

Biometric study, sex ratio and potential biological activities of the edible mantis shrimp *Erugosquilla massavensis*

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ABSTRACT

The edible mantis shrimp *Erugosquilla massavensis* is a popular, cheap, and nutritious food source in the Mediterranean coasts. In the present study, sex ratio, morphometric and weight relationships, and the condition factor of the mantis shrimp were studied. Samples were collected from October to December 2021 from the Mediterranean coast of Port Said. In the current study, females (702) dominated over males (610) with the sex ratio in favor of females (1.15:1). Body length-body weight relationship indicated a negative allometric growth pattern for both sexes ($b < 3$). The findings of this study showed well-being and good health condition for both sexes since the condition factor was higher than one (2.576 and 2.424 for males and females, respectively). In addition, the *in vitro* antiproliferative, antioxidant, and antimicrobial activities of hepatopancreas (HP), muscles (M), and hemolymph (HL) extracts of *E. massavensis* were evaluated. All extracts exhibited a significant cytotoxic effect on the breast (MCF-7) and liver (HepG2) cancer cell lines, especially at higher concentrations. Significant preference for HP was observed on HepG2 cells, with IC_{50} dose of 1.14 mg/ml ($P < 0.001$). However, M extract possessed higher antioxidant properties (49.3%) than HL and HP (37.14% and 26.37%, respectively). Moreover, these extracts showed a high degree of antimicrobial activity, especially against *E. coli* with an inhibition zone of 38, 31, and 31 mm for M, HP, and HL, respectively.

INTRODUCTION

The marine mantis shrimp *Erugosquilla massavensis* (Kosmann, 1880) is a benthic stomatopod crustacean species belonging to Superfamily Squilloidea. *E. massavensis* is a native species to the Red Sea and the Persian Gulf (Froglia & Manning, 1989). However, it migrated through the Suez Canal into the Mediterranean Sea where it was firstly recorded in the Mediterranean coast of Egypt in 1933 and misidentified as *Squilla africana* (Calman, 1917) (Steuer, 1936; Ounifi-Ben Amor *et al.*, 2015). Accordingly, the distribution of this mantis shrimp has expanded along the Mediterranean Sea, and it has been introduced and recorded in different Mediterranean

coasts of Turkey, Italy, Libya, Tunisia, and Greece (**Shakman & Kinzelbach, 2007; Corsini-Foka *et al.*, 2017; Dimitriadis *et al.*, 2019; Gianguzza *et al.*, 2019; Amor & Amor, 2021**).

The stomatopod *E. massavensis* is characterized by 2 raptorial claws with 6 or 7 teeth on each one, telson lacks large black spots with a clear external morphological difference between male and female on thoracic somites (**Abo-Hashesh *et al.*, 2020**). The last pair of walking legs in males bears a pair of penes on the 8th thoracic segment (**Wortham-Neal, 2002**).

The mantis shrimp *E. massavensis* is a common edible marine crustacean distributed along the Mediterranean coast of Port Said, Egypt. Nowadays, it is considered as a commercial valuable constituent of local seafood that possesses a minor yet growing economic importance in the markets (**Hamdi, 2011**). This is due to its nutritional value as its muscles are enriched with proteins, vitamins, amino acids, minerals, carbohydrates, omega-3, and healthy fatty acids (**Hamdi, 2011; Göçer *et al.*, 2018**). The potential economical and nutritional values of this edible stomatopod have been attracted the consideration of several researchers to investigate its biology and ecology worldwide, especially in Egypt. Biological information such as length-weight relationship, sex ratio, condition factor, and length frequency distribution can serve as a database to monitor *E. massavensis* stocks, population dynamics, good exploitation, resource management, and successful culture of this species in the Mediterranean Sea, Port Said, Egypt (**Mon, 2020; Aminisarteshnizi, 2021**). Such parameters indicate the healthiness state and adaptation degree of a species in its environment (**Araújo & Lira, 2012**).

Like invertebrates, mantis shrimps lack adaptive immunity and rely mainly on powerful innate immune system to protect themselves from pathogens (**Sánchez- Salgado *et al.*, 2017; Huang *et al.*, 2020**). Hemolymph and hepatopancreas serve as the main components of immunity in the bodies of crustaceans (**Rószler, 2014**). Hemolymph is a crucial defense line as it contains the major immune cells or hemocytes that perform a variety of immunological functions such as lysis of foreign substances, phagocytosis, the release of humoral proteins and encapsulation (**Wu *et al.*, 2019; Zakzok *et al.*, 2021**). Likewise, hepatopancreas plays vital defensive and immunological roles besides its digestive function. It is the chief organ for xenobiotic detoxification and synthesis of many crucial immune molecules, such as hemocyanin, lectins, stress proteins, antibacterial, and apoptotic peptides (**Ortega *et al.*, 2011; Wei *et al.*, 2020**). Significant anticancer, antibacterial, and antioxidant activities of different extracts of some mantis shrimps species have been reported (**Qi *et al.*, 2013; DeVries *et al.*, 2016; Elkhodary *et al.*, 2017**).

Therefore, the current study is aimed to study some biological information and determine the condition factor and length-weight relationship of the mantis shrimp *E. massavensis* at Port Said. Furthermore, this study is also carried out to assess the potential

cytotoxic, antioxidant, and antimicrobial activities of muscles, hepatopancreas, and hemolymph extracted from this species.

MATERIALS AND METHODS

1. Sample collection

Random samples of the mantis shrimp *E. massavensis* were collected from the Mediterranean coast of Port Said, between October 2021 to December 2021. Live specimens of *E. massavensis* samples were transported in plastic tanks containing ice to the laboratory of the Faculty of Science, Port Said University for further investigation. Sexes were differentiated externally according to presence of male's genital organ (penis). A total of 1312 individuals of *E. massavensis* (610 males and 702 females) were used in this study.

