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Seasonal Variation of Biochemical Composition of *Penaeus Semisulcatus* (Decapoda: Penaeidae) and the Effect of Its Shell Extract on Bacteria, Fungi and Cancer

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ABSTRACT

Background: shrimps are extremely good source of high nutritive value making them a very healthy choice for human food. **Aim of the work:** to examine the changes induced by seasonal variation in *Peanuts semisulcatus* biochemical composition and nutritive value. The present work aimed also to screening of crude shell extract for bioactivity and economic importance. **Materials and methods**: The proximate composition and biochemical constituents were analyzed seasonally in the muscle tissue of *Penaeus semisulcatus*. The Crude protein, lipid, carbohydrate, calorific value, moisture and ash were quantified in the Penaeid Prawn. Extraction, isolation, and identification of some bioactive marine natural products were done for study its effect on bacteria, fungi and Larynx cancer. **Results**: there is seasonal variation of the nutritional status of edible Portion of prawn. Statistical analysis for the measured results of the correlation values for each of measurements with the seasons of the year. The correlation was a positive weak. The results of Antimicrobial activity of *Penaeus semisulcatus* shell extract indicated a significant effect differences between the groups treated with shell extract (1.5, 2.5, and 5 mg/mL) compared to the control group. Also, evaluation of viability of Hep -2 cell line post treatment with shell extract of *Penaeus semisulcatus* using Sufranin uptake assay as (M T T) uptake and Microscopic examination indicated a significant difference between the groups treated with shell extract compared to the control group. Cell viability decreased depending on dose or concentration. The shell extract inhibited the proliferation of a Larynx cancer cell line in a dose dependent manner.

Conclusion: the results of the present study reported that, the nutritional values of shrimp muscle constituents were different from season to the other, with positive weak correlation. Also, Shell extract can be used as antibacterial, antifungal and anticancer.

Keywords: Seasonal variation - edible Portion - Proximate composition -seafood – Marine biotechnology- Marine ecology - biochemical composition - *Penaeus semisulcatus* - Decapoda –Benthos - Shell - extract – Bacteria- Fungi – Cancer.

INTRODUCTION

Marine food constitutes an important part of the diet and health of the world's population [(1)]. Various indigenous allergenic or toxic nitrogen compounds can be found. The occurrence of these compounds is not only species characteristic but is also affected by environmental and seasonal factors. Both aspects have been extensively investigated during the recent two decades[(2)]. Marine invertebrates are an important part of our diet as they contribute to the intake of health[(3)] . Marine natural products have also been submitted to general screening assays, as exemplified by the search for anticancer compounds, anti-AIDS compounds, anti-biotics, and enzyme inhibitors.Despite the current interest in bioactive marine compounds, our knowledge is limited because of the short history of this area of research Moreover, the difficulties associated with the collection and isolation [(4,5,6)]. Also the identification of new therapies is still required for treating cancer

So that the ***aim of this work* is**: to identify the changes induced by seasonal variation in *Penaeus semisulcatus* biochemical composition and nutritive value.The present work aimed also to study extraction, isolation, and identification of some bioactive marine natural products and screening of crude extracts for bioactivity to determine its effects on bacteria, fungi and Larynx cancer (HepII CelL line).

MATERIALS AND METHODS

Sampling :

For the present work, seasonally sampling was carried out for *Penaeus semisulcatus*, during a period of one year, from January 2012 to January

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2013 , along Suez Canal and the Northwest coast of Suez Gulf (Suez Governorate). specimens stated as XL size were taken Fresh from fishermen when they reach the shore, or were from different fishing centers . The collected specimens were transported to the laboratory to study them.

Identification of benthic crustaceans species:

For identification, the following reference were consulted: FAO species identification sheets for fishery purposes [(7)] .

Determination of total protein:

The total protein was determined using the Folin-Cioclten method described by Lawery *et al.* [(8)] with its modification suggested by Ansell and Lander [(9)].

Determination of total lipid:

Lipids determination was performed according to the method described by Knight *et al.* [(10)] .

Determination of carbohydrate content:

Glycogen was measured according to the method of Carrol, *et al.* [(11)].

Calculation of calorific value:

The calorific content for each sample (stage) has been calculated in each case for the biochemical composition by multiplying each component by the appropriate calorific equivalents (4.2 kcal for carbohydrates; 9.45 kcal for lipids and 5.7 kcal for protein). The results were expressed as kcal per gram[(1)] .

Water Content :

By weight loss at 105 ºC until constant weight. According Ruiz, Roso *et al.* [(12)].

Ash content :

By incineration in a muffle furnace at 450 – 500 to constant weight. According AOAC [(13)] .

Statistical analysis :

To study the seasons of the year and all of protein, lipid, carbohydrate, calorific value, water content, and ash content of *Penaeus semisulcatus* , the data were analyzed by using the coefficient of association which is used to show how strong a relationship between two variables is; a value of 0 indicates no relationship, whereas a value of, normally, 1 represents the maximum (a few coefficients have a maximum lower than 1, some can exceed 1 in particular conditions). Coefficients meant for ordinal or higher levels of measurement are *signed* to indicate a positive or negative association (direction of the relationship): Lahcene [(14)] · Note: All analyses were carried out in triplicate. The results were expressed as mean values ± standard deviation.

