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Morphometric analysis of Echinometra mathaei (Blainville 1825) from the Northern Red Sea, Gulf of Suez, Egypt and the anticancer potential of its petroleum ether extract

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ABSTRACT

Sea urchins are ecologically important marine invertebrates with recognized medical, nutritional, and ecological significance. This study aimed to investigate the morphological characteristics of the sea urchin *Echinometra mathaei* collected from the Northern Red Sea, Gulf of Suez, Egypt, in September 2022. Simultaneously, the anticancer activity of a petroleum ether extract derived from the exoskeleton of *E. mathaei* was evaluated against the human liver cancer cell line HepG2. The results showed significant variation in weight, with the largest specimen weighing 80.4 g and the smallest 18.6 g. Diameter ranged from 3 to 7 cm and height varied from 1.5 to 3.5 cm. The maximum test length and total length were 6 and 9.5 cm, respectively, with minimum values of 2.5 and 5 cm. The length-weight relationship (LWR), diameter-weight relationship (DWR), and height-weight relationship (HWR) indicated a negative allometric growth trend ($b \neq 3$, $p > 0.001$). The extract exhibited cytotoxic activity against HepG2 cells, with IC_{50} values of 65.58 µg/ml at 24 hours and 23.21 µg/ml at 48 hours. Using flow cytometry analysis, the extract caused significant enrichment of G1 and S phases in HepG2 cells, confirming cell cycle arrest and apoptosis induction. These findings demonstrate that the petroleum ether extract from the exoskeleton of *E. mathaei* induces apoptosis in HepG2 cells, confirming its cytotoxic effect. Therefore, it emphasizes the potential of this marine-derived extract as a source of novel anticancer therapeutics.

Keywords: Sea urchins, *Echinometra mathaei*, Length-weight relationship, Red Sea, Apoptosis

1. INTRODUCTION

The marine environment, encompassing about seventy percent of the Earth's surface, is characterized by a high biodiversity of species and provides a habitat for numerous marine organisms that generate

diverse metabolites as protective mechanisms against predators [1]. Among these marine organisms, invertebrates include cnidarians, lophophorates, mollusks, arthropods and echinoderms [2]. Echinoderms are essential to marine ecosystems and belong to a diverse phylum comprising five classes: Holothuroidea (sea cucumbers), Crinoidea (feather stars), Ophiuroidea (brittle stars), Asteroidea (sea stars) and Echinoidea (sea urchins) [3].

Sea urchins are vital elements of marine ecosystems globally, valued for their medical, nutritional, and ecological significance [4]. Ecologically, they act as cleaners of the ocean floor and contribute significantly to the food chain and substrate modification [4,5]. These grazing animals greatly influence the ecology of coral reef habitats [6]. The diversity of sea urchins can vary considerably across location and time, where their spatiotemporal distribution and diversity are shaped by factors such as substrate characteristics, vegetation, seasonal fluctuations, and climatic events like El Niño [7, 8, 9, 10, 11]. The monograph by Clark and Rowe 1971 [12] is recognized as the first comprehensive study of echinoderms in the Indo-Pacific region, including the Red Sea, where they documented 189 species, with 43 echinoids. Later, Hasan 1995 [13] reported 34 echinoderm species from the Gulf of Suez, of which 17 were sea urchins. Recently eight species of echinoidea out of 33 echinoderm species have been identified along the Egyptian Red Sea Coast, including the Aqaba Gulf [14]. This diversity is likely attributed to their ability to thrive in various Red Sea habitats, such as seagrass, mangroves, sandy and rocky shores, and coral reefs [13, 15, 16, 17]. Echinoids currently dominate the echinoderm fauna of the Egyptian Red Sea coastline, comprising 62% of the recorded species. *Diadema setosum* and *Echinometra mathaei* were identified as eudominant species, while *Tripneustes gratilla* was the dominant species. *Phyllacanthus imperialis* was a resident species. Other notable species include *Echinothrix diadema*, *Heterocentrotus mammillatus*, and *Clypeaster humilis* [14].

Bioactive components derived from sea urchins have extensive medicinal applications and have been employed for a long time to treat various ailments. Isolated compounds from these creatures exhibit antimicrobial, anticancer, anti-inflammatory, antioxidant, anticoagulant, and anti-toxic properties [18]. Bonellinin, a bioactive compound from sea urchins, has significantly inhibited cancer cell growth [19]. Several anticancer compounds, including didemnin, bryostatin, and dolasstatin, have been isolated from sea urchins, showing promising results in clinical trials [18].

Shells of sea urchins such as *Hemicentrotus pulcherrimus*, *Hemicentrotus mamillatus*, *Strongylocentrotus droebachiensis*, *Glyptocidariscre nularis* and*. Anthocidaris crassispina* are considered effective anti-inflammatory agents [18]. Extracts from various organs of sea urchins, such as the gonads, gut, spines, and mouthparts of *T. gratilla*, demonstrate diverse biological activities, including antimicrobial and hemolytic effects [20]. Peptide fractions from the gonads of *S. nudus* exhibited strong antioxidant activity in both DPPH and reducing power assays [21]. Liu *et al.* 2006 [22] reported significant inhibitory effects of *S. nudus* eggs on tumor cells in mice, attributing this to the polysaccharide D-glucan. Furthermore, dichloromethane extracts and steroids from *D. savignyi* have demonstrated potent in vitro cytotoxic activity against various human cancer cells [1]. Among sea urchin species, *E. mathaei* is notably widespread along the Egyptian Red Sea coast and is recognized as the most abundant echinoid globally, particularly in shallow reef habitats [23].