2. Sample measurements

Total body weight (BW) of *E. massavensis* was weighed to the nearest gram (0.001 g) using a digital balance (IIAXIS model ATZ520). In addition, body length (BL) was measured dorsally along the midline from posterior of eyes to end of the telson. Body length was measured to the nearest centimeter.

3. Biometric relationships

3.1. Body length-body weight relationship

This relationship was determined for each sex separately and combined sexes according to the following formula (Hartnoll, 1978):

$$BW = a BL^b$$

Where BW is the total body weight in (g), BL is the total body length (cm), *a* is a constant and *b* is the slope.

3.2. Condition factor

Condition factors (K) of each sex and combined sexes were calculated by the following equation (Hile, 1936):

$$K = 100*(BW/BL^b)$$

Where BW is the total body weight in grams, BL is the total body length in cm and *b* is the power of the body length– body weight equation.

4. Hemolymph, hepatopancreas, and muscles collection

Using fine sterile syringes, hemolymph was collected from the ventral hemal sinus of each mantis shrimp and then mixed with a sodium citrate solution (3:1 v/v, pH 4.6) as an anticoagulant to avoid hemocytes degranulation (Shields, 2017). Following that, the specimens were dissected to collect hepatopancreas and muscles. Then, samples were homogenized on ice with a 50 mM Tris/HCl solution (4 ml/g, pH 8.0) (Srivastava *et al.*, 2017). After that, hemolymph and tissues homogenates were centrifuged at 1×10^4 rpm for 15 minutes at 4°C. The hemolymph and tissues supernatant were collected after centrifugation and kept at -20 °C until being lyophilized.

5. Total protein concentration

After lyophilization, the sample extracts were weighed and dissolved in dimethyl sulfoxide (DMSO). The total protein concentration in all extracts was assayed according to Lowery method (Lowry *et al.*, 1951).

6. Cells and cell cultures

Human hepatocellular (HepG2) and human breast (MCF-7) cancer cell lines were grown in RPMI-1640 media supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic (penicillin) before being incubated in 5% CO₂ at 37°C. The cells were then planted at a concentration of 1×10^4 cells/well (100 µl/well) for 24 hours on 96 well plates. After seeding, cells were treated with serial concentrations (ranging from 0.25 to 8 mg/ml) of hemolymph, hepatopancreas and muscles extracts and incubated for 48 hours at 37°C and 5% CO₂.

7. Cell proliferation by MTT assay

The 3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to assess the antiproliferative effects of the previous extracts (Mosmann, 1983). After 48 h treatment, the media were discarded and cells were washed with PBS twice and supplemented with fresh medium. After that, 20 µl MTT solution were added to each well and the cells were incubated at 37°C and 5% CO₂ for 4 h. Then, 100 µl of DMSO was added to each well in order to solubilize formazan crystals that were formed in viable cells only, then the plates were centrifuged for 5 minutes at 4000 rpm. The absorbance was measured at 560 nm using a Bio-Tek microplate reader ELISA. The experiment was performed in triplicates. The percentage of cell viability was calculated according to the following formula:

$$\text{Cell viability (\%)} = (\text{AT} / \text{AC}) \times 100.$$

Where AT denotes the absorbance of treated cells and AC denotes the absorbance of the control (untreated cells).

The IC₅₀ (the concentration that inhibits the growth of 50% of cells) values of each sample were determined from a plot of dose-response curve between dose concentration on X-axis and cell inhibition percentage on the Y-axis.

8. DPPH radical scavenging assay

The free radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was used in order to evaluate the radical scavenging capacity of the investigated extracts according to the method of **Brand-Williams *et al.* (1995)**. A mixture of 100 µl DPPH solution (0.004% in 95% methanol) and 300 µl of each extract at the desired concentration (2 mg/ml) were incubated in the dark for 30-60 min at 25°C. Vitamin C was used as a reference (positive control), and the changes in color were determined using a spectrophotometer (UV-VIS Milton Roy) at 517 nm. The DPPH scavenging activity (%) was measured using the following equation:

$$\text{Antioxidant activity (\%)} = 100 \times [(A_C - A_E)/A_C]$$

Where A_C is the mean absorbance of negative control and A_E is the mean absorbance of each extract or reference.

9. Antimicrobial activity

The antimicrobial activity of mantis shrimp extracts was assessed using well diffusion method (**Magaldi *et al.*, 2004; Mercan *et al.*, 2006**). Antibacterial activity was determined against four bacterial strains (*Bacillus Subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 90274) and one fungal species (*Candida albicans* ATCC 10221). Briefly, a volume of microbial suspension was spreads over on the agar plate surface. Then, a 6 mm hole is punched aseptically with a sterile cork borer, and 100 µL of each tested extract (3 mg/ml) or controls is introduced into the well. Gentamycin and fluconazole were used as positive controls for bacteria and fungi, respectively. While, DMSO was used as a negative control. Then, agar plates are incubated under suitable conditions depending upon the test microorganisms. After incubation, *in vitro* antimicrobial activity was expressed in terms of diameter of inhibition zone in mm.

10. SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate protein bands of all extracts using **Laemmli (1970)** method with some modifications. In brief, equal volumes of each supernatant (40 µg protein) and solubilizing buffer (62.5 mM Tris-HCl (pH 6.8), 20% glycerol, 3% SDS, 0.5% 2-mercaptoethanol and 0.01% bromophenol blue) were heated at 95 °C for 4 min. Then, samples were immediately loaded into wells of a 12% SDS-PAGE separating gel.

Electrophoresis was carried out at a constant 35 mA for 2 h using Consort N.V. (Belgium) mini vertical electrophoresis system with running buffer. Coomassie Brilliant Blue R-250 (0.1%) was used to stain the gel overnight, and then de-stained with 5% methanol and 10% acetic acid until the bands became clear.

II. Statistical analysis

Statistical analysis of data was performed using SPSS software version 22.0. The results were represented as a mean value \pm SE. Student's t-test and One-way ANOVA followed by the Tukey's test were used to analyze the data. A statistically significant difference was reported, when P -value was higher than 0.05.