Bioactivity studies

Preparation of the volatile constituents :

The volatile constituents were obtained by hydro distillation extract using the E.P apparatus. The distill oil was extracted with ether after saturation with sodium chloride. Ether extract was dehydrated.

Over anhydrous sodium sulphate.solvent was removed under reduced pressure at low temperature, .Oil was kept in cold place in dark container tell analysis.According to Masada [(15)].

Identification of the constituents:

Qualitative identification of different constituents were done by TLC,Silica gel using Benzene EtAc(93:7) for one dimentional TLC and CHCL3 EtAc(93:7) then Benzene:EtAc(93:7) for two dimentional TLC.VanilineH2SO4. 1% was used as a spray Reagent.GC/MS was done using the apparatus GC/MS system.

Anti-microbial activity :

Most of the synthesized compounds were evaluated for their anti-microbial activity using the agar diffusion technique . According Cooper [(16)] .

Microbial Organisms :

Escherichia coli (NCTC10418):

gram –ve straight rods, occurs in the lower part of the intestine .

***Bacillus subtilis* (NCIMB8054):**

Endospore-forming, gram +ve rods.

Klebsilla pneumonia :

gram –ve bacteria ,straight rods and normal in habitants of the intestinal tract and causes the urinary respiratory tract infection .

Pseudomonas aeruginosa :

gr. –ve rods , often can cause respiratory and urinary tract infection.

***Staphylococcus aureus* (ATCC 29737):**

gram +ve , potential pathogen causing a wild range of infections e.g. pneumonia, various abscesses.

Bacillus pumilus :

Gram positive (gram +ve) bacteria , rods shape ,endospore forming .

Micrococcus luteus :

Gram positive (gram +ve) bacteria , cocci primary habitat is mammalian skin.

***Candidal albicans* :**

Yeast –like microorganism pathogen causes Candidasis .

***Aspergillus niger* :**

Multi-cellular fungi ,causes disturbance in respiratory system and lung .

***Aspergillus flavus*:**
Multi-cellular fungi ,causes disturbance in respiratory system and lung .
The inoculum prepared from a typical colony grown overnight on nutrient agar. Colony sampled less than 24 hours old transfer the growth to a tube of sterile saline.

3- Anti-Cancer Activity :

CELL CULTURE TECHNIQUES

This methods carried according to Mohamed *et al.*[17] for making **Subculture of a cell line:** Hep II cell line (Larynex Cancer).

RESULTS

Seasonal variation of biochemical composition of *Penaeus semisulcatus*

Table (1) demonstrates the protein, lipid and carbohydrate contents (g /100 g tissue) of *Penaeus semisulcatus*.

The data showed that the protein contents differed from season to another. The highest value was 23 g /100 g observed in spring season**.**
But the lowest one was recorded in winter season being 16.6. g/100 g. While recorded 22.6 and 20.3 g/100 g, for summer and autumn respectively.
Regarding lipid content The highest value was calculated in summer season (1.7 g/100 g) , while the lowest one was measured in winter season (0 .8 g/100 g) . While recorded (1.3 and 1 g/100 g) for spring and autumn respectively.
On the other hand the highest value of carbohydrate was recorded in spring season (2 .9 g/100 g), but the lowest one was calculated in winter season (0.2 g/100 g). It recorded (0.9 and 0.5 g/100 g) for summer and autumn respectively.

In the same time, the data of Calorific Value (K.cal / 100g) showed that the values differed from season to another. The highest value was 155.6 (K.cal / 100g) observed in spring season**.** But the lowest one was recorded in winter season being 101.6 (K.cal / 100g). While recorded 144.8 (K.cal / 100g) and 126 (K.cal / 100g) for summer and autumn respectively.
Table (1) demonstrates the water and ash contents (g /100 g) of *Penaeus semisulcatus*. Regarding water content, the highest value was calculated in winter season (75.2 g/100 g), while the lowest one was measured in summer season (70 g/100 g) . It recorded (73 and 72 g/100 g) for spring and autumn respectively.

On the other hand the highest value of ash was recorded in winter season (1 .9 g/100 g), but the lowest one was calculated in autumn season (1 .2 g/100 g). While recorded (1.5 and 1.7 g/100 g) for spring and summer respectively.

Statistical analysis :

Table. (1). shows the measured results of the correlation values for each of them with the seasons of the year. As shown in Table.1. The correlation was a positive weak correlation. Therefore the presence of different amounts of protein, lipid, carbohydrate, calorific value, water content, and ash did not depend on the change in different seasons only of the year.

Biochemical compounds from G C-Mass spectrometry:

The result in table (2 & 3): recorded the chemical Compound (by G C-Mass spectrometry analysis) *of Penaeus semisulcatus* shell extract, which extracted through hydro distillation, and consist of volatile constituents.

Antimicrobial Activity

Antimicrobial activity of *Penaeus semisulcatus* shell extract:

The result in table (4) indicated a significant difference between the groups which treated with shell extract (1.5, 2.5, and 5 mg/mL) compared to the control group.