Previous studies have shown that various solvent extracts from the sea urchin *E. mathaei* (Blainville 1825) [26] have distinct effects on different types of cancer cells. For instance, Vaseghi *et al*. 2018 [24] investigated the cytotoxic potential of dichloromethane (and butanol crude extracts from the entire body of *E. mathaei*, finding significant toxic activity against the HeLa cell line. Similarly, Abbass *et al*. 2024 [25] highlighted the potent cytotoxic effects of a methanolic extract from the exoskeleton of *E. mathaei* against liver cancer cells. These discoveries highlight the promising anti-cancer properties exhibited by *E. mathaei* extracts. Consequently, this investigation is focused on exploring the cytotoxic effects of the petroleum ether extract derived from the exoskeleton (shell and spines) of *E. mathaei* on HepG2 cancer cells, aiming to elucidate its potential mechanism of action.

2. MATERIALS AND METHODS

2.1 study area: The coastal zone of the study area at Al-Ain Al-Sokhna (29°53.572' N and 32°37.227' E) is predominantly characterized by rocky shores. The rocky substrate provides a stable habitat for various marine organisms, including a rich variety of echinoderms. The area is characterized by a high abundance of *E. mathaei*, which frequently occupies crevices and rock surfaces. grazing on algae and contributes to the ecological balance of the intertidal zone. The collection site was characterized by a mean sea surface temperature (SST) of 31°C, pH of 7.64, dissolved oxygen (DO) levels of 5.22 mg/l, electrical conductivity of 62.3 mS, and total dissolved solids (TDS) of 42.5 g/l, that measured with using electronic digital thermometers, a pH meter, a dissolved oxygen meter, a TDS meter, and a conductivity meter, respectively during samples collection in September 2022. Samples of *E. mathaei* were collected from the intertidal zone of a depth range 0.5-1m.

Fig (1): The study site at the Gulf of Suez, Egypt.

2.2 Samples collection: Samples of *E. mathaei* (Fig 2) were collected with a hook and immediately placed in an icebox and transported to the Medical Malacology Department Laboratories, TBRI, Giza, Egypt. The internal organs of the collected samples were removed using a sharp knife, after that the shell and spines of the exoskeleton were homogenized using a grinder and kept at -20°C until subsequent analyses.

Fig (2): The sea urchin *E. mathaei*.

2.3 Biometric analysis: Prior to morphometric analysis, samples were meticulously rinsed with seawater to remove any adhering debris, such as sand, dirt, or epibionts. Whole-body weight was measured using a sensitive balance. Diameter and height were determined with a caliper, while total length and test length were measured with a ruler (Fig. 3). The formula $W=aL^b$ was employed to estimate length-weight relationships [27]. Through linear regression analysis on $\log_{10}$ transformed weight and diameter values, coefficients a and b were derived and represented by $\log_{10}W = a + b \log_{10}L$ [28]. According to this methodology, growth patterns are classified as isometric when b=3 [29].

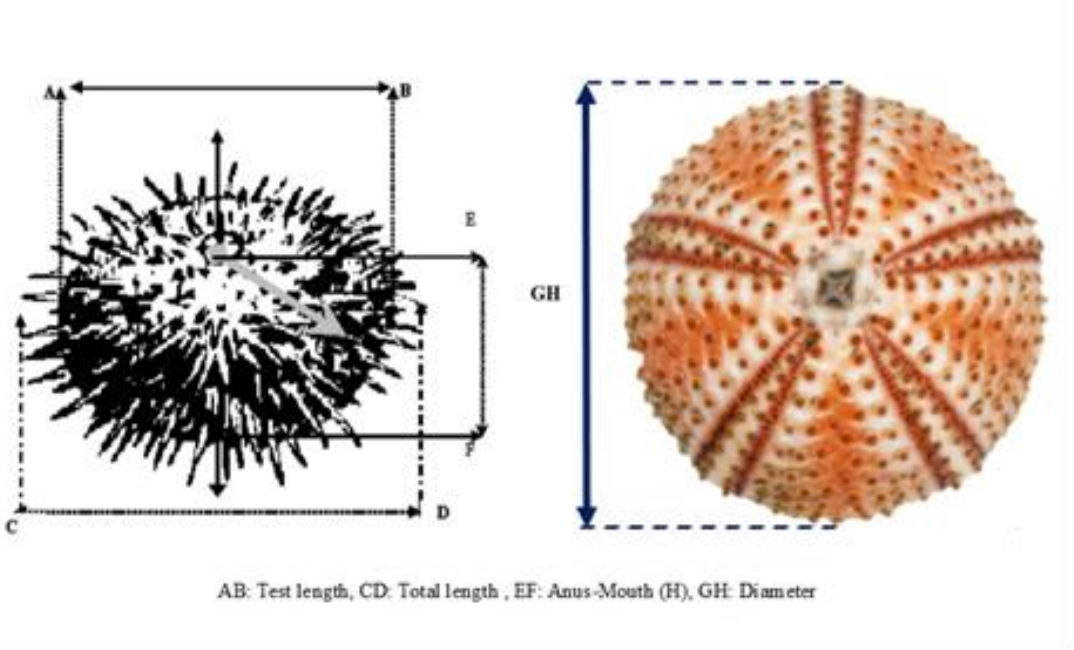


Fig (3): Morphometric parameters and measurement points of sea urchin.