RESULTS

I. Size composition

Descriptive analysis of total body length (BL) and total body weight (BW) for both sexes and combined sexes of the mantis shrimp *E. massavensis* are presented in Table 1. Considering the overall specimens (1312), females (702) outnumbered males (610) during the study period. The length and weight of females ranged from 8.0 to 16.9 cm and 5.9 to 51 g, respectively. On the other hand, BL ranged from 7.8 to 18 cm and BW varied from 6.0 to 52 g for males. Differences in total length and weight between both sexes were non-significant ($P > 0.05$).

Table 1. Descriptive analysis of *E. massavensis* during the study period.

Sex	N	Body weight (g)			Body length (cm)		
		Min.	Max.	Mean \pm SE	Min.	Max.	Mean \pm SE
Female	702	5.9	51	21.054 \pm 0.31	8	16.9	12.084 \pm 0.063
Male	610	6	52	23.139 \pm 0.358	7.8	18	12.229 \pm 0.066
Combined sexes	1312	5.9	52	22.023 \pm 0.237	7.8	18	12.152 \pm 0.046

N: Counts, Min: Minimum, Max: Maximum and SE: Standard error.

2. Sex ratio

The abundance of both sexes of *E. massavensis* is shown in Fig.1. During the present study, females dominated the population with abundance of 54%, while males represented about 46% of the whole specimens, giving a female-biased sex ratio for the entire population (1.1:

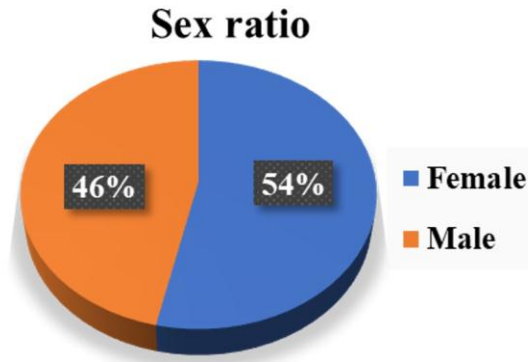


Fig. 1. Sex ratio for both sexes of *E. massavensis*.

3. Body length frequency distribution

The percentage of total body length frequency distribution for both sexes of *E. massavensis* was represented in Fig. 2. Male mantis shrimps dominated the population within the highest two class sizes (15-17 and 17-19 mm) and exhibited bimodal structure (23 and 1.0 individuals, respectively). Moreover, the maximum (261) and minimum (1) frequency of males were recorded within 11-13 cm and 17-19 cm class groups, respectively. On the other hand, females exhibited multimodal with the maximum frequency (298) was within the class size 11-13 cm. A highly significant difference ($P < 0.001$) was registered between both sexes in all size classes except 13-15 cm size group.

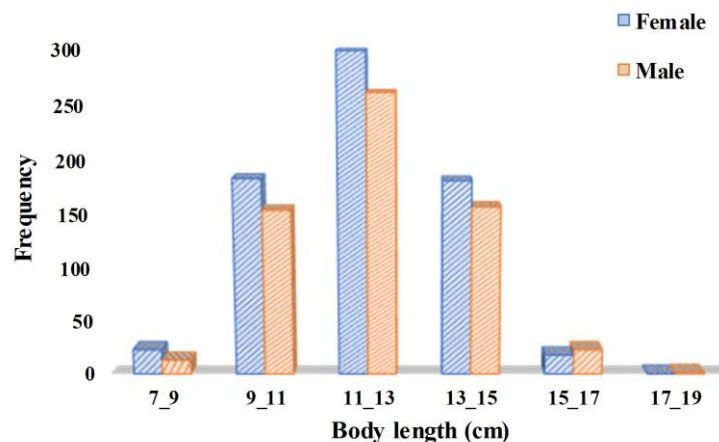


Fig. 2. Total body length frequency distribution for both sexes of *E. massavensis* collected during the study period.

4. Biometric relationships

4.1. Body length-body weight relationship

Fig. 3 displays the relationships between total body length (BL) and total body weight (BW) for females, males, and combined sexes of *E. massavensis*. The regression equations for these relationships were as the following:

$$BW = 0.023 BL^{2.716} \quad \text{for females.}$$

$$BW = 0.0245 BL^{2.716} \quad \text{for males.}$$

$$BW = 0.0231 BL^{2.726} \quad \text{for combined sexes.}$$

The estimated (b) values for females, males and combined sexes were 2.716, 2.716 and 2.726, respectively. These values indicated negative allometric growth pattern for all of them. The correlation coefficient (r) values were high (>0.9) indicating very strong correlations Table 2.

Table 2. Coefficient of regression and correlation for total body length (BL) and total body weight (BW) relationship of *E. massavensis*.

Relationship	Sex	b value	Growth pattern	r	R ²
BL-BW	Female	2.716	-ve	0.947	0.897
	Male	2.716	-ve	0.937	0.878
	Combined sexes	2.726	-ve	0.94	0.884

BL: total body length, BW: total body weight, b value: regression coefficient, -ve: negative allometry, r: correlation coefficient and R²: coefficient of determination.