The result shows the this extract of *Penaeus semisulcatus* has high antibacterial activity against tested bacterial (*Klebsilla pneumonia*) and *Bacillus subtitles*. While has moderate effect on *Micrococcus latus, , Pseudomonas aeruginosa, staphylococcus aureus and Candidal albicans* . In the same time , weak effect of the tested extract appear with tested bacteria (*Escherichia coli* and *Bacillus pumilus).*

This extract has weak effect with tested fungus (*Aspergillus niger*), and has no effect on (*Aspergillus val*es).

The effect of *Penaeus semisulcatus* shell extract on (Hep 2) cell line (Microscopic examination):

The effect of *Penaeus semisulcatus* shell extract on larynx Cancer cell line (Hep 2) through culture assay (Table:5) indicated a significant difference between the groups treated with shell extract (20, 40, 80, and 160,320 μg/mL) compared to the control group.

The results revealed that the effect of *Penaeus semisulcatus* shell extract was clear especially with dilution 20, while with dilution 40 the effect of *Penaeus semisulcatus* shell extract was good, in the same time the effect of *Penaeus semisulcatus* shell extract was weak with dilution 80. In spite of the above mentioned results, the *Penaeus semisulcatus* shell extract don't affect on (Hep 2)

Cancer larynx cell line through culture assay with another dilution specially with 160 and 320 dilutions, (table:5 & Figure:1) .

Tissue Culture Toxic Dose 50 (mg) (The concentration of inhibition or toxicity)

The result revealed that toxicity of biological extracts using cell culture assay revealed that the effect of *Penaeus semisulcatus* shell crude extract showed toxic (or inhibition) effect on larynx cancer cell line (Hep 2).

The toxic (or inhibition) concentration varied according to the sample; the toxic concentration for larynx cancer cell line (Hep 2) was 1.52 mg through the shell crude extract of *Penaeus semisulcatus*.

Evaluation of viability % of Hep -2 cell line post treatment with studied Crustacean extracts using Sufranin uptake assay as (M T T) uptake:

The effect of biological extracts on larynx cancer cell line (Hep 2) through culture assay (by the viability of cell) (table 6 & fig. 2) indicated a significant difference between the groups treated with shell extract (20 , 40, 60, 80, and 160,320, 640, 1280 and 2560 µg/mL) compared to the control group.

The evaluation revealed that the effect of *Penaeus semisulcatus* shell extract was clear specially with dilution 20 , while with dilution 40 the effect of *Penaeus semisulcatus* shell extract were good, in the same time the effect of *Penaeus semisulcatus* shell extract was weak with dilution 80, (through the range of viability of cell). In spite of the above mentioned results, the *Penaeus semisulcatus* shell extract don't affect on (Hep 2) cancer larynx cell line through culture assay with another dilution specially with 160 and 320 dilutions, through culture assay, i.e. Cell viability decreased depending on dose or concentration.

DISCUSSION

The knowledge about *Penaeus semisulcatus* (Decapods: Penaeidae), is very important from the scientific point of view. Consequently, this animal plays an important role in the food chain, the diet and health of the population.

There is alteration in environmental condition of sea that influences all physical, chemical and biological processes [18,19] .

Abd-Elaziz[3] stated that the alteration of the environmental factors in marine habitat affect on biochemical composition of marine animals.

Regarding seasons , The fluctuations in the crustaceans might related not only to water temperature but also to its indirect influences on their food items [20] . It was noticed that , the percentage of each food item taken by the studied species was changed from season to another . This agrees Farina *et al*.[21] who reported that the feeding of some species can exert strong effects on ecosystem process .

These changes are due to fluctuation of temperature or Salinity,[22] then the changes in food composition of the studied species reflect the changes in the availability of food type [23].

The present study appeared that there is differentiation in biochemical composition of *Penaeus semisulcatus* edible portion, which differed from season to another.

Studied *Penaeus semisulcatus*, have high ratios of total protein than total lipid and carbohydrates. This is in agreement with Amer *et al*.[24] Hashem [25] and Abd-Elaziz [1].

Regarding biochemical composition of *Penaeus semisulcatus* the data showed that the protein contents differ from season to another, the highest value was observed in spring season**,** but the lowest one was recorded in winter season.

The mentioned decrease in protein levels, perhaps owing to moulting, ovarian development and egg formation, regeneration for the lost limbs and deposition of protein in chitin which perform the carapace. This agrees with the results of Highnam and Hill [26] and Subramoniam[27].

The comparison between total protein and total lipid levels of *Penaeus semisulcatus* showed that the later were the lowest . This is in agreement with Hashem [25] And Venugopal and Shahidi [28] whom recorded that the total lipid is low in crustacean Regarding lipid content The highest value was calculated in summer season, while the lowest one was calculated in winter .

The changes of lipid levels may be due to the morphological and physiological changes of the studied species .This agrees with , Akpan [29] and Abd-Elaziz [3] .

On the other hand, the highest value of carbohydrate was recorded in spring season , but the lowest one was calculated in winter season**.**

This is in agreement with, Schmitt and Santos [30]. The increase of carbohydrate levels may be due to the high activity (glycogenolysis) and accumulation of carbohydrates in the new tissues of moulted and post-moulted crabs . This is in

agreement with Siu-Ming chan *et al*.[31]. and Abd-Elaziz.[3]

In the same time, the data of Calorific Value (K.cal / 100g tissue) showed that the values differed from season to another. The highest value was observed in spring season**,** but the lowest one was recorded in winter season.