2.4 Preparation of *E. mathaei* extracts: The exoskeleton (shell and spines) of sea urchin *E. mathaei* were isolated according to Fadl Allah *et al.* 2023 [30] with slight modification. The spine and shell (2300g) were homogenized using electric mil and dissolved in 3 L of methanol (MeOH) and allowed to sit for one week at room temperature. Then it evaporated under reduced pressure using a rotatory evaporator under vacuum till dryness at a temperature of 50 ˚C. This process was repeated three times and obtained 85 g of MeOH extract. A semi-dry MeOH extract (50 g) was dissolved in less amount of distilled water and then fractionated using organic solvents including petroleum ether 60-80˚ C, the fraction was dried utilizing a rotary evaporator, and the remaining water residue was thoroughly dried to yield 1.4 g of the petroleum ether fraction.

2.5 Cell lines and cell culture: The human cell line hepatocytes and three human carcinoma cell lines HepG2 were obtained from the American-type culture collection, which was frozen in liquid nitrogen. Serial sub-culturing was used to keep these cell lines alive in the VACSERA cell culture unit.

2.6 Assay of viability/cytotoxicity using MTT test: The cytotoxic effects of *E. mathaei* petroleum ether extract against different cell lines were measured by the MTT assay, following the principles described by Mosmann in 1983 [31]. Briefly, in the experiment, the HepG2 ($1x10^5$ cells/well) were seeded in 96 well tissue culture plates (100 µl /well) and allowed to reach 80% confluence at 37°C for 24 h. Subsequently, varying concentrations of *E. mathaei* petroleum ether extract (12.5 to 80 µg) in DMSO were added in triplicates and incubated for 24 h and 48 h. After incubation, MTT solution was added, and formazan crystals were dissolved using DMSO. The optical density at 570 nm was measured with a microplate reader. The experiment was repeated three times, and results were presented as Mean ± SD.
The percentage of surviving cells was calculated as a percentage of untreated control cells: Survival = (A treated cells / A control cells) X 100% Percent viability and the IC_{50} values (the concentration of *E. mathaei* petroleum ether extract required to inhibit cell growth by 50%) were assessed using nonlinear regression analysis (GraphPad Software Instant, version 10; Inc., La Jolla, CA, USA).

2.7 Flow cytometry-based Apoptosis Assay (Annexin V-FITC/PI Staining): Apoptosis was assessed in cells using annexin V-FITC/PI double labeling detection kit (Bio Vision Research Products, CA 94,043, USA) by flow cytometer (FACSCalibur, Becton-Dickinson, USA). HepG2 cells were cultured overnight in 6-well plates, treated with the IC_{50} of *E. mathaei* petroleum ether extract and incubated for 48 h at 37 °C with 5% CO_2. After trypsinization and centrifugation, pellets were washed with PBS, resuspended in 1X Binding Buffer, and stained with Annexin V-FITC and PI. Following 5 minutes of incubation in the dark at room temperature, mean fluorescence intensities were measured.

2.8 Cell cycle analysis by flow cytometry: HepG2 cells were seeded overnight in 6-well plates (~100,000–500,000 cells) and treated with the IC_{50} of *E. mathaei* petroleum ether extract for 48 h. Floating cells were collected from the culture media and adherent cells were harvested by trypsinization. Cells were centrifuged, washed with ice-cold PBS containing 1% FBS, and fixed with cold ethanol (66% followed by 100%) overnight at 4°C. After fixation, cells were washed to remove residual ethanol, resuspended in PI + RNase staining solution, and incubated at 37°C in the dark for 20–30 minutes. Data analysis was performed using CellQuest software.

2.9 Statistical analysis: All results were presented as mean ± SD. GraphPad Prism software, version 10 free trial, was used to analyze data using linear regression between size distributions (weight, diameter, height and total length) of sea urchins and student's t-test. The correlation coefficient (r) was used to measure the correlation strength and direction between the different variables. Differences at $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Size distribution of the *E. mathaei*: Samples of *E. mathaei* (n=30) exhibited a range of characteristics: The weight (W) of the sea urchin varied from the largest was 80.4g and the smallest was 18.6g with an average of (42.53 ± 17.93) g. The diameter (D) length ranged from 3–7cm with an average of (5.16 ±0.92) cm, while the height (H) varied from the smallest of 1.5 to the largest of 3.5cm with a mean of (2.62 ± 0.52) cm. The maximum values of test length (TEL) and total length (TL) were 6 and 9.5cm while the minimum values were 2.5 and 5cm with a mean (4.03 ± 0.84) cm and (7.22 ± 1.24) cm, respectively (Table 1). Similar studies reported that *E. mathaei* specimens from the Buleji rocky shore in Karachi, Pakistan, had shell lengths ranging from 19 to 77.2 mm and total weights between 13.3 and 121.6 g [32]. In other studies, the sea urchin *S. variolaris* from the Visakhapatnam Coast, India, was

reported to have a maximum shell length of 110 mm [33], while in Sri Lanka, *S. variolaris* was observed to have a maximum shell length of 89 mm [34]. *P. lividus* sea urchins from Tunisia's shallow coasts were found to have a shell length of 67.9 mm [35]. In another study, the shell length and total weight of *A. lixula* and *P. lividus* sea urchins from Urla-Iskele in Izmir Bay were reported to be 55.6 mm and 73.78 g, respectively, and 73.3 mm and 96.12 g, respectively [36].