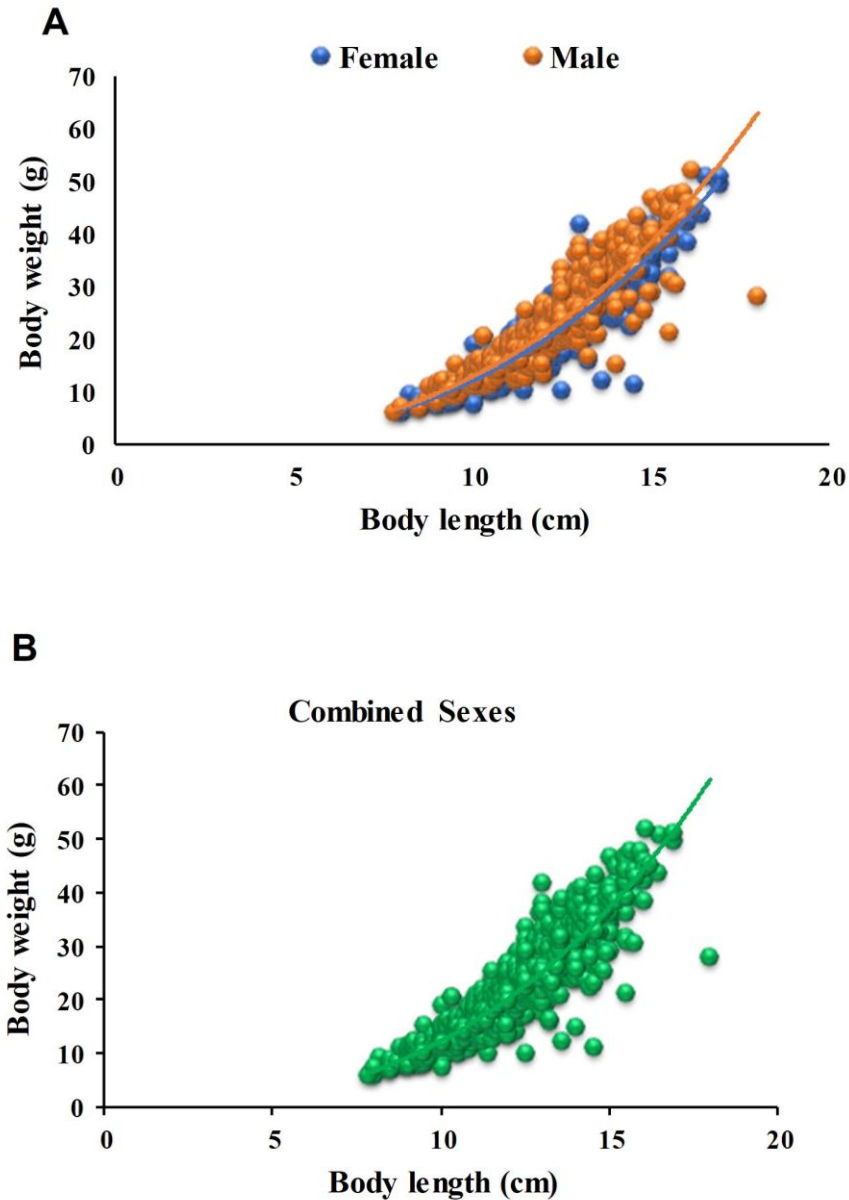


Fig. 3. Relationships between A; total body length and B; total body weight for females, males, and combined sexes of *E. massavensis*.

4.2. Condition factor

The condition factor values for females, males, and combined sexes were calculated during the study period. These results clarified that males exhibited higher K value than females Fig. 4. The K value was 2.576 for males, 2.424 for females, and 2.43 for combined sexes. The results revealed that K values for both sexes were higher than two.

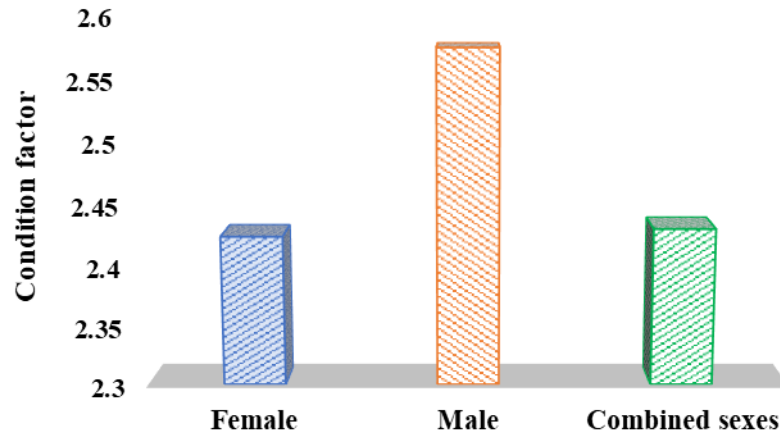


Fig. 4. Condition factor (K) for females, males and combined sexes of *E. massavensis*.

5. Cell proliferation by MTT assay

The viability of two human cancer cell lines (HepG2 and MCF-7) was inhibited in a dose-dependent manner after being treated with muscles (M), hepatopancreas (HP), and hemolymph (HL) extracts of *E. massavensis* for 48 h Fig. 5. Overall, HP possessed more cytotoxic activity than other extracts against both tested cancer cells. In terms of HepG2 cells, HP showed the highest antiproliferative effect against these cells with a significantly lowest IC_{50} dose of 1.14 mg/ml Fig. 5A. However, the IC_{50} values of HL and M were 2 and 2.66 mg/ml, respectively Fig. 6. The difference between IC_{50} doses of extracts was highly significant ($P < 0.001$). On the other hands, growth of more than 95% of MCF-7 cells was inhibited after being treated with the previous extracts at 8 mg/ml Fig. 5B, with IC_{50} doses of 1.6, 1.1 and 1.76 mg/ml for M, HP and HL, respectively. A non-significant difference between extracts was observed Fig. 6.