With regard to water content of *Penaeus semisulcatus* the highest value was calculated in winter season, (75.2 g/100 g) , while the lowest one was measured in summer season.

On the other hand the highest value of ash was recorded in winter season, but the lowest one was calculated in autumn season.

This with an agreement with the results of King *et al.* [32] and Naczk *et al.*,[33] which emphasized that these differences are apparently associated with variations between species, nutrient composition of the diet [34], the surrounding medium [35] and other environmental factors (e.g., season, location, substrate, depth, water salinity, temperature and anthropogenic influence) [36, 3].

The effect of season in lipid and nutrient compositions have been studied for some marine animals [37, 38] , but interpretation is difficult and depends on numerous factors [39].

Also with an agreement with the results of this study, The fluctuations in biochemical composition is related mainly to the food composition of marine organisms which are greatly influenced by the changes in environmental factors, particularly temperature, where food intake being lower during colder months [23,40].

Suzuki and Shibrata[41] reported that the chemical composition differ slightly because of the differences in size, age season of sampling. However, seasonal changes in food items were greatly noticed in temperate [23] , as well effects human impacts, pollution and severe physical conditions[42,43] .

Banu *et al.* [44] investigated evaluation of nutritional status of Penaeid Prawns through Proximate Composition Studies, they emphasized that all the Penaeid prawns are supposed to be a source of food for human consumption. Also the variation in Biochemical composition is may be due to enzyme activety during catching shellfish generally are low [6].

As shown in Table.1. The correlation was a positive weak correlation. Therefore the presence of different amounts of protein, lipid, carbohydrate, calorific value, water content, and ash does not only depend on the change in different seasons of the year.

The findings obtained in the present study are similar to those obtained previously, and confirm the findings regarding different circumstances and diversity.

Shrimp is compiled a major portion of the processed seafood industry. Its exoskeleton (shell) and cephalothorax consist of about 30–40% of raw shrimp weight and are discarded as waste [45]. Approximately 60% of the drugs approved for cancer treatment are derived from natural sources [46] . An earlier review of antitumor and cytotoxic compounds from marine organisms which covered the literature into early 1986 was published in 1987 by Munro *et al* . [4]. The primary aim was to include all the marine natural,[47] and Antimicrobials [48, 49] .

Tor Haug *et al.* [50] studied the Antibacterial activity in four marine crustacean decapods, they reported antibacterial activity of different body-parts of shrimp and crab.

In the first record through the present work, crud extract from the shell of *Penaeus semisulcatus* give good effect on microbial organisms (Bacteria and Fungi) : *E. Coli* , *Bacillus subtillus , Klebsilla sp., Pseudomonas aeruginosa , staphylococcus, B.p , M.latus , Candidal , A niger* and *A. Valv.*

El-Mehdawy *et al.* [51] stated that shell extract of shrimp shells (*Penaeus semisulcatus*) contains a considerable content of the chitosan and Chitin which give inhibition effect on bacteria specially (-ve gram), this finding is in line with the results of the present study.

On the other hand, by the present work, crud extract give good effect on Hep II cell line - (Cancer larynx), specially with high concentration.

Leila *et al.* [52] reported that crab shell extract inhibited the proliferation of a breast cancer cell line in a dose and time-dependent manner.

The findings obtained in the present study are similar to those obtained previously, and confirm the findings regarding different cancer cell lines, that were developed in collaboration with marine biologists and ecologists as an integral part of marine natural products chemis

University scientists who study marine natural products have already made some significant contributions toward the discovery of new pharmaceutical agents over the past 20 years [5] .

During the present study, although the results provide detailed information about biochemical composition of *Penaeus semisulcatus* the most common in the Suez Canal, Suez Gulf and the western coast of the Red Sea, in addition the reflection of seasonal variation on the biochemical composition of one marine benthic crustacean which are still virgin and required to more studies and researches.

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RESULTS

(Tables and Figures)

Table (1): Seasonal variation of Biochemical composition of *Penaeus semisulcatus* edible Portion (g / 100g tissue) with statistical analysis.

Season / Composition	*Penaeus semisulcatus*					The coefficient of association	
	Winter	Spring	Summer	Autumn	Annual mean ± SD	Association value	Association degree
Protein (g/100g)	16.5	23	22.6	20.3	20.6±2.9	0.06370495	Positive weak
Lipid (g/100g)	0.8	1.3	1.7	1	1.2±0.4	0.0311293	Positive weak
Carbohydrate (g/100g)	0.2	2.9	0.9	0.5	0.8±0.1	0.09948296	Positive weak
Cal. Value (K.cal / 100g)	101.6	155.6	144.8	126	132±23	0.06056528	Positive weak
W. content (g/100g)	75.2	73	70	72	72.5±2.1	0.04193019	Positive weak
ASH (g/100g)	1.9	1.5	1.7	1.2	1.6±0.3	0.02076564	Positive weak

Biochemical compounds from the analysis with gas chromatography-mass spectrometry

Table (2): the chemical composition of of *P. semisulcatus* shell extract :