3.2 Size frequency distribution of *E. mathaei*

3.2.1 Length-frequency distribution: Sea urchins' specimens were divided into six size classes, I, II, III, IV, V and VI. The first size class includes animals with a size smaller than 5 cm, and the fifth class includes animals larger than 9cm. The most frequently recorded size class was class IV (7.1-8 cm) which constituted about 36% of the whole community of the sea urchin. Size classes II (less than 5 cm), and III (6.01-7cm) have almost the same values (approx. 20% of the total population). It is clear that animals smaller than 5 cm (class I) and those larger than 9 cm (class VI) were very rare in the collection with about 8% and 5% for I and VI, respectively.

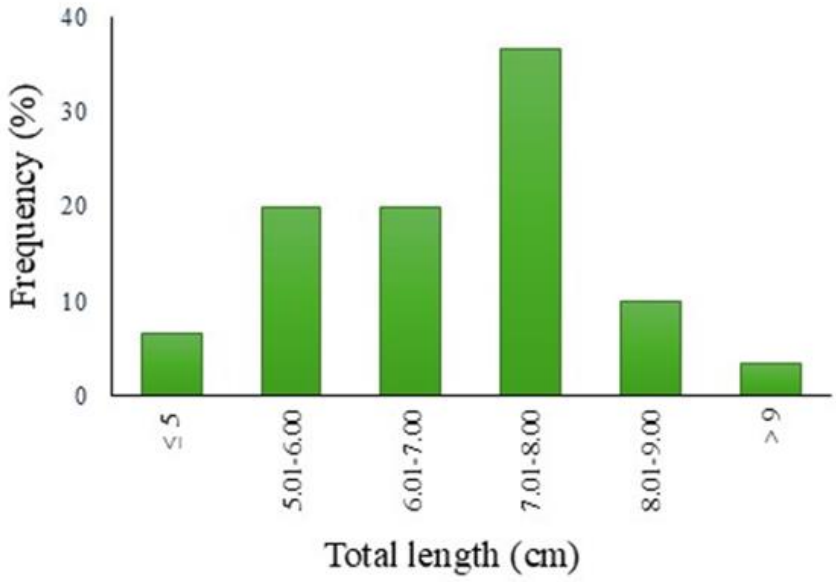


Fig (4): Length frequency distribution of E. mathaei collected from the Red Sea.

3.3 Biometric relationships of *E. mathaei*

3.3.1 Length-Weight Relationship (LWRs): LWRs of *E. mathaei* were estimated as $W = 0.1374*L^{1.7036}$ ($R^2 = 0.4753$) (Table 2). The constant (a) was 0.1374, while the growth coefficient (b) was 1.7036. Fig 5 shows the linear scale LWR curves. The LWR result revealed negative allometric ($b<3$) growth for *E. mathaei*.

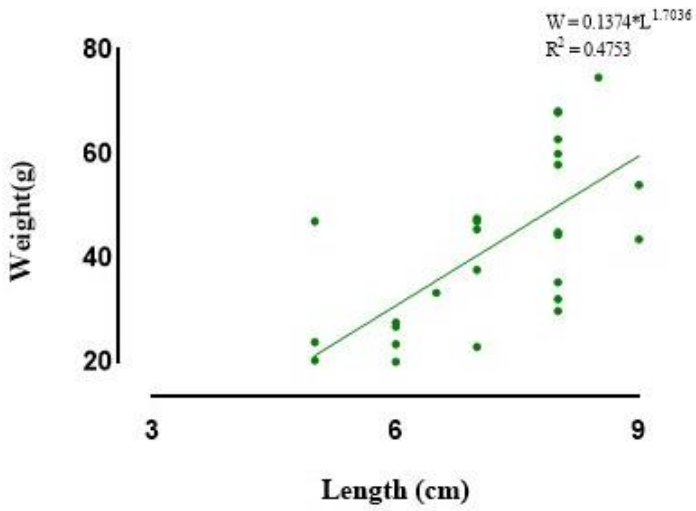


Fig (5): The length and weight relationship of E. mathaei.

3.3.2 Diameter-weight Relationship (DWRs): When estimating the diameter-weight relationship, the exponent 'b' (slope) was 2.003, as indicated in Table 2 and Fig 6. The correlation coefficient 'r' was determined to be 0.86, indicating a high level of significance at $p < 0.0001$ (Table 2). This strong correlation suggests a robust relationship between diameter and weight

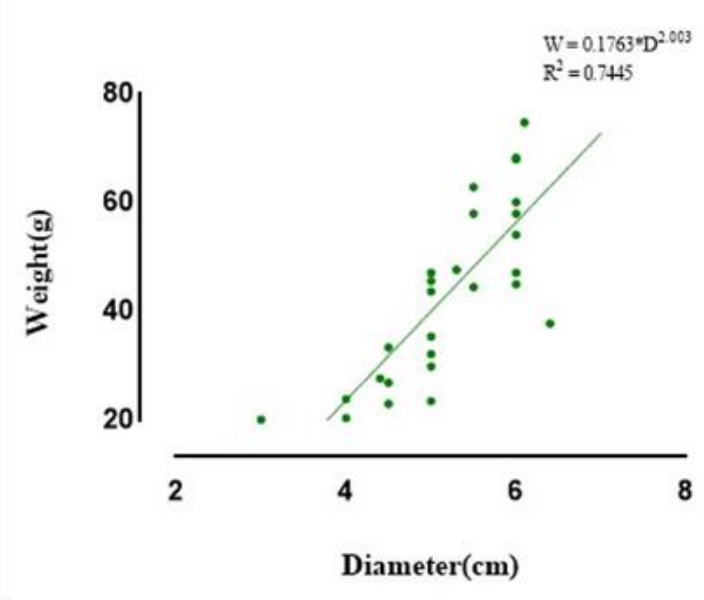


Fig (6): The diameter and weight relationship of E. mathaei.

