5-(2-ethylbutyl) (4.17%), acetyl iodide (4.00%), ethiosuximide (3.85%), dodecane, 5,8-diethyl (2.29%), silane, trichlorooctadecyl (1.88%), 11-tricosene (2.24%), acetic acid, cyano (1.81%), heptadecane, and 9-hexyl- (2.29%).

Antioxidant activity

The antioxidant activity of the ethyl acetate extract of *P. vannamei* shells was detected by DPPH assay and IC_{50} values are calculated as 22.6 μg/ml for standard and 15.03 μg/ml for sample. The percentage inhibition of scavenging activity is revealed in Table 5 and Figure 5. The quenching effect of nitric oxide by sample extract of shows good inhibition activity and it is presented in Table 6 and Figure 6. The IC_{50} value for the standard was 30.97 μg/ml and for the sample extract was noted to be 24.42 μg/ml. The sample extract shows low scavenging activity when compared to the standard which was predicted by superoxide radical scavenging activities which were exposed in Table 7 and Figure 7. Hydrogen peroxide is a weak oxidizing nonreactive agent and has an ability to across cellular membranes. The involvement of hydrogen peroxide in the generation of hydroxyl radicals plays a prominent role in initiating cytotoxicity. The scavenging of H_2O_2 by the ethyl acetate extract of the sample is tabulated in Table 8 and presented in Figure 8. IC_{50} values are 33.09 μg/ml for standard and 27.6 μg/ml for the sample extract.

DISCUSSION

An extensive proportion of biological substance includes carbohydrates, mainly cellulose in the earth. Heidelberger *et al.*, 1950 reported the biological functions of carbohydrates from his studies on the capsular antigens of different strains of *Streptococcus pneumonia*.[21] Carbohydrates are the significant basis of energy used by living things which can also act as structural components in the plant and bacterial cell wall shows its existence by qualitatively in this study.

Currently, the tannins have intent scientific interest and are utilized in many industries, specifically dyestuff industry, and in the food industry.[22] In Asian (Japanese and Chinese) medicinal field, tannins are used as a natural curating agent and also they are used as astringents. Tannins also proved to be good anti-inflammatory, antiseptic agents, and hemostatic pharmaceuticals.[23]

Flavonoids are the active bio-compound synthesized from phenylalanine.[24] Flavonoids play a key role in the handling of various diseases such as cardiovascular diseases, cancer and disorders of duodenal and gastric ulcers, vascular fragility, allergies, and viral and bacterial infections.[25] Thus, this study shows the quantitative analysis of tannins and flavonoids in enough amount which can be further utilized for therapeutic purposes. Saponins are the strong antifungal agent which was reported by Cook & Samman 1996.[26] Chen and Chen, 1997 describes that flavonoids can improve the respiration and metabolism rate in *Penaeus japonicas* when exposed to the concentration of 20 mg saponins/l for 24 h.[27]

The GC-MS analysis of the *P. vannamei* shell shows the various numbers of compounds. Some of them are timonacic which has the powerful antioxidant property,[28] octadecane, 3-ethyl-5-(2-ethylbutyl) which acts as the antifungal agent,[29] and acetamide which possesses antioxidant and anti-inflammatory property.[30] Ethiosuximide is used in the congenital adrenal hyperplasia treatment.[31] 2,3-hexane dione acts against neuroblastoma cells,[32] and 1-tricosanol is stated to possess antifungal and antibacterial properties.[33] Cycloheptasiloxane possesses antimicrobial property.[34] Dodecane is used in tetany, pulmonary edema, and muscle weakness treatment.[35] Clocortolone pivalate is used to treat skin conditions such as eczema, dermatitis, allergies, and rashes.[36] Cholesterol is required for normal cell growth and repair of tissue, which is found to be present in the high peak area.[37]