N	R . T	Molecular weight	Base Peak	Another Fragment	Compound Name
1-	21.032	188	83.00	41-55-70-96-112	3-methylthio nonanal C10 H20 OS
2-	20.517	144	60	127-115-84-101-41-73-60	Caprylic acid C8 H16 O2
3-	21.025	158	60	141-158-87-129-98-115-41-73-60	Nonanoic acid C9 H 18 O2
4-	22.813	273	43.00	65-69-152-83-193-258-135-273	1,3,5-Triazine.2,4.diamine, N,N-bis(1-methylethyl)-6-(methylsulfonyl) C10H19N5O2S
5-	28.433	297	149.10	41-96-55-69-124-84-167-149-110	6-Nitro-cylohexadecane.1,3-dione C16H27NO4
6-	28.039	324	83	43-55-69-107-137-149-187-167-199	Akuammilan-17-ol,10-methoxy. C20H24N2O2
7-	22.20	222	95.15	150-43-135-81-107-121-55-165-207-177-189-208-22	Alpha.-Cedrol C15H26O
8-	28.430	268	41	137-123-111-97-82-69-55	9-Octadecan -1-ol C 18 H36 O
9-	24.57	228	41	60-73-129 -185-87-97-115 143-228- 157-171-199-212	Tetradecanoic Acid C14H28O2
10-	25.68	284	43.05	284-241-199-185-171-157-143-129-111-97-83-73-55-43	Octadecanoic acid C 18 H36 O2
11-	25.683	242	43.05	73-60-43-129-83-199-97-115-143-185-157	Pentadecanoic Acid C15H30O2
12-	26.833	256	4/3.05	43-73-60-129-83-97-115-143 -157-171-185-213-256-199	Hexadecanoic Acid C16H32O2
13-	28.843	282	43.05	73-55-83-129-97-111-185-143-157-199-171	9- octdecanoic acid C18 H34O2

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The chemical composition of *Penaeus semisulcatus* shell extract.

Table (3): The chemical Compound of *Penaeus semisulcatus* shell extract :

1	**3-methylthio nonanal C10 H20 OS**
2	**Caprylic acid C8 H16 O2**
3	**Nonanoic acid C9 H 18 O2**
4	**1,3,5-Triazine.2,4.diamine, N,N-bis(1-methylethyl)-6-(methylsulfonyl) C10H19N5O2S**
5	**6-Nitro-cylohexadecane.1,3-dione C16H27NO4**
6	**Akuammilan-17-ol,10-methoxy C20H24N2O2**
7	**Alpha.-Cedrol C15H26O**
8	**9-Octadecan -1-ol C 18 H36O**
9	**Tetradecanoic Acid C14H28O2**
10	**Octadecanoic acid C18H36 O2**
11	**Pentadecanoic Acid C15H30O2**
12	**Hexadecanoic Acid C16H32O2**
13	**9- octdecanoic acid C18 H34O2**

Antimicrobial Activity

Table (4) : Antimicrobial activity of *Penaeus semisulcatus* shell extract:

Samples / Test Organisms	Concentrations (mg/ml)		
	1	**2.5**	**5**
Escherichia coli	+	+	+
Bacillus subtillus	++	++	++
Klebsilla pneumonia	+++	+++	+++
Pseudomonas aeruginosa	++	++	++
staphylococcus aureus	++	++	++
Bacillus pumilus	+++	+++	+++
Micrococcus luteus	++	++	++
Candidal albicans	++	++	++
Aspergillus niger	+	+	+
Aspergillus valvus	-	-	-

The effect of *Penaeus semisulcatus* shell extract on (Hep 2) cell line (Microscopic examination):

Table (5) : The effect of *Penaeus semisulcatus* shell extract on (Hep 2) cell line (Microscopic examination):

Dilution	**20**	**40**	**80**	**160**	**320**
The effect of *Penaeus semisulcatus* shell extract on (Hep 2) cell line microscopic examination	+++	++	+	-	-

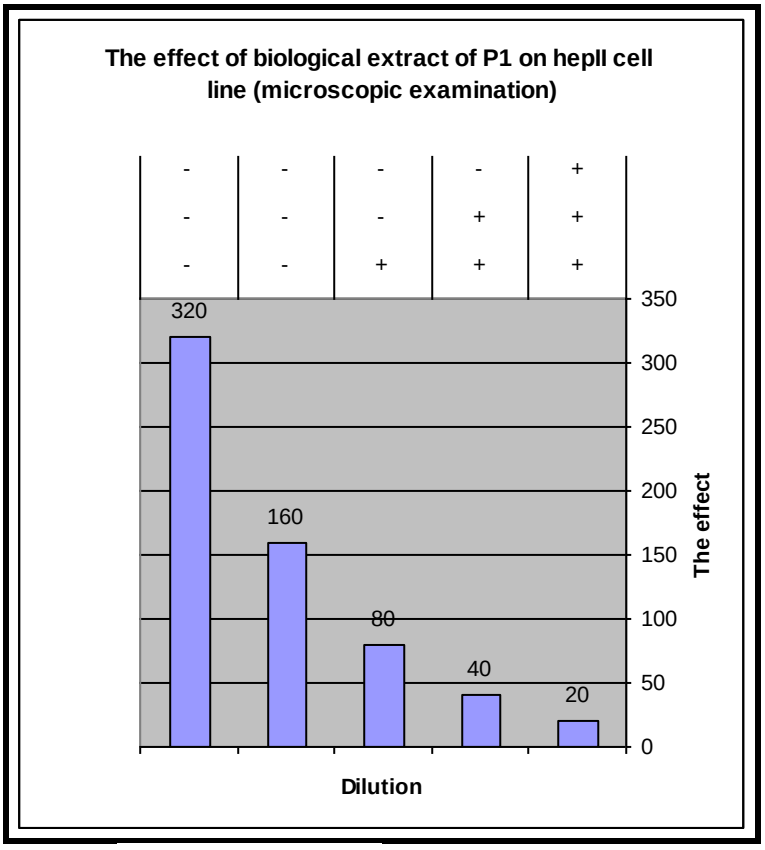

Figure (1): The effect of *Penaeus semisulcatus* shell extract on (Hep 2) cell line (Microscopic examination).