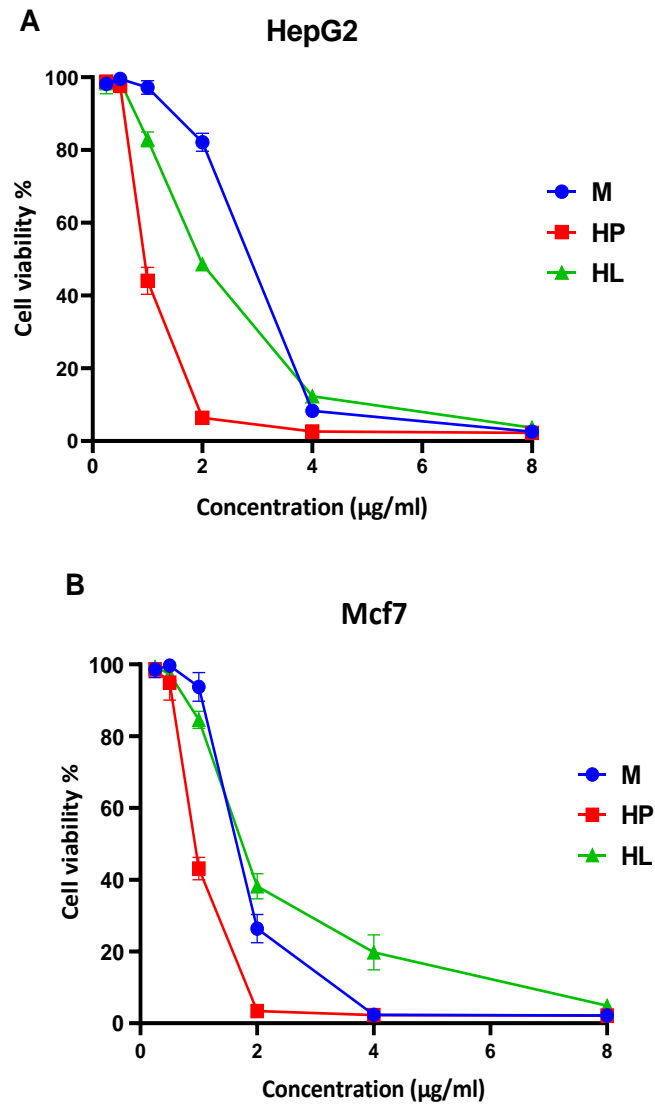


Fig. 5. Effects of muscles (M), hepatopancreas (HP), and hemolymph (HL) extracts of *E. massavensis* on cell proliferation of two human cell lines at different concentrations. A: liver (HepG2) and B: breast (MCF-7) cell lines.

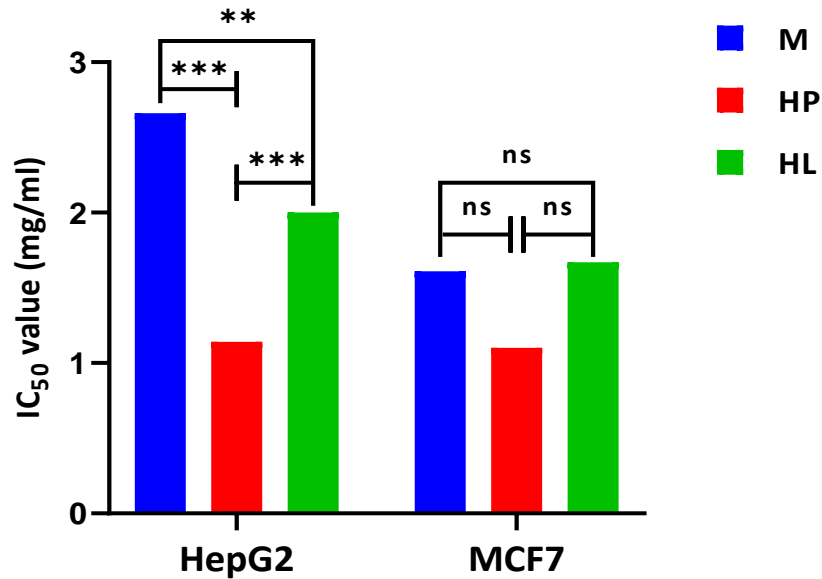


Fig. 6. IC_{50} values of muscles (M), hepatopancreas (HP), and hemolymph (HL) extracts of *E. massavensis* on HepG2 and MCF-7 cell lines. Bars sharing superscript of stars (*) differ significantly (** $P < 0.01$, *** $P < 0.001$), while ns means non-significant using a One-way ANOVA followed by the Tukey's test.

6. DPPH radical scavenging assay

Muscles, hepatopancreas and hemolymph extracts of *E. massavensis* showed a potent radical scavenging activity Fig. 7. Overall, muscles extract (M) exhibited the highest antioxidant properties with a significantly scavenging activity (49.3%) than that of HL and HP (37.14% and 26.37%, respectively) ($P < 0.001$).

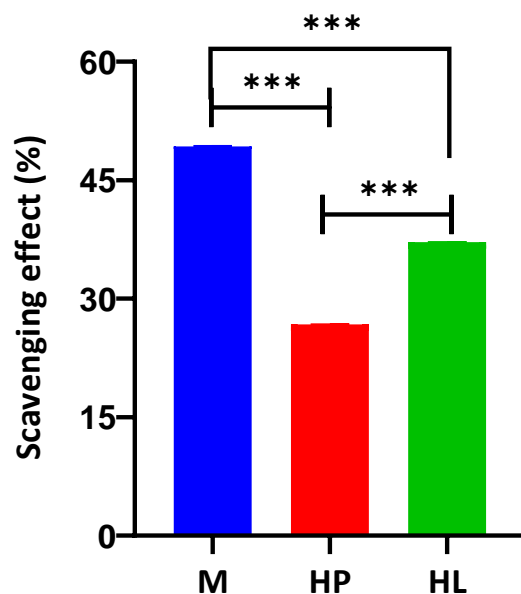


Fig. 7. DPPH radical-scavenging activity of muscles (M), hepatopancreas (HP), and hemolymph (HL) extracts of *E. massavensis*. Bars sharing superscript of stars (*) differ significantly (***) $P < 0.001$) using a One-way ANOVA followed by the Tukey's test.

7. Antimicrobial activity

The three extracts (M, HP, and HL) of *E. massavensis* exhibited a high antimicrobial activity against two Gram-positive and two Gram-negative bacteria and one fungal species Fig. 8. These extracts showed the same antibacterial activity as gentamycin Fig.9. The highest antibacterial activity of these extracts was observed against *E. coli*, while the minimum activity was observed against *S. aureus* and *P. aeruginosa*. Among these extracts, HP extract possessed significant antibacterial activity against *B. subtilis* with 30 mm inhibition zone. While, M extract showed the highest antibacterial activity against *E. coli* (38 mm). Similarly, the three extracts possessed high antifungal activity against *C. albicans* with significant largest inhibition zone (24, 25, and 21 mm for M, HP, and HL, respectively) than fluconazole (17 mm) Fig. 9.