3.3.3 Height-weight Relationship (HWRs): The linear regression was conducted between height and weight. The height-weight relationships in *E. mathaei* corresponding to the logarithmic form was log W= 0.9426+1.5813 log H (R^2= 0.5696) (Table 2 & Fig 7). The value of b was 1.513, indicating a negative allometric growth trend in *E. mathaei*.

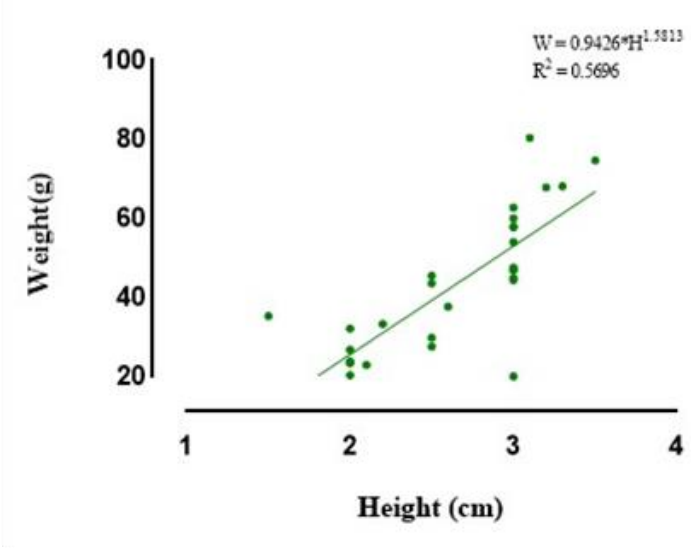
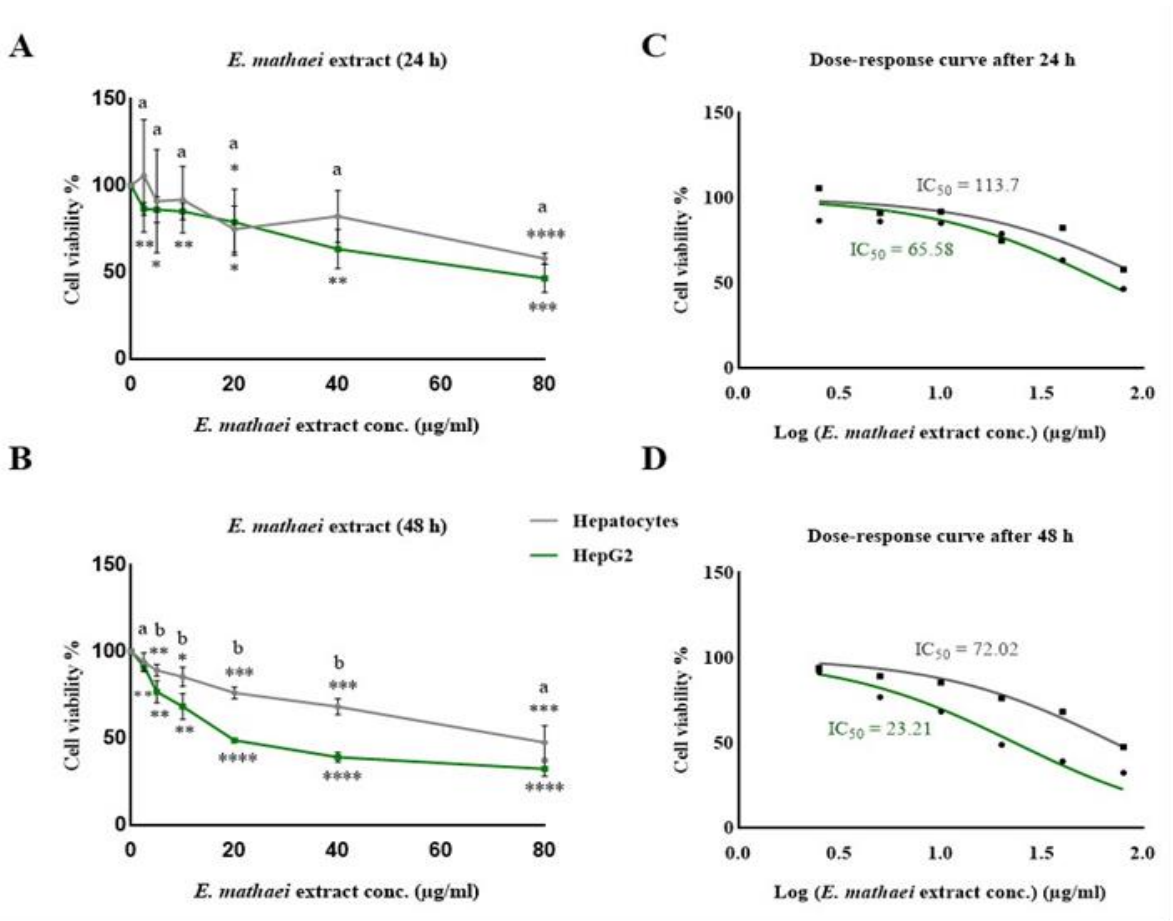


Fig (7): The height and weight relationship of E. mathaei.