Dilution of *P. semisulcatus* shell extract	viability % of **Hep -2 cell**
1/20	16.7
1/40.	21.1
1/80	98.7
1/160	84.9
1/320	90.9
1/640	97.3
1/1280	98.2
1/2560.	98.4

Table (6): Evalution of vaiability % of Hep -2 cell line post treatment with *Penaeus semisulcatus* crud shell extract using Cell Viability.

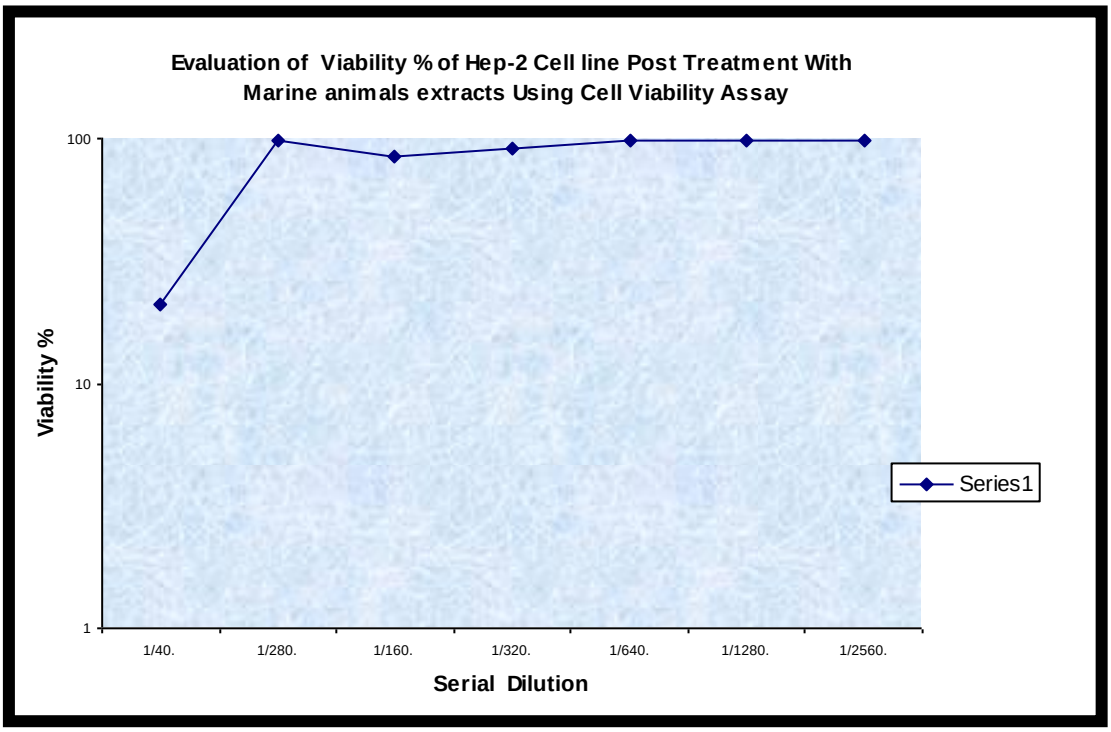

Figure (2): Evalution of vaiability % of Hep -2 cell line post treatment with *Penaeus semisulcatus* shell extract using Cell Viability Assay .