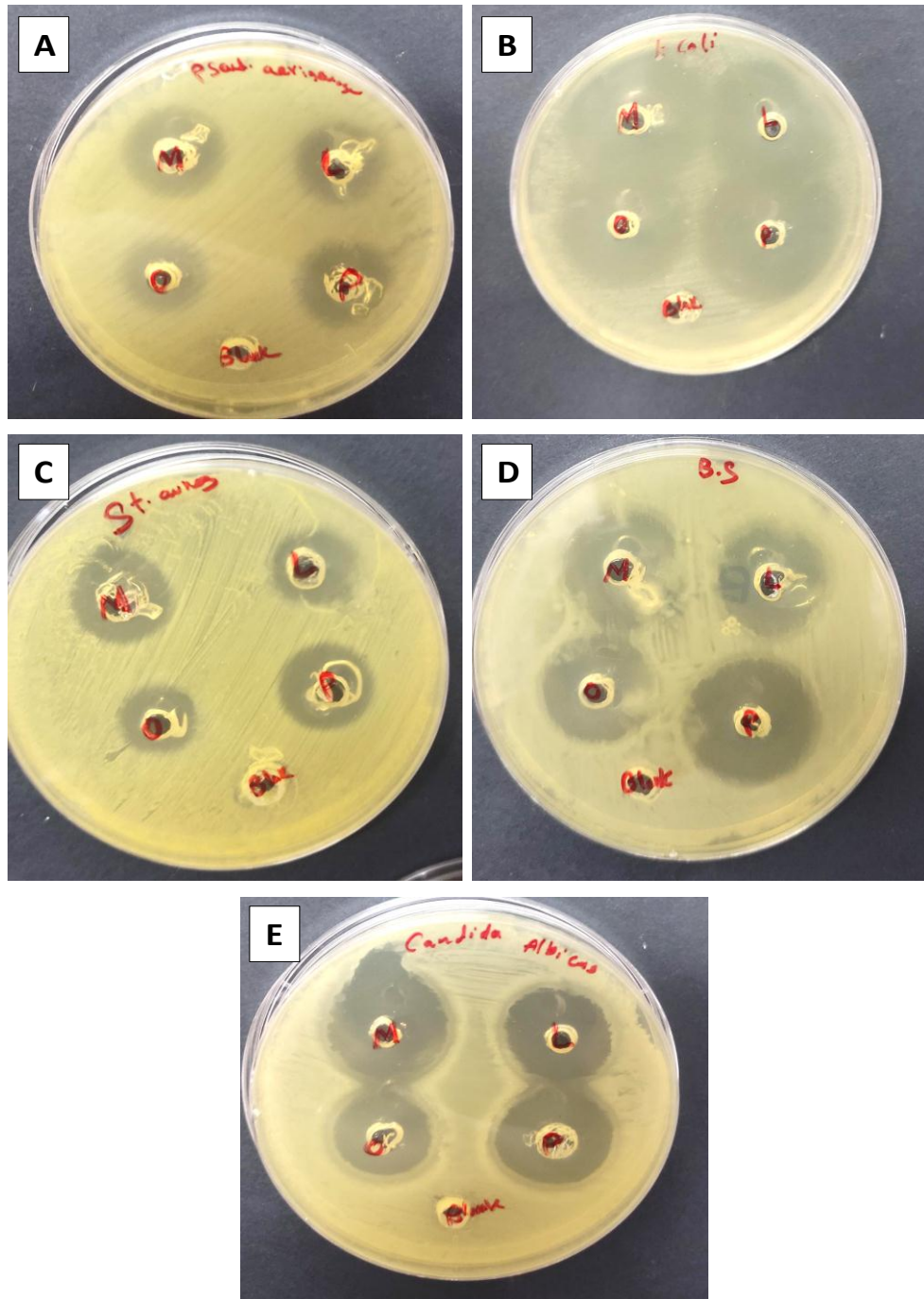


Fig. 8. Antimicrobial activity of muscles (M), hepatopancreas (HP), and hemolymph (HL) extracts of *E. massavensis* on five different microorganisms. A: *P. aeruginosa*, B: *E. coli*, C: *S. aureus*, D: *B. subtilis* and E: *C. albicans*.