Many antioxidant potentials have been examined from the natural product which is a fast-growing research area and is done by various methods. Thus, the results of different antioxidant assays such as DPPH assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, and hydrogen peroxide scavenging assay show that *P. vannamei* shells possess good scavenging activity. Figure 5 and Table 5 show the total antioxidant scavenging potential of the sample by DPPH assay which was found to be good in quenching activities. DPPH assay is very sensitive and can detect active ingredients at very low concentrations.[38] Nitric oxide (NO) is an effective inhibitor of physiological processes which predicts the good inhibitory activity of the sample. During the nitric oxide assay, $Na_2[Fe(CN)_5NO]_2H_2O$ decomposes in aqueous solution at physiological pH producing NO, making it an ideal assay to mimic the human body system in scavenging the free radical.[39] During this assay, nitrite is formed when NO generated from $Na_2[Fe(CN)_5NO]_2H_2O$ reacts with oxygen. Hence, it can be deduced that the plant fractions inhibit nitrite formation by directly competing with oxygen and other nitrogen oxides such as NO.[40] Table 7 and Figure 7 show the inhibitory effect of the crude ethyl acetate extract which possesses the scavenging activity of superoxide radicals at various concentrations. Scavenging activity of hydrogen peroxide, at various concentrations, is plotted in Figure 8. The inhibition power of *P. vannamei* shells was increased with the increasing concentration.

By interpreting these bioactive compounds, it shows that *P. vannamei* shells possess various therapeutic uses. Furthermore, research investigation is needed to define the nature's impact and synergy between the identified bioactive compounds and other dietary components to determine its capability in decreasing the risk of cancer and other human health issues. This study result predicts that *P. vannamei* shell possesses numerous bioactive components and they comprise many medicinal values which can be sequestered and utilized in future to get the novel drug with less toxic and at reasonable cost from cheap and easily available source.