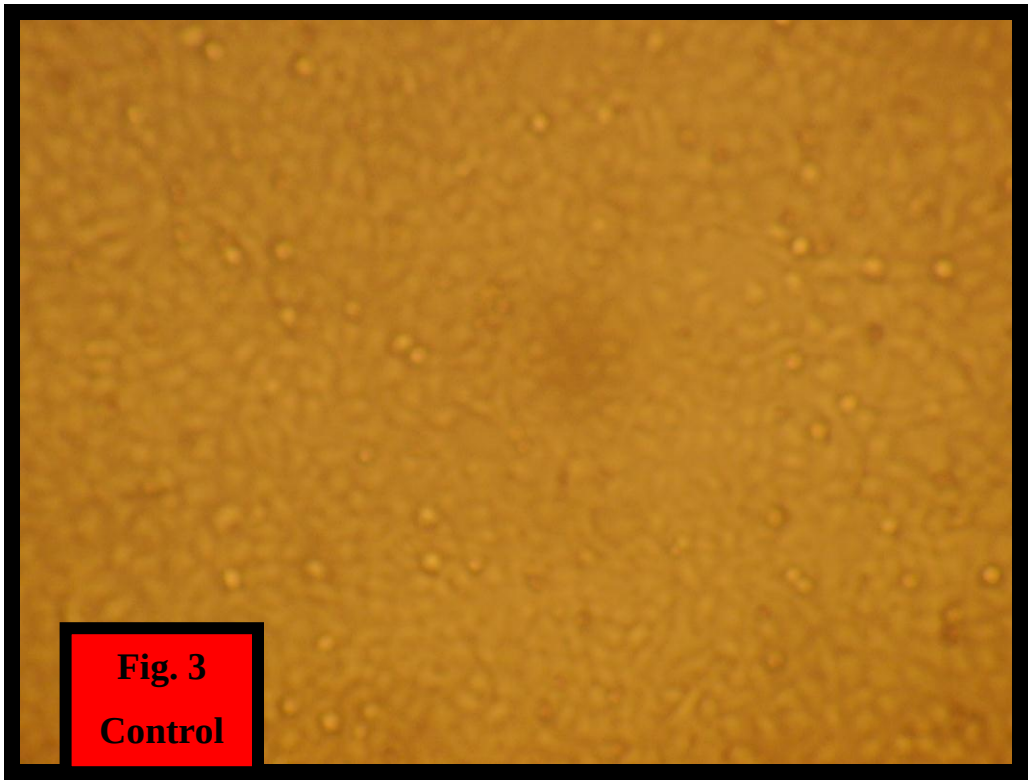

Figure (3): Control of larynx Cancer (Hep -2 cell line) .

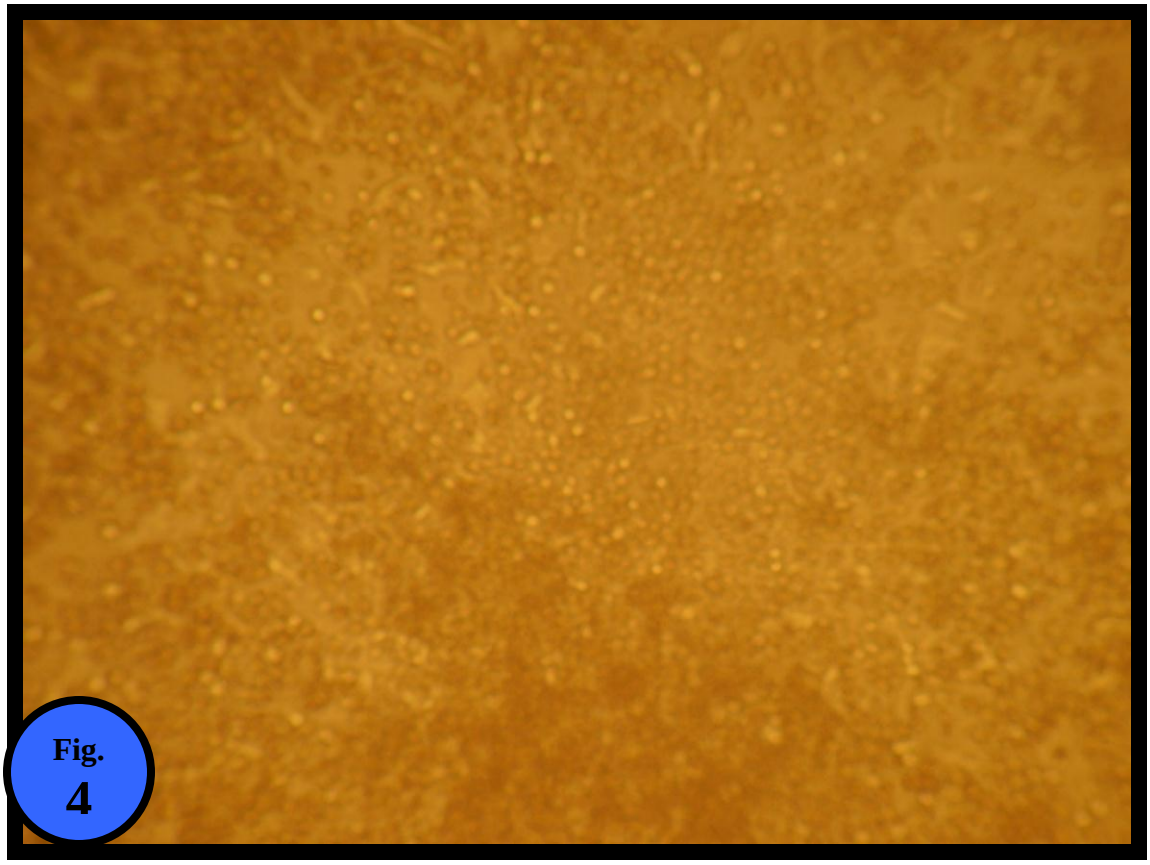

Figure (4) : The effect of *Penaeus semisulcatus* shell extract (dilution 20) on larynx Cancer cell line.