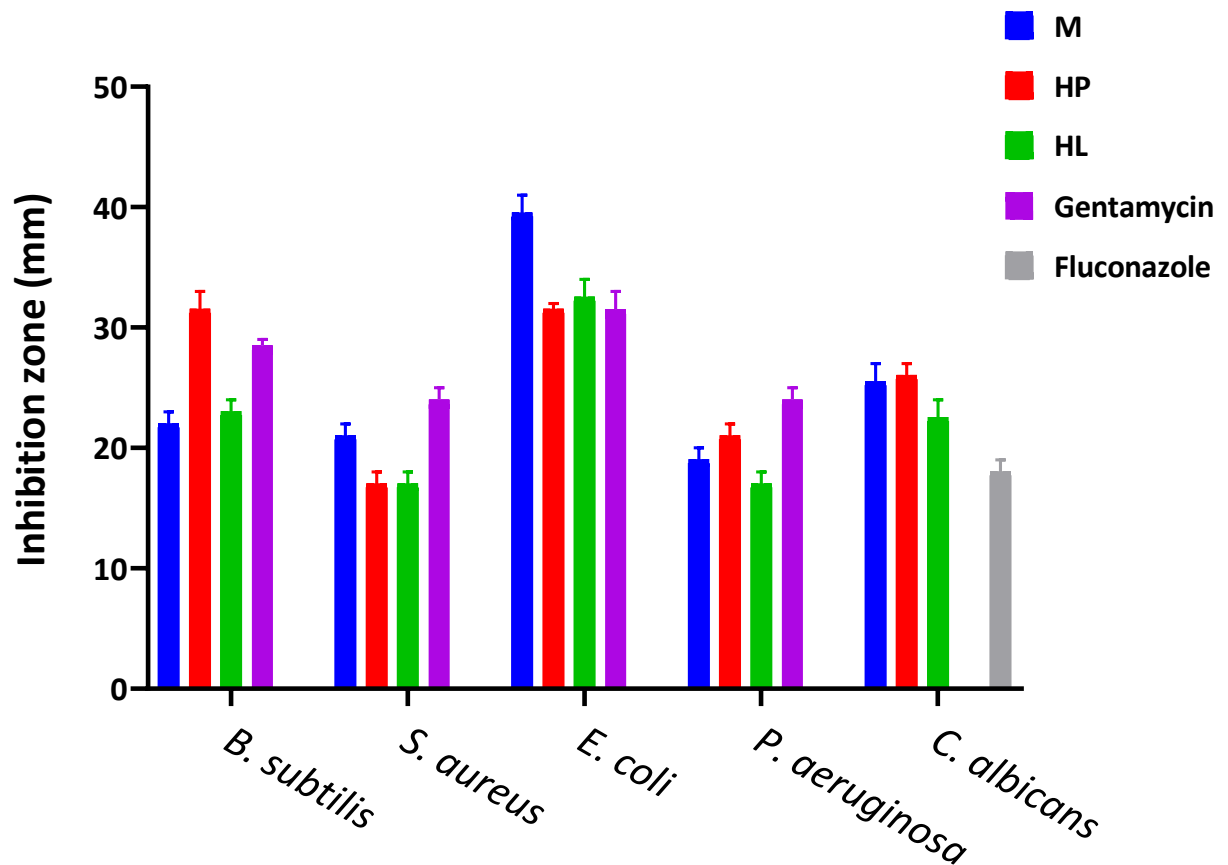


Fig. 9. Zone of inhibition of four bacteria species (*B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*) and one fungus (*C. albicans*) after being treated with muscles (M), hepatopancreas (HP), and hemolymph (HL) extracts of *E. massavensis*. Gentamycin and fluconazole are used as positive controls for bacteria and fungi, respectively.

8. SDS-polyacrylamide gel electrophoresis

Quantitative analysis of proteins from hemolymph, muscles, and hepatopancreas extracts of the mantis shrimp *E. massavensis* was performed using SDS-PAGE Fig.10. The SDS profile represented protein bands found in HL, M, and HP extracts. HL showed an increased number of protein bands than M and HP. For HL, the results revealed the presence of proteins in the range of 13 to ~250 kDa (lane 1). Meanwhile, about eight protein bands with molecular masses of ~ 17, ~ 28, ~ 40, ~ 50, ~ 70, ~ 100, ~ 150 and ~ 250 kDa were detected in the M extract (lane 2). However, the least bands number (7 bands) with molecular weights of ~ 17, 40, 50, 70, 100, 150 and 250 kDa were observed in HP extract (lane 3). About six bands with molecular weight between 40 and 250 kDa were observed commonly in all samples. A 17 KDa protein band was common in M and HP and a 28 KDa protein band was common in M and HL.

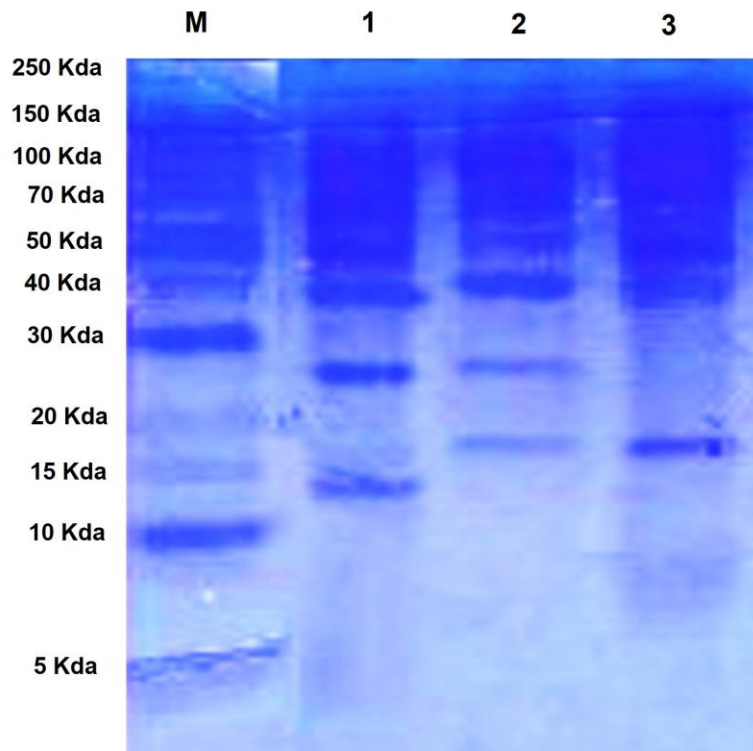


Fig. 10. The SDS-PAGE gel shows the protein profile in the hemolymph, muscles and hepatopancreas extracts of *E. massavensis*. M: marker, 1: protein extracts from hemolymph (HL), 2: protein extracts from muscles (M) and 3: protein extracts from hepatopancreas (HP).

DISCUSSION

The mantis shrimp *E. massavensis* is an important and abundant marine crustacean species in Port Said. In the current study, both sexes of *E. massavensis* exhibited similar length and body weight ($P > 0.05$). The overall sex ratio of *E. massavensis* was in favor of females (1.15:1). Similar results were reported for *Squilla mantis* in Turkey (Sağlam *et al.*, 2018), *Oratosquilla oratoria* in Korea (Kim *et al.*, 2017) and *Harpisquilla raphidea* in Indonesia (Mulyono *et al.*, 2016). The low abundance of males due to their increased mortality rates that may be because males of shrimps search for a new cavity every time they breed (Sallani, 2005). On the other hand, males of *Harpisquilla spp.* dominated over females (1.54:1) in Thailand (Samphan & Ratanamusik, 2018). Such studies explained that males outnumbered females during the breeding season because females may remain in their burrows after spawning (Hernández *et al.*, 2011; Türeli and Yeşilyurt, 2017). While, the numbers of both sexes of *Erugosquilla massavensis* in Turkey were the same (1:1) (Gökoğlu *et al.*, 2008).