In the current investigation, the b value of 2.00 for *E. mathaei* indicates negative allometric growth Among fishes and invertebrates, an exponent value (b) of 3 for the length-weight relationship indicates isometric growth in weight relative to length. When the b value deviates from 3, it indicates allometric growth. This type of growth can either be negative (b < 3) or positive (b > 3)[32]. Similar findings were reported by Siddique and Ayub 2016 [32] who also observed a b value of 2.4, indicating negative allometric growth in *E. mathaei*. Furthermore, prior studies on the LWRs of various sea urchin species such as *P. lividus, A. lixula, S. granularis* [37], *D. setosum* [29], *S. variolaris* [38], *P. lividus* [39] and *S. variolaris* [34] showed also a negative allometric growth. Factors contributing to this phenomenon in sea urchins are likely to involve environmental parameters and feeding habits [34].

3.4 Cytotoxic effect of the *E. mathaei* petroleum ether extract on HepG2 cancer cell line: The MTT assay was employed to assess whether the petroleum ether extract could impede the proliferation of cancerous HepG2 cells in contrast to normal hepatocytes. Various concentrations of the petroleum ether extract (2.5, 5, 10, 20, 40, and 80 µg/ml) were evaluated over 24 and 48-hour incubation periods. As depicted in Figure 8, exposure of hepatocytes and HepG2 cells to the petroleum ether extract resulted in a marked decline in HepG2 cell viability, particularly evident when treated with 5 µg/ml for 48 h. This reduction in cell viability exhibited a concentration-dependent trend (Fig 8 A and B). Furthermore, the dose-response curve illustrated that the IC_{50} of the petroleum ether extract for HepG2 cells was 65.58 and 23.21 µg/ml after 24 h and 48 h respectively (Fig 8 C and D).



	24 h		48 h	
	HepG2	Hepatocytes	HepG2	Hepatocytes
Log IC50	1.817	2.056	1.366	1.857
IC50	65.58	113.7	23.21	72.02
R squared	0.8817	0.8110	0.9394	0.9655

Fig (8): Cytotoxic effects of the *E. mathaei* petroleum ether extract on HepG2 liver cancer cells after 24- and 48-h incubation time. (**A)** Cytotoxic effects of the *E. mathaei* extract after 24h. **(B)** Cytotoxic effects of the *E. mathaei* extract after 48h. **(C)** Dose-response curve showing IC_{50} values after 24h calculated by non-linear regression (curve fit). **(D)** Dose-response curve showing IC_{50} values after 48h calculated by non-linear regression (curve fit). Data are presented as mean± SD, *p≤0.05 (according to two-tailed unpaired Student's t-test). (a) represents that there is no significant difference between hepatocytes and HepG2 cells and (b) represents that there is a significant difference between hepatocytes and HepG2 (according to two-tailed unpaired Student's t-test) cells (n=3 independent replicates).

Previous research has highlighted the anticancer properties of sea urchin extracts. For instance, Vaseghi *et al*. 2018 [24] reported that extracts from *E. mathaei* collected from the southwest coastline of the Persian Gulf exhibited notable anticancer properties. Similarly, Abbass *et al*. 2024 [25] demonstrated the potential of *E. mathaei* extracts from the Red Sea in Al-Ain Al-Sokhna, Egypt, in exhibiting cytotoxic effects. In the present study, we tested the petroleum ether extract obtained from *E. mathaei* collected from the Red Sea in Al-Ain Al-Sokhna, Egypt, on the HepG2 human liver cancer cell line. The cytotoxicity of the *E. mathaei* petroleum ether extract was evaluated using the MTT assay, assessing both HepG2 cells and healthy hepatocytes after 24 h and 48 h- exposure periods. The results revealed that the extract significantly suppressed HepG2 cell viability in a concentration-dependent manner after 48 h-exposures. In contrast, the cytotoxic effect on normal hepatocytes was less pronounced, showing concentration-dependent inhibition only after the 48-hour exposure period.

In agreement with Vaseghi *et al*. 2018 [24] found that both dichloromethane (CH_2Cl_2) and butanol crude extracts from the whole body of *E. mathaei* exhibited toxic activity against the HeLa cell line, further supporting the anticancer potential of *E. mathaei* extracts. Additionally, Abbass *et al*. 2024 [25], reported that methanolic extracts from the exoskeleton of *E. mathaei* exhibited potent cytotoxic effects against liver cancer cells. Furthermore, studies on other sea urchins have also demonstrated similar anticancer properties Such as, Abdelkarem *et al*. 2022 [40] reported that the ethyl acetate (EtOAc) crude extract from the exoskeleton of *Diadema setosum* exhibited inhibitory effects against the HeLa cancer cell line, reinforcing the potential of sea urchin-derived extracts in cancer treatment.

3.5 Apoptosis induced by *E. mathaei* petroleum ether extract on HepG2 cancer cell line: The Annexin V-FITC Apoptosis Staining/Detection Kit was utilized to identify cells undergoing apoptotic stages. HepG2 cells were treated with petroleum ether extract IC_{50} which demonstrated a significant suppression of cell growth. After the double staining analysis, cell population percentages were measured and divided into four classes as shown in Fig 9A. In recent study, treatment of HepG2 cells with IC_{50} concentration (23.21 µg/ml) of petroleum ether extract resulted in a significant increase in the percentage of cells undergoing early apoptosis. Specifically, the percentage of cells undergoing early apoptosis increased from 0.46 % to 19.56%. Furthermore, a substantial increase in the percentage of cells undergoing late apoptosis was observed, with the percentage rising from 0.19% to 7.13%. Moreover, there was a noticeable difference in necrotic cell counts from 1.86% to 2.48% (Fig 9 B and C). These results indicate a highly significant increase in the cell population at the early apoptosis stage.