CONCLUSION

Every year, 60,000–80,000 tons of waste are made by the shellfish industry. The proper utilization of these bio wastes will provide us valuable by-products. Drug discoveries of marine species have an important research field for decades. Nowadays, keen interest is increased in assessing the marine products to detect the new potential disease preventive drugs with no side effects. Thus, the present study reveals that the *P. vannamei* shells contain the range of biological active molecules which can be utilized as a source of antibiotics.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sawadogo WR, Schumacher M, Teiten MH, Cerella C, Dicato M, Diederich M, *et al*. A survey of marine natural compounds and their derivatives with anti-cancer activity reported in 2011. Molecules 2013;18:3641-73.
2. Abdulla O, Ayse O, Meylut A, Gozde G, Jelena M. A comparative study on proximate, mineral and fatty acid compositions of deep sea water rose shrimp (*Parapenaeus longirostris*, Lucas, 1846) and red shrimp (*Plesionika martia*, A. Milne Edwards, 1883). J Anim Vet Adv 2009;8:183-9.
3. Jayalakshmi M, Vanitha V, Amudha P, Pushpabharathi N. Assesment of minerals from shell waste of *Penaeus indicus*. Int J Res Pharm Sci 2017;8:194-7.
4. Connor WE, Neuringer M, Reisbick S. Essential fatty acids: The importance of n-3 fatty acids in the retina and brain. Nutr Rev 1992;50:21-9.
5. Arvanitoyannis IS, Kassaveti A. Fish industry waste: Treatments, environmental impacts, current and potential uses. Int J Food Sci Technol 2008;43:726-45.
6. Heu MS, Kim JS, Shahidi F. Components and nutritional quality of shrimp processing by-products. Food Chem 2003;82:235-42.
7. Briggs M, Funge Smith S, Subasinghe R, Philips M. Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. RAP Publication. 2004;10:92.
8. Neethu PV, Suthindhiran K, Jayasri MA. Antioxidant and antiproliferative activity of *Asparagopsis taxiformis*. Pharmacognosy Res 2017;9:238-46.
9. Harborne JB. Phytochemical Methods – A Guide to Modern Techniques of Plant Analysis, and Ed. London: Chapman and Hall; 1984. p. 4-16.
10. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
11. Amadi BA, Agomuo EN, Ibegbulem CO. Research Methods in Biochemistry. Vol. 31. Owerri, Nigeria: Supreme Publishers; 2004. p. 50-9.
12. Srinivasan K, Sivasubramanian S, Kumaravel S. Phytochemical profiling and GC-MS study of Adhatoda vasica leaves. Int J Pharm Bio Sci 2013;5:714-20.
13. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-200.
14. Liu F, Ooi VE, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sci 1997;60:763-71.
15. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR, *et al*. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982;126:131-8.
16. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10:1003-8.
17. Ravichandran S, Ramesh Kumar G, Rosario Prince A. Biochemical composition of shell and flesh of the Indian white shrimp *Penaeus indicus* (H. milne Edwards 1837). Am Eurasian J Sci Res 2009;4:191-4.
18. Solanki R, Khanna M, Lal R. Bioactive compounds from marine actinomycetes. Indian J Microbiol 2008;48:410-31.
19. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio RG, *et al*. Flavonoids: Prospective drug candidates. Mini Rev Med Chem 2008;8:1429-40.
20. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother 2001;48:487-91.
21. Heidelberger M, Dilapi MM, Siegel M, Walter AW. Presence of antibodies in human subjects injected with pneumococcal polysaccharides. J Immunol 1950;65:535-541.
22. Hatano T, Yazaki K, Okonogi A, Okuda T. Tannins of *Stahcyurus* species II praccoxins A, B, Cand D four new hydrolyzable tannins from leaves of *Stachyurus praecox* leaves. Chem Pharm Bull 1991;39:1689-93.
23. Saijo R, Nonaka G, Nishioka I. Tannins and related compounds. LXXXIV. Isolation and characterization of five new hydrolyzable tannins from the bark of *Mallotus japonicus*. Chem Pharm Bull (Tokyo) 1989;37:2063-70.
24. Harborne JB, Turner BL. Plant Chemosystematics. London: Academic Press; 1984.
25. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995;22:375-83.
26. Cook NC, Samman S. Review: Flavonoids chemistry, metabolism, cardio protective effects, and dietary sources. J Nutr Biochem 1996;7:66-76.
27. Chen JC, Chen KW. Oxygen uptake and ammonia-N excretion of juvenile *Penaeus japonicus* during depuration following one-day exposure to different concentrations of saponin at different salinity levels. Aquaculture 1997;156:77-83.
28. Reynolds JE. Martindale: The Extra Pharmacopoeia. 31st ed. London: Royal Pharma ceutical Society; 1996. p. 621.
29. Abubacker MN, Kamala Devi P. *In vitro* antifungal efficacy of bioactive compounds heptadecane, 9- hexyl and octadecane, 3-ethyl-5-(2- ethylbutyl) from *Lepidagathis cristata* willd.(*Acanthaceae*) root extract. Eur J Pharm Med Res 2015;2:1779-8.
30. Sashidhara KV, Palnati GR, Dodda RP, Sonkar R, Khanna AK, Bhatia G, *et al*. Discovery of amide based fibrates as possible antidyslipidemic and antioxidant agents. Eur J Med Chem 2012;57:302-10.
31. Yau M, Rao N, Nimkarn S, Vogiatzi M. Effect of ethosuximide on cortisol metabolism in the treatment of congenital adrenal hyperplasia. J Pediatr Endocrinol Metab 2014;27:549-54.
32. Zilz TR, Griffiths HR, Coleman MD. Apoptotic and necrotic effects of hexanedione derivatives on the human neuroblastoma line SK-N-SH. Toxicology 2007;231:210-4.
33. Tayade AB, Dhar P, Kumar J, Sharma M, Chauhan RS, Chaurasia OP, *et al*. Chemometric profile of root extracts of *Rhodiola imbricata* edgew. With hyphenated gas chromatography mass spectrometric technique. PLoS One 2013;8:e52797.
34. Sheeba Gnanadeebam D, Viswanathan P. GC-MS analysis of phytocomponents in *Spermacoce articularis* L.f. leaf. Res Pharm 2014;4.
35. Babu, Johnson M, Raja DP, Arockiaraj AA, Vinnarasi J. Chemical constituents and their biological activity of *Ulva lactuca* linn. Int J Pharm Drug Anal 2014;2:595-600.
36. Del Rosso JQ, Kircik L. A comprehensive review of clocortolone pivalate 0.1% cream: Structural development, formulation characteristics, and studies supporting treatment of corticosteroid-responsive dermatoses. J Clin Aesthet Dermatol 2012;5:20-4.
37. Incardona JP, Eaton S. Cholesterol in signal transduction. Curr Opin Cell Biol 2000;12:193-203.
38. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014;22:296-302.
39. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87:315-424.
40. Boora F, Chirisa E, Mukanganyama S. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. J Food Process 2014;7.