The length-weight relationship is used to indicate the growth pattern of an organism either isometric or allometric growth. This can be determined using a mathematical equation to show relationship between length and weight. Isometric growth is observed when the regression coefficient (b) equals 3, otherwise allometric growth pattern is recorded (Ayodele & Fafioye, 2019). In the present study, both sexes of *E. massavensis* exhibited negative allometric growth as the b value was less than 3 (2.716 for females and males). The same growth pattern was recorded for the same species in Turkey (Gökoğlu *et al.*, 2008; Türeli & Yeşilyurt, 2017).

The condition factor (K) is an important index for indicating if the conditions are favorable or not in the environment of the marine creatures from the same or different habitats and related with mean body length. It is a crucial parameter used to assess the well-being and physiological state of any organism (Anene, 2005; Araújo & Lira, 2012). It can also be used for comparing the health or fattening of the marine organisms (Iftitah *et al.*, 2017; Famoofo & Abdul, 2020). During the current study, the condition factor of females and males was high than two indicating perfect health condition for both sexes in the study area (Moslen & Miebaka, 2018). In addition, the condition factor of males was higher than females. This may be related to the high length of males. Similar result was reported by Arshad *et al.* (2015) for *Harpiosquilla harpax* in Malaysia. Meanwhile, both sexes of *Harpiosquilla raphidea* exhibited the same condition factor value (Mulyono *et al.*, 2016).

E. massavensis is a rich source of protein, vitamins and minerals that are necessary for human body (Salam & Hamdi, 2015). In the present study, hemolymph, muscle, and hepatopancreas extracts of *E. massavensis* possessed a moderate antiproliferative activity in a dose-dependent manner against both tested cell lines (Mcf7 and HepG2). Among these extracts, HP exhibited the highest cytotoxicity against cancer cells. As other invertebrates, hepatopancreas is a crucial immune organ responsible for synthesis of numerous immune proteins that then are circulated with the hemolymph upon immune stimulation (Rószter, 2014; Zakzok *et al.*, 2021). The SDS-PAGE gel revealed the presence several protein bands that may possess anticancer activity. In addition, protein bands with molecular weight of 17 KDa and 198 KDa, similar to small and large classes of lectins, have been detected in HL and HP extracts. Such lectins possess antiproliferative activities and are considered as potential natural anticancer agents (Fujii *et al.*, 2012; Cheung *et al.*, 2015; Yau *et al.*, 2015). Interestingly, the antiproliferative activities of HL and HP may be attributed to hemocyanin with 70 KDa molecular size. Hemocyanin is an oxygen-transport protein that have a wide spectrum of biological activities such as antioxidant, antiparasitic, antimicrobial, and anticancer activities (Zhang *et al.*, 2004; Guo *et al.*, 2011; Zakzok *et al.*, 2021).

In the current study, M, HP, and HL extracts of *E. massavensis* showed relative degree of antioxidant activity. This revealed the capacity of these extracts to reduce

oxidative stress by scavenging reactive oxygen species such as DPPH. DPPH is a free radical that can be used to assess *in vitro* antioxidant properties of any extracts (Kedare & Singh, 2011). The antioxidant capacity of extracts was measured as their abilities to scavenge and convert DPPH (diphenylpicrylhydrazyl) radicals into the reduced form (diphenylpicrylhydrazine) by receiving a hydrogen atom from extracts (Molyneux, 2004). This revealed that DPPH radical scavenging activity of extracts may be related to their hydrogen donating ability. Among extracts, M extract possessed significant stronger antioxidant properties than HL and HP ($P < 0.001$). Muscles of *E. massavensis* are rich source of healthy fatty acids and vitamins that may have the ability to act as antioxidants or prooxidants (Abdel-Salam, 2013). These results of the present study are consistent with previous studies that have been reported the radical scavenging activities of *Erugosquilla massavensis* (Elkhodary *et al.*, 2017), *Harpiosquilla raphidea* (Noorani & Nazeer, 2020), *Oratosquilla woodmasoni* (Joshi *et al.*, 2020), *Odontodactylus scyllarus* (Scherbaum *et al.*, 2010) and *Oratosquilla nepa* (Chaijan & Panpipat, 2019).

Like invertebrates, *E. massavensis* possess a powerful innate immune system to fight pathogens and microbes. Antimicrobial peptides (AMPs) are secreted from hepatopancreas and hemocytes in order to slow microbial growth leading to the death of pathogens (Olatunde *et al.*, 2020). The findings of this study revealed that M, HL, and HP extracts of *E. massavensis* had high antimicrobial activity against different bacterial species and fungi. Such activity may be attributed to hemocyanins (70 KDa) that release histidine-rich AMPs and antifungal peptides in response to microbial infection. Such peptides have the ability to selectively bind to cell wall of microbes leading to the loss of plasma membrane integrity and pathogen clearance and death (Destoumieux-Garzón *et al.*, 2016).

CONCLUSION

The current study showed that females outnumbered males with sex ratio of 1.15:1 (F : M). Both sexes exhibited negative allometric growth pattern and condition factor indicated good health condition of both sexes of *Erugosquilla massavensis*. The present study revealed for the first time to the best of our knowledge the antiproliferative, antimicrobial, and antioxidant activities for muscles, hepatopancreas, and hemolymph of the edible mantis shrimp *E. massavensis*. This study showed that the hepatopancreas extract exhibited more antiproliferative activities than other extracts. However, detailed investigation of these biological activities for these extracts is needed to carry out to establish whether these extracts could be effectively used as potential sources for drugs discovery.

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