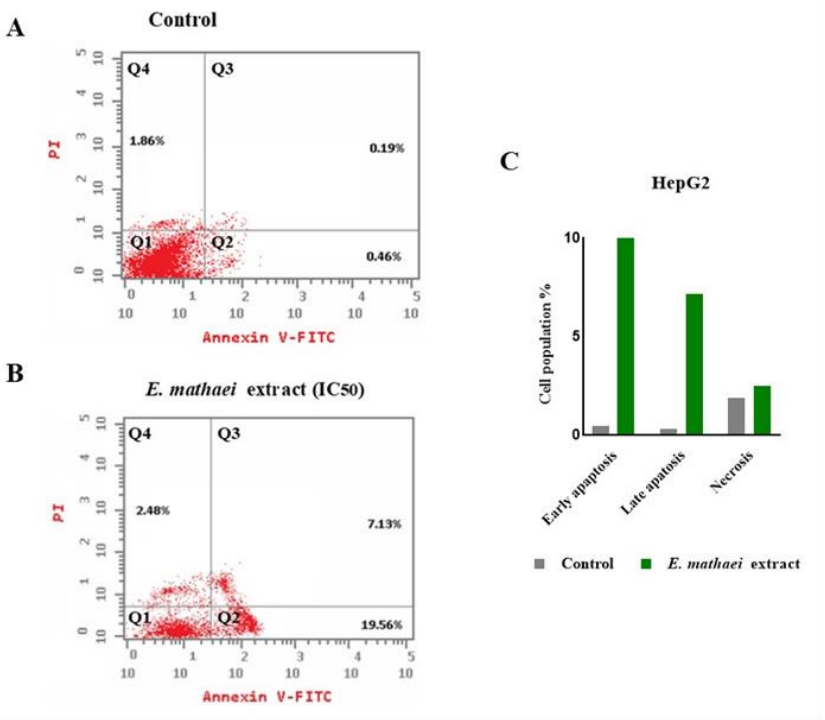


Fig (9): Flow cytometric analysis of FITC Annexin/PI apoptosis assay in HepG2 cells following treatment with *E. mathaei* petroleum ether extract. **(A)** Dot plots representation of flow cytometry apoptosis assay of control cells. **(B)** Dot plots representation of flow cytometry apoptosis assay of HepG2 cells after the treatment with IC_{50} value (23.21µg/ml) of *E. mathaei* extract after 48 h**. (C)** Bar graph representation of each cell death status of HepG2 cells compared to control cells.

3.6 Effect of *E. mathaei* petroleum ether extract on cell cycle progression of HepG2 cancer cell line: The inhibitory effect of petroleum ether extract on HepG2 cells was investigated by the cell cycle distribution in the tested cells after exposure to IC_{50} concentration (23.21µg/ml) for 48h. The results showed that the cell cycle in HepG2 was significantly impacted by the petroleum ether extract as compared to the control. It was observed that cell G1/S cycle arrest was induced in HepG2 cells by the

IC_{50} concentration of petroleum ether extract. This was manifested by a significant increase in the percentage of cells in the G1 phase and S phase by 1.54% and 5.13%, respectively. This was accompanied by a corresponding decrease in the G2/M phase of 6.67 % compared to the control as shown in (Fig 10 B and C). The result demonstrates that petroleum ether extract inhibits cell proliferation of HepG2 cells which is mediated by G1/S cell cycle arrest and induces apoptosis.