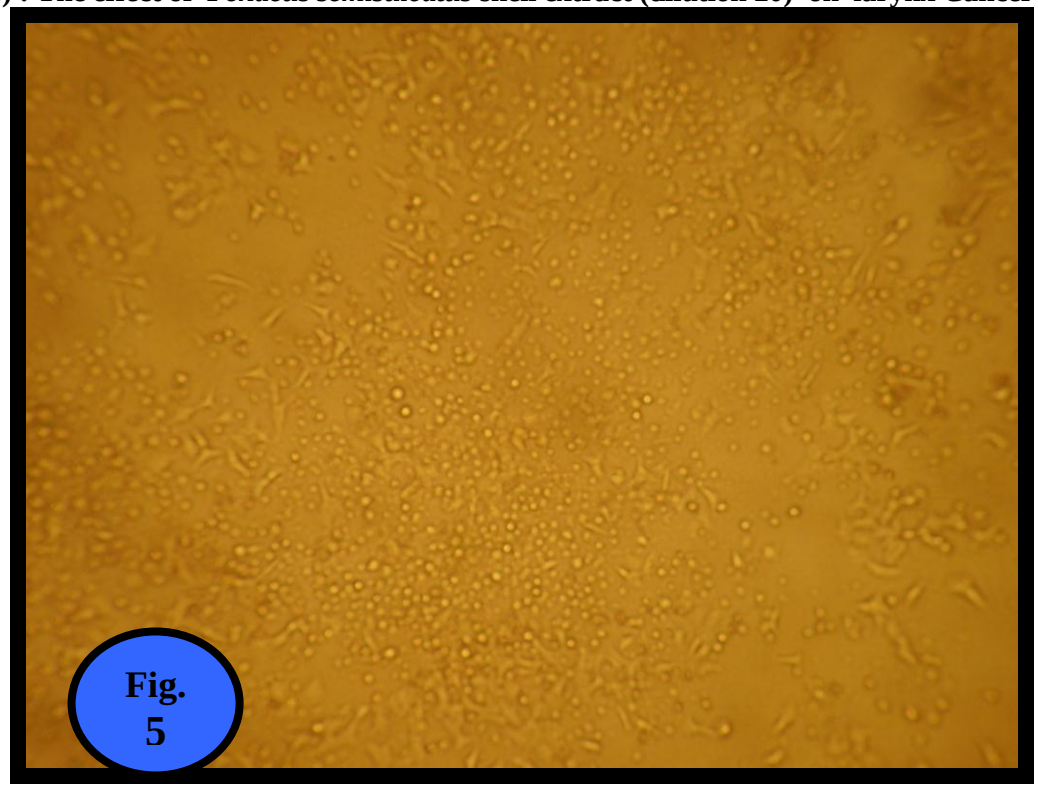

Figure (5) : The effect of *Penaeus semisulcatus* shell extract (dilution 40) on larynx Cancer cell line.

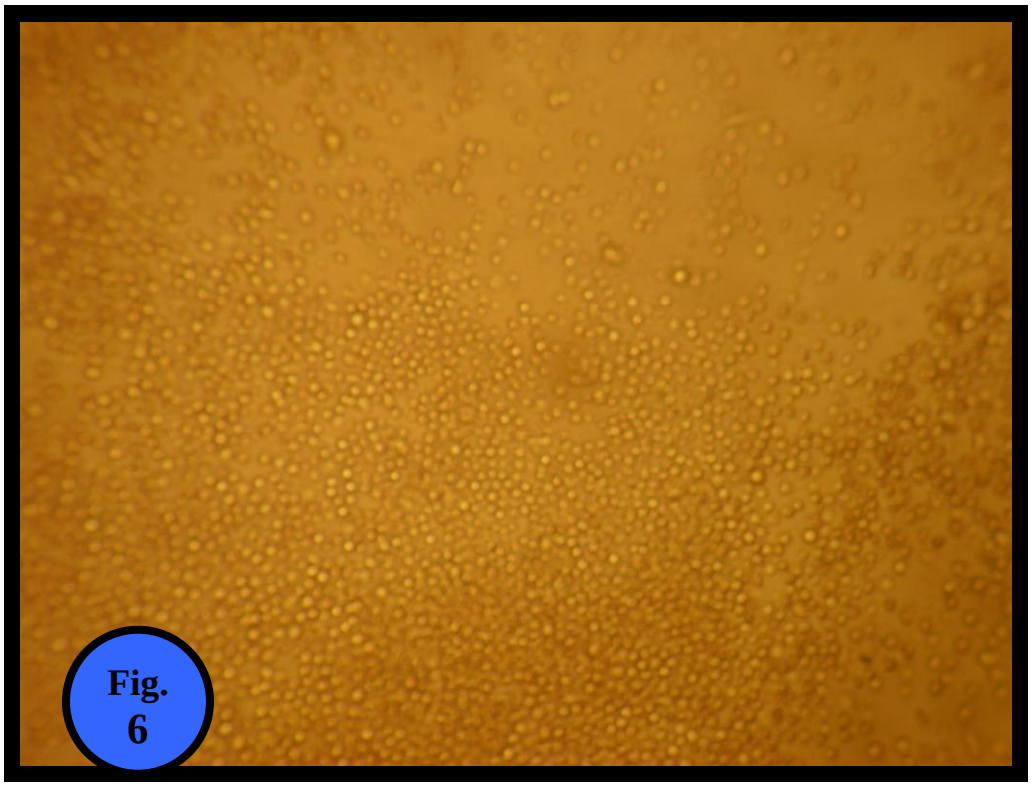

Figure (6): The effect of *Penaeus semisulcatus* shell extract (dilution 80) on larynx Cancer cell line.