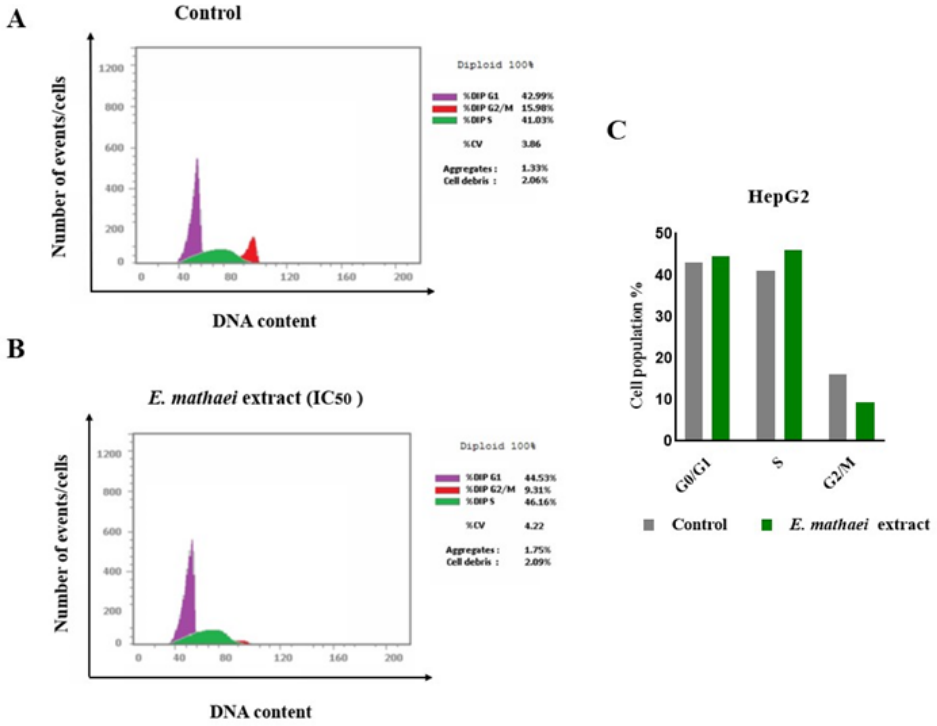


Fig (10): Cell cycle distribution in HepG2 cells after incubation with IC_{50} concentration of petroleum ether extract. HepG2 and control cells were stained by Propidium Iodide Flow Cytometry Kit after 48 h-incubation time. **(A)** Representative flow cytometry histogram of cell cycle distribution for control cells. **(B)** Representative flow cytometry histogram of cell cycle distribution for HepG2 cells after treatment with *E. mathaei* extract. **(C)** Percentage of HepG2 cells in cell cycle phases.

Inducing apoptosis through cytotoxic natural substances is considered a vital strategy in cancer treatment and drug development [41]. To evaluate whether *E. mathaei* petroleum ether extract inhibits liver cancer cell proliferation by promoting apoptosis, treated cells were analyzed using dual staining (annexin and PI) along with cell cycle assessment. This dual staining method allows for the differentiation between living cells, early and late apoptotic cells, and necrotic cells [42]. In this study, HepG2 cells exhibited an increase in both early apoptotic and late apoptotic/necrotic cells, indicating that the petroleum ether extract of *E. mathaei* induces apoptosis in liver cancer cell lines. Notably, the extract had a more pronounced effect on early apoptosis compared to late apoptosis. These results are consistent with the findings of Abbass *et al*. 2024 [25], methanolic extracts from the exoskeleton of *E. mathaei* were reported to induce apoptosis in HepG2 cells. Likewise, CH_2CH_2 extracts from D. *savignyi* were shown to induce apoptosis in HL-60, PC-3, and SN cell lines [1]. Additionally, lipids extracted from *S. nudus* (SUL) have been found to promote apoptosis in human hepatocellular carcinoma cells [43].

Additionally, *E. mathaei* petroleum ether extract was found to enrich the G1 and S phases in treated HepG2 cells, confirming its role in inducing cell cycle arrest at these phases and promoting apoptosis. Similarly, the coelomic fluid extract of *A. lixula* was reported to inhibit the cell cycle at the S phase in triple-negative breast cancer cells [44].

4. CONCLUSION

This study highlights the morphological characteristics of the sea urchin *E. mathaei* from the Red Sea and demonstrates the significant anticancer potential of the petroleum ether extract derived from its exoskeleton against the HepG2 human liver cancer cell line, promoting cell cycle arrest followed by apoptosis. Moreover, the negative allometric growth trends observed in the sea urchin specimens provide insights into their ecological adaptability, which may influence the bioactive properties of the extract. These findings suggest that *E. mathaei* may serve as a valuable source of novel anticancer agents, warranting further investigation into its bioactive compounds and their mechanisms.

Table 1: The weight (g), diameter (cm), height (cm), test length (cm) and total length (cm) of the sea urchin *E. mathaei* collected in the intertidal zone of the Red Sea, Al-Ain Al-Sokhna City, Egypt.

N	Weight (g)	Diameter (cm)	Height (cm)	Test Length (cm)	Total Length (cm)
1	62.8	5.5	3	6	8
2	37.7	6.4	2.6	4	7
3	27.6	4.4	2.5	3.5	6
4	47.6	5.3	3	4.5	7
5	47.02	6	3	5	7
6	47	5	3	4	5
7	57.9	6	3	4.5	8
8	23.4	5	2	3	6
9	80.4	7	3.1	5	8
10	58.6	6	3	4.5	8
11	23.8	4	2	3	5
12	18.6	4	2	3	6
13	43.6	5	2.5	4	9
14	67.9	6	3.2	5	8
15	56.7	6	3	4	9
16	74.7	6.1	3.5	5	8.5
17	33.3	4.5	2.2	3.5	6.5
18	68.2	6	3.3	4	8
19	57.9	5.5	3	5	9.5
20	44.4	5.5	3	4.5	8
21	22.9	4.5	2.1	3	7
22	45.5	5	2.5	4.5	7
23	26.8	4.5	2	3.5	6
24	44.9	6	3	5	8
25	32.1	5	2	3.5	8
26	29.8	5	2.5	4	8
27	19.1	3.5	2	3	6
28	20.3	4	2	2.5	5
29	20	3	3	3	6
30	35.3	5	1.5	4	8
MEAN	42.53	5.16	2.62	4.03	7.22

Table 2: The results of linear regression analysis and correlation for the different measurements and weight relationships of *E. mathaei*.

Relationship	Logarithmic transformation	Regression Coefficient		r	R^2	p-value
		b value	Iso- allometric growth			
LWRs	Log W= 0.1374+1.7036 log D	1.70	-ve	0.68	0.47	0.0001
DWRs	Log W= 0.1763+2.003 log D	2.00	-ve	0.82	0.74	0.0001
HWRs	Log W= 0.9426+1.5813 log H	1.58	-ve	0.75	0.56	0.0001
*Regression is significant at the 0.05 level						

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